1. NUTRIENT AGAR

Peptone	5.0	g
Meat extract	3.0	g
Agar, if necessary	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0. For Bacillus strains the addition of 10.0 mg $MnSO_4$ x H_2O is recommended for sporulation.

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2. BACILLUS PASTEURII MEDIUM

To medium 1 add 20 g/l urea before autoclaving. Do not adjust pH; pH raises to about 8 due to heat degraded urea.
For sporulation enhancement add 10 mg/l $MnSO_4$ x H_2O.

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3. AZOTOBACTER MEDIUM

Glucose	5.00	g
Mannitol	5.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
Na_2MoO_4 x 2 H_2O	5.00	mg
K_2HPO_4	0.90	g
KH_2PO_4	0.10	g
$FeSO_4$ x 7 H_2O	0.01	g
$CaCO_3$	5.00	g
Agar	15.00	g
Distilled water	950.00	ml

Adjust pH to 7.3.
Sterilize glucose and mannitol separately (in 50 ml H_2O) and add to the medium after autoclaving. Calcium carbonate in the medium serves as a buffer. The calcium carbonate will settle in agar plates before the agar has set, producing an opaque layer in the bottom. As the strain grows and acid is produced this will react with the calcium carbonate, causing it to dissolve and form zones of clearing immediately below the colonies.

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6. ALLANTOIN MINERAL MEDIUM

K_2HPO_4	0.8	g
KH_2PO_4	0.2	g
$MgSO_4$ x 7 H_2O	0.5	g
$CaCl_2$ x 2 H_2O	0.05	g
$FeSO_4$ x 7 H_2O	0.01	g
$MnSO_4$ x H_2O	1.0	mg
Allantoin	20.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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7. ANCYLOBACTER - SPIROSOMA MEDIUM

Glucose	1.0	g
Peptone	1.0	g
Yeast extract	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0

For DSM 27188 use washed agar and adjust to pH 5.0-5.5.

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8. BACILLUS "RACEMILACTICUS" MEDIUM

Glucose	5.0	g
Peptone	5.0	g
Yeast extract	5.0	g
$CaCO_3$	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 6.8.

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9. VY/2 AGAR

Baker's yeast	5.00	g
$CaCl_2$ x 2 H_2O	1.36	g
Vitamin B_{12}	0.50	mg
Agar (Difco)	15.00	g
Distilled water	1000.00	ml

Sterilize vitamin B_{12} separately by filtration. Prepare and store yeast cells as autoclaved stock suspension (5 g baker's yeast/100 ml distilled water, adjust pH to 6.5 and autoclave). Adjust pH of medium to 7.2 with KOH before, and after autoclaving and cooling to 50°C (use pH-indicator paper).

For DSM 11116 reduce amount of vitamin B_{12} to 0.05 mg/l.

Cultures of myxobacteria delivered freeze-dried:
Please see our video tutorial and follow the special instructions: 'Reactivation of Myxobacteria' given with the strain entry of our catalogue. For suspending the freeze-dried cells from ampoules, add about 0.5 - 1.0 ml medium MD1 (per liter: casiton 3.0 g; calciumchloride dihydrate 0.7 g; magnesiumsulphate heptahydrate 2.0 g) to the vial with freeze dried material.

Cultures of myxobacteria delivered as active cultures (growing on agar plates): Always use the rim of the swarm as inoculum for fresh media. If the swarms are creamy, transfer high amounts of cell mass to several spots on fresh VY/2 agar medium. If the swarm adheres to the agar or grows within the agar, cut small agar cubes from the rim of the swarm and place onto a fresh agar plate using an appropriate tool such as a lancet. Make sure that the pieces of swarm colonies grown on the agar are transferred to the agar plate. Attempt to place the inoculum in such a way that the swarms are in contact with the fresh agar plate.

Incubate for up to 3 weeks (in particular *Sorangium* and *Nannocystis* strains) at the temperature given for the strain, taking measures against desiccation. If there is no growth after ten days, carefully split up the agar-culture-cubes and squeeze the material to the agar plate and reincubate.

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10. ZYMOMONAS MEDIUM

Bacto peptone	10.0	g
Yeast extract	10.0	g
Glucose	20.0	g
Agar if required	15.0	g
Distilled water	1000.0	ml

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11. MRS MEDIUM

Casein peptone, tryptic digest	10.00	g
Meat extract	10.00	g
Yeast extract	5.00	g
Glucose	20.00	g
Tween 80	1.00	g
K_2HPO_4	2.00	g
Na-acetate	5.00	g
$(NH_4)_3$ citrate	2.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$MnSO_4$ x H_2O	0.05	g
Distilled water	1000.00	ml

Adjust pH to 6.2 - 6.5.

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12. SOIL EXTRACT MEDIUM

Sterilize 400.0 g of air-dried garden soil (with high content of organic matter) in 1000 ml tap water for one hour at 121°C. Allow it to sediment for a few hours at room temperature. Centrifuge the supernatant. Add 15.0 g agar per 1000 ml to the clear supernatant solution thus obtained. Adjust pH to 6.8 - 7.0 and sterilize.

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13. BACILLUS ACIDOCALDARIUS MEDIUM

Solution A:

Yeast extract	1.00	g
$(NH_4)_2SO_4$	0.20	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
KH_2PO_4	0.60	g
Distilled water	500.00	ml

Adjust pH to 3.0 - 4.0.

Solution B:

Glucose	1.00	g
Agar	20.00	g
Distilled water	500.00	ml

Sterilize solution A and B separately at 121°C for 15 min. Cool to 50°C and then combine.

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14: CLOSTRIDIUM FORMICACETICUM MEDIUM

Yeast extract	5.00	g
K_2HPO_4	10.00	g
Trace element solution SL-4	10.00	ml
Pyridoxine hydrochloride (0.1% w/v)	1.00	ml
Na-thioglycolate	0.75	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
D-Fructose	5.00	g
Agar, for solid medium (optional)	15.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate and fructose) and sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fructose from a sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Adjust pH of complete medium to 8.0.

Trace element solution SL-4 (from medium 14)

Na_2-EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.

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21. SARCINA MEDIUM

Glucose	30.0	g
Peptone	5.0	g
Yeast extract	5.0	g
Distilled water	1000.0	ml

Adjust pH to 6.0.

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25. RHODOPSEUDOMONAS GLOBIFORMIS MEDIUM

Yeast extract	0.25	g
Mannitol	1.50	g
Na-gluconate	0.50	g
KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NH_4Cl	0.40	g
NaCl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-6 (see medium 27)	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 4.9, then add:

Fe(III) citrate solution (0.1% in H_2O)	5.00	ml
Biotin solution (0.002% in H_2O)	1.00	ml
p-Aminobenzoic acid solution (0.01% in H_2O)	1.00	ml

Sterilize in screw-capped bottles. Cool to room temperature; to each 100 ml medium add 0.2 ml of a sterile 10% $Na_2S_20_3$ solution. Incubate in the light using a tungsten lamp.

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26. ACID RHODOSPIRILLACEAE MEDIUM

Use medium 27 and reduce the yeast extract concentration to 0.2 g/l. Adjust pH to 5.7. Incubate in the light using a tungsten lamp.

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27. RHODOSPIRILLACEAE MEDIUM (modified)

Yeast extract	0.30	g
Na_2-succinate	1.00	g
(NH_4)-acetate	0.50	g
Fe(III) citrate solution (0.1% in H_2O)	5.00	ml
KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Vitamin B_{12} solution (10 mg in 100 ml H_2O)	0.40	ml
Trace element solution SL-6 (see below)	1.00	ml
L-Cysteiniumchloride	0.30	g
Resazurin(0,1%)	0.50	ml
Distilled water	1000.00	ml

Adjust pH to 6.8.
Boil the medium for a few minute. Bubble the medium with nitrogen gas and fill 10 ml in 15 ml tubes with a rubber septum under a stream of nitrogen gas. Autoclave at 121°C for 15 min. Sterile syringes are used to inoculate and remove samples.
Incubate in the light using a tungsten lamp.

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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28. PFENNIG'S MEDIUM I

Solution A:

$CaCl_2$ x 2 H_2O	0.25	g
Yeast extrakt	0.25	g
Distilled water	460.00	ml

Fill 10x 46ml in 100 ml screw-cap bottles. Bubble with N_2/CO_2 and autoclave 121°C 15 min.
(For marine or estuarine isolates add 100.0 g NaCl to this solution and increase the $MgSO_4$ x 7 H_2O to 15.0 g).

Solution B:

Na_2S x 9 H_2O	2.00	g
Distilled water	135.00	ml

Prepare in a screw-cap bottle, bubble with N_2 to replace air, close tightly and autoclave.

Solution C:

$NaHCO_3$	1.50	g
H_2O	50.00	ml

Bubble with CO_2 and filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution D

Resazurin (0,1%)	0.5	ml
Distilled water	450.00	ml

Autoclave in a cotton-stoppered Erlenmeyer flask with an outlet tube for medium, connected to a glass outlet at the bottom of the vessel and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles.
Cool to room temperature under an atmosphere of N_2/CO_2 in an ice bath.

Solution E:

Ammonium chloride	0.35	g
Ammonium acetate	0.25	g
Pyruvic acid sodium salt	0.25	g
Dextrose	0.25	g
$MgSO_4x7H_2O$	0.50	g
KCL	0.35	g
KH_2PO_4	0.35	g
Trace element solution SL-12 B	1.00	ml
Distilled water	25	ml

Filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

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Solution F:

Vitamin B_{12}	0.01	g
Distilled water	100.00	ml

Filter sterilized

Trace element solution SL-12 B:

Distilled water	1000.00	ml
Na_2-EDTA	3.00	g
$FeSO_4$ x 7 H_2O	1.10	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 2 H_2O	50.00	mg
$ZnCl_2$	42.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	18.00	mg
H_3BO_3	300.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg

Adjust pH to 6.0.

Mix solution D, C and E. Bubble with CO_2 in an ice bath under sterile conditions.
Fill 50 ml in each bottle of solution A. Before using add 4 ml solution B and 0.1 ml solution F.
Adjust the pH with filter-sterilised 1M Na_2CO_3 to 7.1-7.3.
Fill in sterile, N_2 gassed screw-cap tubes under N_2 gas.

During the first 24 h, the iron of the medium precipitates in the form of black flocks. No other sediment should arise in the otherwise clear medium. Feed periodically with neutralized 3% solution of sodium sulfide to replenish sulfide and with other supplement solutions (see Ref. 3365).

Neutralized sulfide solution:

Distilled water	100.00	ml
Na_2S x 9 H_2O	3.00	g

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.
Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

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29. PFENNIG'S MEDIUM II

Solution A:

$CaCl_2$ x 2 H_2O	0.25	g
Yeast extrakt	0.25	g
Distilled water	460.00	ml

Fill 10x 46ml in 100 ml screw-cap bottles. Bubble with N_2/CO_2 and autoclave 121°C 15 min.
(For marine or estuarine isolates add 100.0 g NaCl to this solution and increase the $MgSO_4$ x 7 H_2O to 15.0 g).

Solution B:

Na_2S x 9 H_2O	2.00	g
Distilled water	135.00	ml

Prepare in a screw-cap bottle, bubble with N_2 to replace air, close tightly and autoclave.

Solution C:

$NaHCO_3$	1.50	g
H_2O	50.00	ml

Bubble with CO_2 and filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution D

Resazurin (0,1%)	0.5	ml
Distilled water	450.00	ml

Autoclave in a cotton-stoppered Erlenmeyer flask with an outlet tube for medium, connected to a glass outlet at the bottom of the vessel and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles.
Cool to room temperature under an atmosphere of N_2/CO_2 in an ice bath.

Solution E:

Ammonium chloride	0.35	g
Ammonium acetate	0.25	g
Pyruvic acid sodium salt	0.25	g
Dextrose	0.25	g
$MgSO_4x7H_2O$	0.50	g
KCL	0.35	g
KH_2PO_4	0.35	g
Trace element solution SL-10 B	1.00	ml
Distilled water	25	ml

Filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

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Solution F:

Vitamin B_{12}	0.01	g
Distilled water	100.00	ml
Filter sterilized		

Trace element solution SL-10 B:

Distilled water	1000.0	ml
HCl (25%)	7.7	ml
$FeSO_4$ x 7 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	300.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg

Mix solution D, C and E. Bubble with CO_2 in an ice bath under sterile conditions.
Fill 50 ml in each bottle of solution A. Before using add 4 ml solution B and 0.1 ml solution F.
Adjust the pH with filter-sterilised 1M Na_2CO_3 to 6.8 -7.1.
Fill in sterile, N_2 gassed screw-cap tubes under N_2 gas.

During the first 24 h, the iron of the medium precipitates in the form of black flocks. No other sediment should arise in the otherwise clear medium. Feed periodically with neutralized 3% solution of sodium sulfide to replenish sulfide and with other supplement solutions (see Ref. 3365).

Neutralized sulfide solution:

Distilled water	100.00	ml
Na_2S x 9 H_2O	3.00	g

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 N H_2SO_4 drop-wise with a syringe without opening the bottle.
Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

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31. ALKALINE NUTRIENT AGAR

Same as medium 1. After sterilization add sterile 1 M Na-sesquicarbonate solution (1 ml in 10 ml) to achieve a pH of 9.7.

Na-sesquicarbonate solution:

$NaHCO_3$	4.2	g
Na_2CO_3 anhydrous	5.3	g
Distilled water	100.0	ml

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34. CARYOPHANON LATUM MEDIUM

Yeast extract	2.00	g
Trypticase (BBL)	2.00	g
Soya peptone	2.00	g
Na-acetate	1.00	g
Na-glutamate	0.10	g
Thiamine-HCl x 2 H_2O	0.20	mg
Biotin	0.05	mg
K_2HPO_4	1.00	g
$MgSO_4$ x 7 H_2O	0.27	g
Tris/HCl-buffer 10mM, pH 7.8	1000.00	ml

Adjust pH to 7.8.

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35: ACIDITHIOBACILLUS MEDIUM

NH_4Cl	0.10	g
KH_2PO_4	3.00	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.14	g
Sulfur (powdered)	10.00	g
Distilled water	1000.00	ml

1. Dissolve all ingredients, except the sulfur and adjust the pH to 4.2, then autoclave. For sterilization place the sulfur in screw-capped tubes or bottles, wet with a few drops of water and heat for 3 hours to 90 - 100°C in a water bath on each of three successive days. Before use, aseptically layer the sulfur onto the surface of autoclaved liquid basal medium.

2. Note: Incubate statically without shaking.

For DSM 612: Supplement medium with 0.10 g/l yeast extract (sterilized separately).

For DSM 105150: Adjust pH of complete medium to 3.0.

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36: THIOBACILLUS MEDIUM

$(NH_4)_2SO_4$	0.10	g
K_2HPO_4	4.00	g
KH_2PO_4	4.00	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$	0.10	g
$FeCl_3$ x 6 H_2O	0.02	g
$MnSO_4$ x H_2O	0.02	g
Agar, for solid medium (optional)	12.00	g
$Na_2S_2O_3$ x 5 H_2O	10.00	g
Distilled water	1000.00	ml

Dissolve ingredients except thiosulfate and adjust the pH to 6.6. Dispense in suitable culivation vials and autoclave. Add thiosulfate to the autoclaved medium from a sterile stock solution.

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37. SPIRILLUM MEDIUM

Peptone (BD)	10.000	g
Succinic acid	1.000	g
$(NH_4)_2SO_4$	1.000	g
$MgSO_4$ x 7 H_20	1.000	g
$FeCL_3$ x 6 H_20	0.002	g
$MnSO_4$ x H_20	0.002	ml
Distilled water	1000.0	ml

Adjust pH to 6.8

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38: ACETOANAEROBIUM MEDIUM

Tryptone	20.00	g
Yeast extract	10.00	g
K_2HPO_4	1.04	g
KH_2PO_4	0.68	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2S x 9 H_2O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfide), adjust pH to 7.0, bring medium to the boil, then cool to room temperature under 100% N_2 gas. Dispense under same gas atmosphere in culture vessels and autoclave. After sterilization add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas.

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40. PFENNIG'S MEDIUM II WITH SALT

To medium 29 add 1% NaCl.

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48: TRYPTONE THIOGLYCOLATE MEDIUM

K_2HPO_4	5.45	g
KH_2PO_4	1.20	g
$MgSO_4$ x 7 H_2O	25.00	mg
$CaCl_2$ x 2 H_2O	15.00	mg
$FeSO_4$ x 7 H_2O	10.00	mg
$MnCl_2$ x 4 H_2O	2.00	mg
$CoCl_2$ x 6 H_2O	2.50	mg
Na_2MoO_4 x 2 H_2O	2.50	mg
Glucose	20.00	g
Peptone	2.00	g
Tryptone	2.00	g
Yeast extract	6.00	g
Na-thioglycolate	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except glucose), adjust pH to 7.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

2. Add glucose to the medium after autoclaving from a sterile stock solution prepared under 100% N_2 gas.

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49. C/10 MEDIUM

Casitone	3.00	g
$CaCl_2$ x 2 H_2O	1.36	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2 before adding agar.

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51. SPHAEROTILUS MEDIUM

Beef extract (Lab Lemco, Oxoid)	5.0	g
Agar (if necessary)	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

Prepare sterile agar slants by autoclaving. Cool the slants in a sloping position. Cover solid slants with 2 ml sterile tap water. Inoculate into the covering tap water and incubate at 20 to 25°C for at least 48 h.

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52: CLOSTRIDIUM KLUYVERI MEDIUM

K-acetate	10.00	g
K_2HPO_4	0.31	g
KH_2PO_4	0.23	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Ethanol absolute	20.00	ml
Na_2CO_3	1.00	g
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

1. Dissolve all ingredients (except ethanol, carbonate, vitamins, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add ethanol, then dispense medium under same gas atmosphere in Hungate-type tubes or serum vials and autoclave. After autoclaving, add carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere and vitamins from an anoxic and filter-sterilized stock solution prepared under 100% N_2 gas. Before inoculation, add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of medium to 6.8 - 7.0, if necessary.

2. Note: If reduction of medium is not complete after inoculation, add 10 - 20 mg/l sodium dithionite from a 5% w/v solution freshly prepared under 100% N_2 gas and filter sterilized.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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53. CORYNEBACTERIUM AGAR

Casein peptone, tryptic digest	10.0	g
Yeast extract	5.0	g
Glucose	5.0	g
NaCl	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2 - 7.4.

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54. GLUCOSE YEAST EXTRACT MEDIUM

Glucose	20.0	g
Yeast extract	10.0	g
$CaCO_3$ (light precipitate)	20.0	g
Distilled water	1000.0	ml

For solid medium add 17.0 g/l agar.

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55. FRUCTOSE MINERAL MEDIUM

Solution A:		
Na_2HPO_4 x 12 H_2O	9.000	g
KH_2PO_4	1.500	g
NH_4Cl	1.000	g
$MgSO_4$ x 7 H_2O	0.200	g
Trace element solution SL-6 (see medium 27)	0.300	ml
Distilled water	500.000	ml
Solution B:		
$CaCl_2$ x 2 H_2O	0.010	g
Ferric ammonium citrate	0.005	g
Distilled water	300.000	ml
Solution C:		
Fructose	5.000	g
Distilled water	200.000	ml

Autoclave solutions 1 and 2 separately, filter sterilize solution C. Add 1.5% agar if necessary.

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56. TRYPTICASE STARCH AGAR

Trypticase soy broth	30.0	g
Yeast extract	2.0	g
Soluble starch	1.0	g
Agar	15.0	g
Distilled water	1000.0	g

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57. AAM MEDIUM

Tryptone	5.0	g
Meat extract	5.0	g
Yeast extract	7.0	g
Tween 80	1.0	ml
Na-acetate	2.5	g
$MgSO_4$ x 7 H_2O	200.0	mg
$MnSO_4$ x H_2O	50.0	mg
Casamino acids	7.0	g
Distilled water	1000.0	ml

Adjust pH to 5.4.

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58. BIFIDOBACTERIUM MEDIUM

Casein peptone, tryptic digest	10.00	g
Yeast extract	5.00	g
Meat extract	5.00	g
Bacto Soytone	5.00	g
Glucose	10.00	g
K_2HPO_4	2.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$MnSO_4$ x H_20	0.05	g
Tween 80	1.00	ml
NaCl	5.00	g
Cysteine-HCl x H_2O	0.50	g
Salt solution (see below)	40.00	ml
Resazurin (25 mg/100ml)	4.00	ml
Distilled water	950.00	ml

The cysteine are added after the medium has been boiled and cooled under CO_2. Adjust pH to 6.8 using 8 N NaOH. Distribute under N_2 and autoclave.

Salt solution:

$CaCl_2$ x 2 H_2O	0.25	g
$MgSO_4$ x 7 H_2O	0.50	g
K_2HPO_4	1.00	g
KH_2PO_4	1.00	g
$NaHCO_3$	10.00	g
NaCl	2.00	g
Distilled water	1000.00	ml

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59. LEUCONOSTOC OENOS MEDIUM

Casein peptone, tryptic digest	10.00	g
Yeast extract	5.00	g
Glucose	10.00	g
Fructose	5.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$MnSO_4$ x H_2O	0.05	g
(NH_4) citrate	3.50	g
Tween 80	1.00	ml
Tomato juice, filtered	100.00	ml
Cysteine-HCl x H_2O	0.50	g
Distilled water	900.00	ml

Adjust pH to 4.8.

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60: MOORELLA MEDIUM

Solution A	843.00	ml
Solution B	50.00	ml
Solution C	50.00	ml
Solution D	40.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

Sparge solution A with 100% CO_2 gas for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Autoclave separately solutions B, C, E and F under 100% N_2 gas, and solution D under 80% N_2 and 20% CO_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 6.9, if necessary.

Solution A

$MgSO_4$ x 7 H_2O	0.10	g
$(NH_4)_2SO_4$	0.50	g
$Fe(NH_4)_2(SO_4)_2$	0.04	g
Na_2MoO_4 x 2 H_2O (0.1% w/v)	2.40	ml
Na_2SeO_3 x 5 H_2O (0.1% w/v)	0.15	ml
Tryptone	5.00	g
Yeast extract	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	840.00	ml

Solution B

K_2HPO_4	7.00	g
KH_2PO_4	4.50	g
Distilled water	50.00	ml

Solution C

Glucose	18.00	g
Distilled water	50.00	ml

Solution D

Na_2CO_3	2.00	g
Distilled water	40.00	ml

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Solution E

L-Cysteine HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution F

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

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61: THERMOANAEROBACTER MEDIUM

Final pH: 6.8 - 7.5
Final volume: 1000 ml

Tryptone (BD BBL)	10.00	g
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$FeSO_4$ x 7 H_2O	0.20	g
Sucrose	10.00	g
Na_2SO_3	0.20	g
$Na_2S_2O_3$ x 5 H_2O	0.08	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferrous sulfate, sucrose, sulfite, and thiosulfate), adjust pH to 6.8 - 7.5, and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add ferrous sulfate, then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add sucrose, sulfite, and thiosulfate from anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration. The stock solution of sulfite should be freshly prepared.

For DSM 8686, DSM 8690: Replace sucrose with 10.00 g/l D-xylose as substrate and adjust pH of medium to 5.2.

For DSM 15493: Replace sucrose with 3.00 g/l D-maltose.

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63: DESULFOVIBRIO (POSTGATE) MEDIUM

Solution A	980.00	ml
Solution B	10.00	ml
Solution C	10.00	ml

Dissolve ingredients of solution A, bring to the boil, then cool to room temperature while sparging with 100% N_2 gas. Add solutions B and C, adjust pH to 7.8 with NaOH, and distribute under 100% N_2 gas atmosphere into anoxic Hungate-type tubes. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave 15 min at 121 °C. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

For DSM 12838: Supplement medium with 3.0 g/l meat extract.

For DSM 109475: Adjust pH of complete medium to 6.0-6.5.

Solution A

K_2HPO_4	0.50	g
NH_4Cl	1.00	g
Na_2SO_4	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	2.00	g
Na-DL-lactate	2.00	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	980.00	ml

Solution B

$FeSO_4$ x 7 H_2O	0.50	g
Distilled water	10.00	ml

Solution C

Na-thioglycolate	0.10	g
Ascorbic acid	0.10	g
Distilled water	10.00	ml

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65. GYM STREPTOMYCES MEDIUM

Glucose	4.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
$CaCO_3$	2.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2 before adding agar. Delete $CaCO_3$ if liquid medium is used.

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67. CY-AGAR

Casitone	3.00	g
$CaCl_2$ x 2 H_2O	1.36	g
Yeast extract	1.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2.

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68: HALOTHIOBACILLUS NEAPOLITANUS MEDIUM

KH_2PO_4	4.00	g
K_2HPO_4	4.00	g
$MgSO_4$ x 7 H_2O	0.80	g
NH_4Cl	0.40	g
Trace element solution (Vishniac and Santer, 1957)	5.00	ml
Bromocresol purple (saturated aqueous solution)	0.50	ml
Agar (Bacto), for solid medium (optional)	15.00	g
$Na_2S_2O_3$ x 5 H_2O	10.00	g
Distilled water	1000.00	ml

Dissolve ingredients except thiosulfate, dispense into suitable cultivation vessels and autoclave. Add thiosulfate to the autoclaved medium from a sterile stock solution. Adjust pH of complete medium to 6.6 - 7.0.

Trace element solution (Vishniac and Santer, 1957) (from medium 69)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	22.00	g
$CaCl_2$ x 2 H_2O	5.54	g
$MnCl_2$ x 4 H_2O	5.06	g
$FeSO_4$ x 7 H_2O	5.00	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1.10	g
$CuSO_4$ x 5 H_2O	1.57	g
$CoCl_2$ x 6 H_2O	1.61	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water, adjust pH to 7 using 2 N KOH, then add remaining compounds. Adjust pH of final solution to 6.0 with KOH.

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69: STARKEYA NOVELLA MEDIUM

Solution A	900.00	ml
Solution B	50.00	ml
Solution C	5.00	ml
Solution D	50.00	ml

Solutions A, B, C and D are sterilized separately by autoclaving and mixed when cooled to room temperature. Adjust pH of complete medium to 8.5 with sterile 0.5 N NaOH.

Solution A

Na_2HPO_4 x 12 H_2O	10.60	g
KH_2PO_4	1.50	g
NH_4Cl	0.30	g
Yeast extract	0.30	g
Phenol red (0.1% w/v)	2.00	ml
Agar (Bacto), for solid medium (optional)	15.00	g
Distilled water	900.00	ml

Solution B

$MgSO_4$ x 7 H_2O	0.10	g
Distilled water	50.00	ml

Solution C

Trace element solution (Vishniac and Santer, 1957)	5.00	ml

Solution D

$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	50.00	ml

Trace element solution (Vishniac and Santer, 1957) (from medium 69)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	22.00	g
$CaCl_2$ x 2 H_2O	5.54	g
$MnCl_2$ x 4 H_2O	5.06	g
$FeSO_4$ x 7 H_2O	5.00	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1.10	g
$CuSO_4$ x 5 H_2O	1.57	g
$CoCl_2$ x 6 H_2O	1.61	g
Distilled water	1000.00	ml

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69: STARKEYA NOVELLA MEDIUM

Dissolve EDTA in distilled water, adjust pH to 7 using 2 N KOH, then add remaining compounds. Adjust pH of final solution to 6.0 with KOH.

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70: ACIDITHIOBACILLUS FERROOXIDANS MEDIUM

Final pH: 1.8
Final volume: 1000 ml

KH_2PO_4	0.40	g
$MgSO_4$ x 7 H_2O	0.40	g
$(NH_4)_2SO_4$	0.40	g
$FeSO_4$ x 7 H_2O	33.30	g
H_2SO_4 (0.1 N)	1000.00	ml

Adjust pH to 1.8 with 2N sulfuric acid. Sterilize by autoclaving for 15 min at 121°C.

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71: ACIDITHIOBACILLUS THIOOXIDANS MEDIUM

KH_2PO_4	3.00	g
$MgSO_4$ x 7 H_2O	0.50	g
$(NH_4)_2SO_4$	3.00	g
$CaCl_2$ x 2 H_2O	0.25	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	1000.00	ml

1. Prepare the medium without thiosulfate, adjust pH to 4.4 - 4.7 and autoclave at 121°C for 15 min. Sterilize thiosulfate separately by filtration and add after autoclaving.

2. Note: Incubate statically without shaking.

For DSM 585: Replace thiosulfate with 5.00 g/l tetrathionate ($K_2S_4O_6$) added from a stock solution sterilized by filtration.

For DSM 14366: Supplement medium with 10.00 mg/l $FeSO_4$ x 7 H_2O. The final pH of the medium should be 4.4.

For DSM 103717: Omit thiosulfate from the medium and add 10.00 g/l elemental sulfur as substrate. For sterilization place the sulfur in screw-capped tubes, add 1-2 drops of distilled water and incubate on 3 successive days for 3 h at 90-100°C in a water bath. Before use, aseptically layer the sulfur onto the surface of autoclaved liquid basal medium.

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73. MEDIUM FOR HALOPHILIC BACILLI

Casamino acids	10.0	g
Yeast extract	10.0	g
NaCl	100.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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74. THERMUS THERMOPHILUS MEDIUM

Yeast extract (BD Difco)	4.0	g
Proteose peptone Nr. 3 (BD Difco)	8.0	g
NaCl	2.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

The original medium description specifies polypeptone, which appears to be no longer available.

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75. TRYPTICASE PHYTONE MEDIUM

Trypticase peptone	17.0	g
Phytone peptone	3.0	g
NaCl	5.0	g
K_2HPO_4	2.5	g
Glucose	2.5	g
Distilled water	1000.0	ml

Adjust pH to 7.3.

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76: GOTTSCHALKIA MEDIUM

KOH	0.67	g
K_2HPO_4	0.91	g
Uric acid	2.00	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	15.00	mg
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	6.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-thioglycolate	0.50	g
Distilled water	1000.00	ml

First dissolve KOH and K_2HPO_4 in water, then add uric acid and boil until the acid is dissolved. Cool medium to room temperature under 100% N_2 gas atmosphere and add all other compounds, except carbonate and thioglycolate. Dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 15 min at 121°C. Then add carbonate (filter-sterilized stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere) and thioglycolate (stock solution, autoclaved separately under 100% N_2 gas). Adjust pH of complete medium to 7.0 - 7.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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77. LIVER BROTH (Oxoid CM 77)

Prepare the medium according to directions on the bottle under 100% N_2 gas atmosphere.

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78. CHOPPED MEAT MEDIUM

Ground beef (fat free)	500.0	g
Distilled water	1000.0	ml
NaOH 1 N	25.0	ml

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1000 ml, and then add:

Casitone	30.0	g
Yeast extract	5.0	g
K_2HPO_4	5.0	g
Resazurin	1.0	mg

To make medium anoxic bring it to a boil, cool under 100% N_2 gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under 100% N_2 gas atmosphere by filling 7 ml medium into anoxic Hungate-type tubes (for strains demanding meat particles put those first into the tube (use 1 part meat particles to 4 or 5 parts fluid)). Autoclave at 121°C for 30 min. For agar slants use 15.0 g agar per 1000.0 ml medium.

In some cases (as indicated in the catalogue) the addition of Haemin and Vitamin K_1 or Vitamin K_3 is necessary. Add to 1000 ml of medium after autoclaving:

Haemin solution (see below)	10.00	ml
Vitamin K_1 or Vitamin K_3 solution (see below)	10.00	ml

Haemin solution:
Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K_1 solution:
Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K_3 solution:
Dissolve 5 mg/ml of vitamin K_3 in 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.

For DSM 21650 adjust pH of complete medium to 8.5.

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79. LEUCOTHRIX MEDIUM

Tryptone	10.00	g
Synthetic sea water	1000.00	ml

Synthetic sea water:

NaCl	24.00	g
$MgCl_2$ x 6 H_2O	11.00	g
Na_2SO_4	4.00	g
$CaCl_2$ x 6 H_2O	2.00	g
KCl	0.70	g
KBr	0.10	g
H_3BO_3	0.03	g
$NaSiO_3$ x 9 H_2O	5.00	mg
$SrCl_2$ x 6 H_2O	0.04	g
NaF	3.00	mg
NH_4NO_3	2.00	mg
Fe(III)PO_4 x 4 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH to 7.8.

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80. GLYCEROL-SOIL MEDIUM

Peptone	5.0	g
Beef extract	3.0	g
Glycerol	20.0	g
Soil extract	150.0	ml
Distilled water	850.0	ml
Agar	15.0	g

Adjust pH to 7.0. Soil extract is prepared by sieving air dried garden soil through a coarse sieve and autoclaving 400 g with 960 ml of distilled water at 121°C for one hour. After the mixture is cool and settled, the supernatant is carefully decanted, filtered through paper, autoclaved in 200 ml quantities, and stored at room temperature until cleared by sedimentation.

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81. MINERAL MEDIUM FOR CHEMOLITHOTROPHIC GROWTH (H-3)

Solution A:

KH_2PO_4	2.300	g
Na_2HPO_4 x 2 H_2O	2.900	g
Distilled water	50.000	ml

Solution B:

NH_4Cl	1.000	g
$MgSO_4$ x 7 H_2O	0.500	g
$CaCl_2$ x 2 H_2O	0.010	g
$MnCl_2$ x 4 H_2O	0.005	g
$NaVO_3$ x H_2O	0.005	g
Trace element sol. SL-6 (see medium 27)	5.000	ml
Distilled water	915.000	ml
Agar (if necessary)	20.000	g

Solution C:

Ferric ammonium citrate	0.050	g
Distilled water	20.000	ml

Solutions A, B, C are autoclaved separately for 15 min at 121°C, cooled down to 50°C and then mixed aseptically with 5.0 ml filter-sterilized standard vitamin solution (see below).The final pH of this medium should be 6.8 without adjustment.

For chemolithotrophic growth incubate the culture under an atmosphere of 2% (v/v) O_2, 10% CO_2, 60% H_2 and 28% N_2. For heterotrophic growth supplement the mineral medium with an appropriate carbon source (0.2% carbohydrate or 0.1% organic acid). For growth on nitrogen-free medium, omit NH_4Cl and incubate the culture under an atmosphere of 2% (v/v) O_2, 10% CO_2, 10% H_2 and 78% N_2 or heterotrophically under 2% (v/v) O_2 and 98% N_2. For more details see Ref. 1515 and Ref. 3363.

Standard vitamin solution:

Riboflavin	10.000	mg
Thiamine-HCl x 2 H_2O	50.000	mg
Nicotinic acid	50.000	mg
Pyridoxine-HCl	50.000	mg
Ca-pantothenate	50.000	mg
Biotin	0.100	mg
Folic acid	0.200	mg
Vitamin B_{12}	1.000	mg
Distilled water	100.000	ml

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82. BHI-GLUCOSE MEDIUM

Brain heart infusion	18.5	g
Glucose	5.0	g
Agar	12.0	g
Distilled water	1000.0	ml

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83. CZAPEK PEPTONE AGAR

Sucrose	30.00	g
$NaNO_3$	3.00	g
K_2HPO_4	1.00	g
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.50	g
$FeSO_4$ x 7 H_2O	0.01	g
Yeast extract	2.00	g
Peptone	5.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.3.

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84. ROLLED OATS MINERAL MEDIUM

Rolled oats	20.0	g
Agar	20.0	g
Trace element solution (see below)	1.0	ml
Distilled water	1000.0	ml

Trace element solution:

$FeSO_4$ x 7 H_2O	0.1	g
$MnCl_2$ x 4 H_2O	0.1	g
$ZnSO_4$ x 7 H_2O	0.1	g
Distilled water	100.0	ml

pH 7,2

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85. GLUCOSE PEPTONE MEDIUM

Yeast extract	10.0	g
Bacto peptone (Difco)	20.0	g
Glucose	10.0	g
$CaCO_3$	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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86. CASTENHOLZ MEDIUM

Nitrilotriacetic acid (Titriplex I)	100.00	mg
$CaSO_4$ x 2 H_2O	60.00	mg
$MgSO_4$ x 7 H_2O	100.00	mg
NaCl	8.00	mg
KNO_3	103.00	mg
$NaNO_3$	689.00	mg
Na_2HPO_4 x 2 H_2O	140.00	mg
$FeCl_3$ x 6 H_2O	0.47	mg
$MnSO_4$ x H_2O	2.20	mg
$ZnSO_4$ x 7 H_2O	0.50	mg
H_3BO_3	0.50	mg
$CuSO_4$ x 5 H_2O	25.00	µg
Na_2MoO_4 x 2 H_2O	25.00	µg
$CoCl_2$ x 6 H_2O	46.00	µg
Tryptone	1.00	g
Yeast extract	1.00	g
Distilled water	1000.00	ml

Adjust pH to 8.2 with NaOH.

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87. CHLOROFLEXUS MEDIUM (modified)

Yeast extract	1.00	g
Glycyl-glycine	1.00	g
Na_2HPO_4 x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
KNO_3	0.10	g
$NaNO_3$	0.50	g
NaCl	0.10	g
$CaCl_2$ x 2 H_2O	0.05	g
Fe(III) citrate solution (0.1 g in 100 ml H_2O)	5.00	ml
Trace element solution SL-6 (see medium 27)	1.00	ml
Distilled water	1050.00	ml

Adjust pH to 8.2.
Boil the medium under a stream of nitrogen gas for a few minutes and distribute 90 ml medium into 100 ml screw-capped bottles. Bubble each bottle with nitrogen gas and close immediately with a rubber septum and screw tight. Autoclave at 121°C for 15 min. After autoclaving inject 1.0 ml of neutralized sulfide solution (0.03% end concentration) to each bottle. This medium can be stored for several months. Incubate the culture at 50°C at a light intensity of 300 - 500 lux. For heavy cell suspension supplement periodically with sterile yeast extract solution (0.1% end concentration, for more details see Ref. 3365, 3366).

Neutralized sulfide solution:

Distilled water	100.00	ml
Na_2S x 9 H_2O	3.00	g

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.
Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

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88: SULFOLOBUS MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Yeast extract (OXOID)	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except yeast extract or other substrates), adjust pH of the salt solution at room temperature to 2.0 using 10 N H_2SO_4 and autoclave. Yeast extract and other organic substrates are sterilized separately by autoclaving of a 10% (w/v) stock solution at neutral pH.

For DSM 5348: Omit yeast extract and supplement medium with 0.50 g/l powdered sulfur and 20.00 g/l sulfide ore (e.g., pyrite). Sterilize sulfur separately by steaming for 3 hours on each of 3 successive days (see medium 35) and ore by heating at 150°C overnight. Add sulfur and ore aseptically to the autoclaved medium.

For DSM 5389, DSM 7519, DSM 12421: Adjust pH of medium to 3.0 - 3.5.

For DSM 6482, DSM 10039, DSM 104736: Reduce the amount of yeast extract to 0.20 g/l and supplement the medium with 5.00 g/l powdered sulfur. Sterilize sulfur separately by steaming for 3 hours on each of 3 successive days (see medium 35) and add aseptically to the autoclaved medium.

For DSM 9789, DSM 9790: Use 2.00 g/l yeast extract and adjust pH of medium to 1.0 by using 300.00 ml 0.5 M H_2SO_4 and 700.00 ml distilled water for the dissolving of salts.

For DSM 16993: Supplement medium with 1.00 g/l D-glucose and 1.00 g/l Casamino acids. Adjust pH of the completed medium to 3.0.

For DSM 18247: Modified, with 0.5% yeast extract; solidified by 0.7% Gelrite; pH:9.0

For DSM 18786: Use only 0.10 g/l yeast extract and supplement medium with 10.00 g/l sulfide ore (e.g., chalcopyrite). Sterilize ore by heating at 150°C overnight. Adjust the pH of the medium to 0.8.

For DSM 29038: Supplement medium with 3.00 g/l $K_2S_4O_6$ added to the autoclaved medium from a stock solution sterilized by filtration. Adjust pH of completed medium to 2.5.

For DSM 29099: Use only 0.20 g/l yeast extract and supplement medium with 1.00 g/l D-glucose and 10.00 g/l powdered sulfur. Sterilize sulfur separately by steaming for 3 hours on each of 3 successive days (see medium 35) and add aseptically to the autoclaved medium. Adjust pH of final medium to 2.5 - 3.0.

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For DSM 45263: Adjust pH of medium to 7.5

For DSM 111728: Use only 0.50 g/l yeast extract and supplement medium with 10.00 g/l powdered sulfur. Sterilize sulfur separately by steaming for 3 hours on each of 3 successive days (see medium 35) and add aseptically to the autoclaved medium. Adjust the pH of the final medium to 2.5.

For DSM 112778: Supplement medium with 0.50 g/l powdered sulfur and 20.00 g/l sulfide ore (e.g., pyrite). Sterilize sulfur separately by steaming for 3 hours on each of 3 successive days (see medium 35) and ore by heating at 150°C overnight. Add sulfur and ore aseptically to the autoclaved medium. Adjust the pH of the medium to 3.0.

Allen's trace element solution (from medium 88)

$MnCl_2 \times 4\ H_2O$	180.00	mg
$Na_2B_4O_7 \times 10\ H_2O$	450.00	mg
$ZnSO_4 \times 7\ H_2O$	22.00	mg
$CuCl_2 \times 2\ H_2O$	5.00	mg
$Na_2MoO_4 \times 2\ H_2O$	3.00	mg
$VOSO_4 \times 2\ H_2O$	3.00	mg
$CoSO_4 \times 7\ H_2O$	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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90. MALT EXTRACT PEPTONE AGAR

Malt extract	30.0	g
Soya peptone	3.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 5.6. Sterilize at 121°C for 10 min.

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91. PROPIONIBACTERIUM AGAR

Casein peptone, tryptic digest	10.0	g
Yeast extract	5.0	g
Na-lactate	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 - 7.2.

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92. TRYPTICASE SOY YEAST EXTRACT MEDIUM

Trypticase soy broth	30.0	g
Yeast extract	3.0	g
Agar, if necessary	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 - 7.2.

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93. LACTOBACILLUS MEDIUM II

Casein peptone, tryptic digest	10.0	g
Yeast extract	5.0	g
Meat extract	2.0	g
Glucose	20.0	g
KH_2PO_4	0.5	g
K_2HPO_4	0.5	g
$FeSO_4$ x 7 H_2O	10.0	mg
$MgSO_4$ x 7 H_2O	200.0	mg
$MnSO_4$ x H_2O	7.5	mg
Tween 80	1.0	ml
Ethanol	40.0	ml
Na-acetate	20.0	g
DL-mevalonic acid	30.0	mg
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 5.2.

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95: DESULFUROMONAS ACETOXIDANS MEDIUM

Solution A	951.00	ml
Solution B	3.00	ml
Solution C	40.00	ml
Solution D	10.00	ml

Sparge solution A with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense solution under same gas atmosphere into anoxic Hungate-type tubes or serum vials and sterilize by autoclaving. Solutions B and D are autoclaved separately under 100% N_2 gas atmosphere. Solution C is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. The medium is completed before use by adding appropriate amounts of solutions B, C and D to solution A. Adjust pH of complete medium to 7.2, if necessary.

Solution A

KH_2PO_4	1.00	g
NH_4Cl	0.30	g
$MgSO_4$ x 7 H_2O	1.00	g
$MgCl_2$ x 6 H_2O	2.00	g
NaCl	20.00	g
$CaCl_2$ x 2 H_2O	0.10	g
Na_2SO_4	1.77	g
Trace element solution SL-11	1.00	ml
Na_2-DL-malate	2.66	g
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Adjust pH to 6.0 with 2 N NaOH.

Solution B

Ethanol	0.30	ml
Distilled water	2.70	ml

Solution C

$NaHCO_3$	1.85	g
Distilled water	40.00	ml

Solution D

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

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Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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97. HALOBACTERIUM MEDIUM

Casamino acids	7.50	g
Yeast extract	10.00	g
Na_3-citrate	3.00	g
KCl	2.00	g
$MgSO_4$ x 7 H_2O	20.00	g
$FeSO_4$ x 7 H_2O	0.05	g
$MnSO_4$ x H_2O	0.20	mg
NaCl	250.00	g
Agar	20.00	g
Distilled water	1000.00	ml

Adjust pH to 7.4. Add the agar after dissolving all ingredients in the water and adjustment of pH.

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98. RHIZOBIUM MEDIUM

Yeast extract	1.0	g
Mannitol	10.0	g
Agar	15.0	g
Soil extract	200.0	ml
Distilled water	800.0	ml

Adjust pH to 7.0.

Soil extract:

Air-dried garden soil	80.0	g
Na_2CO_3	0.2	g
Distilled water	200.0	ml

Autoclave soil suspension for one hour at 121°C. To obtain a clear supernatant allow to settle and centrifuge. Adjust pH to 7.2.

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101. NUTRIENT AGAR or BROTH WITH NaCl

Peptone	5.0	g
Meat extract	3.0	g
NaCl	30.0	g
Agar, if necessary	15.0	g
Distilled water	1000.0	ml

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104. PYG MEDIUM (modified)

Trypticase peptone	5.00	g
Peptone	5.00	g
Yeast extract	10.00	g
Beef extract	5.00	g
Glucose	5.00	g
K_2HPO_4	2.00	g
Tween 80	1.00	ml
Salt solution (see below)	40.00	ml
Resazurin	1.00	mg
Vitamin K_1 solution (see below)	0.20	ml
Haemin solution (see below)	10.00	ml
Cysteine-HCl x H_2O	0.50	g
Distilled water	950.00	ml

The vitamin K_1, haemin solution and the cysteine are added after the medium has been boiled and cooled under CO_2. Adjust pH to 7.2 using 8 N NaOH. Distribute under N_2 and autoclave.

Salt solution:

$CaCl_2$ x 2 H_2O	0.25	g
$MgSO_4$ x 7 H_2O	0.50	g
K_2HPO_4	1.00	g
KH_2PO_4	1.00	g
$NaHCO_3$	10.00	g
NaCl	2.00	g
Distilled water	1000.00	ml

Haemin solution:
Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water. Store refrigerated.

Vitamin K_1 solution:
Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

For DSM 23669 and DSM 25557 supplement medium after autoclaving with 50.0 ml/l clarified rumen fluid (see medium 1310), 50.0 ml/l horse serum (Bio & Sell PFD.SE.0500) and 0.2 g/l dithiothreitol (DTT) added from filter-sterilized anoxic stock solutions.

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105. GLUCONOBACTER OXYDANS MEDIUM

Glucose	100.0	g
Yeast extract	10.0	g
$CaCO_3$	20.0	g
Distilled water	1000.0	ml

Adjust pH to 6.8.

Add 15 g/l for solid media.

20 g calcium carbonate in the medium serves as a buffer. The calcium carbonate will settle in agar plates before the agar has set, producing an opaque layer in the bottom. As the strain grows and acid is produced this will react with the calcium carbonate, causing it to dissolve and form zones of clearing immediately below the colonies. In liquid culture the calcium carbonate also forms a sediment that clears as acid is produced.

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108: ACIDIPHILIUM ACIDOPHILUM MEDIUM

$(NH_4)_2SO_4$	3.00	g
KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	1.00	g
KCl	0.10	g
$Ca(NO_3)_2$ x 4 H_2O	18.00	mg
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	0.01	ml
Agar, for solid medium (optional)	15.00	g
D-Glucose	10.00	g
Distilled water	1000.00	ml

In liquid medium, the pH should be adjusted to 3.5 with H_2SO_4. In solid medium, the pH should be adjusted to 4.5 with H_2SO_4 after autoclaving the medium. Sterilize the glucose separately and add to the sterile medium.

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109. RHODOPSEUDOMONAS SULFOVIRIDIS MEDIUM

To medium 27 add 0.1% sodium malate and 0.05% sodium thiosulfate. Adjust pH to 6.8 and sterilize at 121°C for 15 min. The liquid medium is sterilized in rubber stoppered or screw-capped bottles under anaerobic conditions (Ref. 3365). After sterilization neutralized sterile sodium sulfide solution is added to a final concentration of 0.03% (see medium 27).

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110. CHOPPED MEAT MEDIUM WITH CARBOHYDRATES

Ground beef (fat free)	500.0	g
Distilled water	1000.0	ml
NaOH 1 N	25.0	ml

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1000 ml, and then add:

Casitone	30.0	g
Yeast extract	5.0	g
K_2HPO_4	5.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
D-Glucose	4.0	g
Cellobiose	1.0	g
Maltose	1.0	g
Starch (soluble)	1.0	g

To make medium anoxic boil it, cool under 100% N_2 gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under same gas atmosphere 7 ml medium into Hungate-type tubes (for strains demanding meat particles put those first into the tube (use 1 part meat particles to 4 or 5 parts fluid)). Autoclave at 121°C for 30 min. For agar slants use 15.0 g agar per 1000.0 ml medium.

In some cases (as indicated in the catalogue) the addition of Haemin and Vitamin K_1 or Vitamin K_3 is necessary. Add to 1000.0 ml of medium after autoclaving:

Haemin solution (see below)	10.00	ml
Vitamin K_1 or Vitamin K_3 solution (see below)	10.00	ml

Haemin solution:
Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K_1 solution:
Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K_3 solution:
Dissolve 5 mg/ml of vitamin K_3 in 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.

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For DSM 22787 supplement medium after autoclaving with 50 ml/l of anoxic sterile horse serum.

For DSM 100320 adjust pH of medium to 7.5 – 8.0.

For DSM 103060 adjust pH of complete medium to 7.5.

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111. BEIJERINCKIA MEDIUM

Glucose	10.0	g
K_2HPO_4	0.8	g
KH_2PO_4	0.2	g
$MgSO_4$ x 7 H_2O	0.1	g
$FeSO_4$ x 7 H_2O	20.0	mg
$MnSO_4$ x 6 H_2O	2.0	mg
$ZnSO_4$ x 6 H_2O	5.0	mg
$CuSO_4$ x 6 H_2O	4.0	mg
Na_2MoO_4 x 2 H_2O	5.0	mg
Agar	15.0	g
Distilled water	950.0	ml

Adjust pH to 6.5. Sterilize glucose separately (10 g in 50 ml H_2O) and mix after cooling.

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113: THIOBACILLUS DENITRIFICANS MEDIUM

Final pH: 7.0 - 7.2
Final volume: 1003 ml

Solution A	962.00	ml
Solution B	20.00	ml
Solution C	20.00	ml
Solution D	1.00	ml

Solutions A, B, and D are sterilized separately by autoclaving at 121°C for 15 min under a 100% N_2 gas atmosphere. Solution C is sterilized by filtration under an atmosphere of 80% N_2 and 20% CO_2 gas mixture. Appropriate amounts of solutions B to D are added to the sterile solution A in the sequence as indicated. Check the pH of the complete medium and adjust it to 7.0 - 7.2, if necessary.

Solution A

KH_2PO_4	2.00	g
KNO_3	2.00	g
NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.80	g
Trace element solution SL-4	2.00	ml
Distilled water	960.00	ml

Solution B

$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	20.00	ml

Solution C

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution D

$FeSO_4$ x 7 H_2O	2.00	mg
H_2SO_4 (0.1 N)	1.00	ml

Trace element solution SL-4 (from medium 14)

Na_2-EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g

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$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.

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115. VIBRIO NATRIEGENS MEDIUM

To medium 1 add 1.5% NaCl.

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118. DAP-NUTRIENT AGAR

To medium 1 add 100 mg DL-diaminopimelic acid.

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119: METHANOBACTERIUM MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1003 ml

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Sludge fluid	50.00	ml
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	930.00	ml

1. Dissolve ingredients except bicarbonate, cysteine and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 6.5 and dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. Note: After growth has started and the culture is becoming turbid add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 - 1 bar overpressure.

For DSM 1093, DSM 1125, DSM 11995, DSM 16643: Supplement medium after autoclaving with 1 ml/l Wolin's vitamin solution (10x, see medium 120), 0.30 g/l DL-dithiothreitol, and 0.15 g/l coenzyme M (2-mercaptoethanesulfonic acid) from anoxic stock solutions sterilized by filtration. Omit Na_2S x 9 H_2O and L-Cysteine HCl x H_2O.

For DSM 1535: Adjust pH of complete medium to 7.6.

For DSM 2030: Adjust pH to 6.5 and add sterile 80% H_2 and 20% CO_2 gas to 2 bar overpressure after inoculation.

For DSM 3267, DSM 11074, DSM 11075: Adjust pH of complete medium to 8.0 - 8.3.

For DSM 6216: Increase amount of Na-acetate to 3.00 g/l.

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For DSM 7057: Supplement medium with 2.00 g/l Na_2SO_4.

For DSM 7095: Supplement medium with 0.3 g DL-Dithiothreitol. Omit Na_2S x 9 H_2O.

For DSM 9575: Add sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure after inoculation.

For DSM 15163: Supplement medium with 0.3 g DL-Dithiothreitol. Omit Na_2S x 9 H_2O. Adjust pH of final medium to 6.0.

For DSM 16632: Replace sludge fluid with the same volume of clarified rumen fluid (see medium 1310) and supplement medium with 2.00 g/l Trypticase peptone and 10.00 ml/l of Wolin's vitamin solution (see medium 141).

For DSM 25824: Supplement medium after autoclaving with 0.10 g/l 2-mercaptoethanesulfonic acid (coenzyme M) added from an anoxic stock solution sterilized by filtration and add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure after inoculation.

For DSM 25939: Adjust pH of the complete medium to 7.2 and add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure after inoculation.

For DSM 25945: Adjust pH of the complete medium to 7.4 and add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure after inoculation.

For DSM 102889: Adjust pH of complete medium to 8.5 - 9.0.

For DSM 108286: Adjust pH of final medium to 6.0.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

Fatty acid mixture (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml
Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

$FeSO_4 \times 7\ H_2O$ solution (0.1% w/v) (from medium 119)

$FeSO_4 \times 7\ H_2O$	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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120: METHANOSARCINA MEDIUM

K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
NaCl	2.25	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	2.00	g
Casitone (BD BBL)	2.00	g
Na-acetate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
Methanol (50% v/v)	20.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Then add and dissolve bicarbonate, adjust pH to 6.5 and dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N_2 gas atmosphere as concentrated solutions in tightly closed tubes. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

For DSM 1538, DSM 7058, DSM 9195: Replace methanol with 5.00 g/l trimethylamine-HCl.

For DSM 1825, DSM 7081, DSM 11855: Supplement medium with 5% (v/v) clarified rumen fluid (see medium 1310) and adjust pH to 6.5 - 6.8.

For DSM 21571: Supplement medium with 6.00 g/l NaCl.

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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122: ACETIVIBRIO MEDIUM

$(NH_4)_2SO_4$	1.30	g
$MgCl_2$ x 6 H_2O	2.60	g
KH_2PO_4	1.43	g
K_2HPO_4	5.50	g
$CaCl_2$ x 2 H_2O	0.13	g
Na_2-ß-glycerophosphate x 5 H_2O (MERCK 35675)	6.00	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.10	ml
L-Glutathione (reduced)	0.25	g
Yeast extract	4.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Cellobiose	5.00	g
Cellulose (Avicel or MN 301) (optional)	10.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients except cellobiose, sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Then adjust pH to 7.0 - 7.2, distribute under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Cellobiose is added to the sterile medium from an anoxic 10% (w/v) stock solution prepared under 100% N_2 gas and sterilized by filtration. Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose (Avicel or MN 301, MACHEREY-NAGEL).

2. Note: A white precipitate forms after mixing the ingredients of this medium, but this has no negative effect on growth.

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123. MARINE AGAR

To 1000 ml "Synthetic sea water" (see medium 79) add 5 g Bacto Peptone, 1 g Bacto Yeast extract, and 15 g agar. Alternatively medium 514 may be used.

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124: DESULFOFARCIMEN MEDIUM

Final pH: 7.0 - 7.2
Final volume: 1003 ml

NaCl	1.17	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.27	g
KH_2PO_4	0.20	g
Na_2SO_4	2.84	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.50	g
Na-acetate	1.40	g
Na-butyrate	1.40	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, butyrate, vitamins, and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 7.0, then dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, butyrate, vitamins, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Before use, check the complete medium's pH and adjust it to 7.0 - 7.2, if necessary.

For DSM 2925: Replace acetate and butyrate with 1.00 g/l ethanol absolute added from a sterile anoxic stock solution after autoclaving.

For DSM 7213: Replace acetate and butyrate with 0.60 g/l Na-benzoate added after autoclaving from an anoxic stock solution sterilized by filtration.

For DSM 100382: Replace acetate and butyrate with 1.60 g/l Na-fumarate added after autoclaving from an anoxic stock solution sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg

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$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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125. METHYLOBACTERIUM MEDIUM

KNO_3	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.02	g
Na_2HPO_4	0.23	g
NaH_2PO_4	0.07	g
$FeSO_4$ x 7 H_2O	1.00	mg
$CuSO_4$ x 5 H_2O	5.00	µg
H_3BO_3	10.00	µg
$MnSO_4$ x 5 H_2O	10.00	µg
$ZnSO_4$ x 7 H_2O	70.00	µg
MoO_3	10.00	µg
Agar	12.00	g
Distilled water	1000.00	ml

Add 5 ml methanol or incubate under methane-air (4:1). Adjust pH to 6.8.

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126. MYCOBACTERIUM INTRACELLULARE MEDIUM

To medium 1 add 20 g glycerol.

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127. MICROMONOSPORA MEGALOMICEA MEDIUM

Glucose	10.0	g
Soluble starch	20.0	g
Yeast extract	5.0	g
Casitone	5.0	g
$CaCO_3$	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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129. POTATO DEXTROSE AGAR

Infusion from potatoes (see below)	1000.0	ml
Glucose	20.0	g
Agar	15.0	g

Potato infusion:
Boil 200 g scrubbed and sliced potatoes in 1000 ml water for 1 hour. Pass through fine sieve. Avoid new potatoes.

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130. CZAPEK-DOX AGAR

Sucrose	30.00	g
$NaNO_3$	3.00	g
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.50	g
$FeSO_4$ x 7 H_2O	0.01	g
K_2HPO_4	1.00	g
Agar	13.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2.

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133: CARBON MONOXIDE OXIDIZER MEDIUM

Na_2HPO_4 x 12 H_2O	4.50	g
KH_2PO_4	0.75	g
NH_4Cl	1.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.03	g
Ferric ammonium citrate	18.00	mg
Trace element solution SL-6	1.00	ml
Na-acetate (optional)	3.00	g
Agar, for solid medium (optional)	12.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients, adjust pH to 7.0 and autoclave.

2. For chemoautotrophic growth incubate under a gas atmosphere of a) 20 - 80% carbon monoxide + 10% O_2 + 70 - 10% N_2 or b) 70% H_2 + 20% O_2 + 10% CO_2 adding 2.50 g $NaHCO_3$ per liter of medium.

3. For chemoorganotrophic growth add 3.00 g sodium acetate and incubate under air atmosphere.

For DSM 1083: The medium has to be supplemented with 10.00 ml/l of the vitamin solution of medium 141, sterilized by filtration. For chemoorganotrophic growth with acetate under air add also 10.00 ml/l of a 5% w/v $NaHCO_3$ solution, sterilized by filtration.

For DSM 1085: The medium has to be supplemented with 20.00 µg/l vitamin B_{12}. For chemoorganotrophic growth with acetate under air add also 20.00 ml/l of a 5% w/v $NaHCO_3$ solution, sterilized by filtration.

For DSM 13294: The medium has to be supplemented with 50.00 µg/l para-aminobenzoic acid. For chemoorganotrophic growth under air add also 2.00 g/l Na-pyruvate and 1.00 g/l yeast extract.

Trace element solution SL-6 (from medium 27)

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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134. HALISCOMENOBACTER MEDIUM

KH_2PO_4	27.00	mg
K_2HPO_4	40.00	mg
Na_2HPO_4 x 2 H_2O	40.00	mg
$CaCl_2$ x 2 H_2O	50.00	mg
$MgSO_4$ x 7 H_2O	75.00	mg
$FeCl_3$ x 6 H_2O	5.00	mg
$MnSO_4$ x H_2O	3.00	mg
Trace element solution SL-6 (see medium 27)	1.00	ml
Glutamic acid	1.31	g
Trypticase soy broth without glucose (BBL)	2.50	mg
Thiamine-HCl x 2 H_2O	0.40	mg
Vitamin B_{12}	0.01	mg
Glucose	2.00	g
Distilled water	1000.00	ml

Adjust pH to 7.5. The vitamins are sterilized by filtration. The glucose is dissolved in 5 ml of distilled water and sterilized separately by autoclaving.

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135: ACETOBACTERIUM MEDIUM

Final pH: 7.5 - 8.0
Final volume: 1021 ml

NH_4Cl	1.00	g
KH_2PO_4	0.33	g
K_2HPO_4	0.45	g
$MgSO_4$ x 7 H_2O	0.10	g
Modified Wolin's mineral solution	20.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	10.00	g
D-Fructose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine, and sulfide, bring to the boil, and cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Add bicarbonate (solid) and equilibrate the medium with the gas until a pH of around 7.4 is reached. Then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Before use adjust the pH to 7.5 - 8.0 by adding a sterile anoxic stock solution of sodium carbonate (5% w/v) prepared under 80% N_2 and 20% CO_2 gas mixture and add fructose, vitamins (sterilized by filtration), cysteine, and sulfide from anoxic sterile stock solutions prepared under 100% N_2.

2. Note: For autotrophic growth D-fructose is omitted and a gas atmosphere of 80% H_2 and 20% CO_2 is used.

For DSM 4132: Use D-fructose as substrate at a final concentration of 1.00 g/l.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g

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$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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136. VEILLONELLA MEDIUM

Trypticase (BBL)	5.00	g
Yeast extract	3.00	g
Na-(DL)-lactate	7.50	g
Na-thioglycolate	0.75	g
Tween 80	1.00	ml
Glucose	1.00	g
Putrescine	3.00	mg
Resazurin	1.00	mg
Distilled water	1000.00	ml

Adjust pH to 7.5 with solid K_2CO_3.

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137. MINERAL LACTATE MEDIUM (low carbon content)

Na-lactate	0.50	g
K_2HPO_4 x 3 H_2O	1.13	g
KH_2PO_4	0.88	g
NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.50	g
NaCl	1.00	g
$CaCl_2$ x 2 H_2O	5.00	mg
Trace element solution (see medium 69)	1.20	ml
Distilled water	1000.00	ml

Adjust pH to 7.0.

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138. LINDANE MEDIUM

Yeast extract	5.0	g
Hexachlorocyclo-hexane (Lindane)	0.1	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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139. HATHEWAYA MEDIUM

Trypticase soy broth (BD BBL)	15.00	g
Proteose peptone (BD BBL)	50.00	g
Na_2HPO_4	9.00	g
KH_2PO_4	1.92	g
$MgSO_4$ x 7 H_2O	0.08	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	1000.00	ml

Dissolve ingredients, adjust pH to 7.2, bring medium to the boil, then cool to room temperature under 100% N_2 gas atmosphere. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

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140: RUMINICLOSTRIDIUM MEDIUM

KH_2PO_4	0.50	g
NaCl	1.00	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
K_2HPO_4	0.50	g
Clarified rumen fluid	300.00	ml
Sludge fluid, alternative	300.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.50	g
Cellobiose	5.00	g
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	700.00	ml

Dissolve ingredients (except carbonate, cellobiose and reducing agents), bring medium to the boil, then cool to room temperature under 100% CO_2 gas atmosphere. Use either clarified rumen fluid or sludge fluid as supplement. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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141: METHANOGENIUM MEDIUM (H2/CO2)

Final pH: 6.8 - 7.0
Final volume: 1013 ml

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Na-acetate	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution	10.00	ml
$NaHCO_3$	5.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.

2. Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.5, then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.

3. For incubation use sterile 80% H_2 and 20% CO_2 gas mixture at two atmospheres of pressure.

For DSM 1498, DSM 22353: Adjust pH to 6.5.

For DSM 2373: Increase the amount of trypticase to 6.00 g/l.

For DSM 4254: Add a filter-sterilized, anoxic solution of L-histidine to a final concentration of 80.00 mg/l.

For DSM 7268, DSM 7466, DSM 14266: Use only one atmosphere overpressure of sterile

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80% H_2 and 20% CO_2 gas mixture.

For DSM 15219, DSM 18860, DSM 21220: Adjust pH to 7.5.

For DSM 15558: Supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N_2 gas. Adjust pH to 6.5 and use only one atmosphere overpressure of sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 16458: Supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N_2 gas. Adjust pH to 7.5 and use only one atmosphere overpressure of sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 22026: Adjust pH to 7.5 - 8.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg

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Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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142: THIOMICROSPIRA PELOPHILA MEDIUM

NaCl	25.00	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_2O	1.50	g
$CaCl_2$ x 2 H_2O	0.42	g
Trace element solution (Vishniac & Santer, 1957)	0.20	ml
Bromothymol blue (0.1% w/v)	4.00	ml
K_2HPO_4	0.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except hydrogenphosphate, thiosulfate and vitamins), adjust pH to 7.2 and autoclave. K_2HPO_4 and $Na_2S_2O_3$ are autoclaved separately each in 10% of the final volume. Filter sterilize the vitamins solution. Adjust pH of the complete medium to 7.2 with sterile 0.4% (w/v) Na_2CO_3 solution. Acidification of the medium during growth causes the pH indicator bromothymol blue to turn from blue to yellow.

2. Note: Growth of most Thiomicrospira strains is more reliable if the medium is prepared under a 80% N_2 and 20% CO_2 gas atmosphere to make it anoxic and then filled under air atmosphere into Hungate-type tubes (5 ml per vial). The pH is adjusted with a sterile stock solution of Na_2CO_3 (5% w/v) after autoclaving.

For DSM 12346, DSM 12350, DSM 12351, DSM 12352, DSM 12353, DSM 12354, DSM 28203: Omit vitamins and adjust pH of complete medium to 7.3 - 7.6.

For DSM 25197, DSM 25194: Omit vitamins and adjust pH of complete medium to 8.0.

For DSM 100274: Adjust pH of complete medium to 6.0 - 6.5.

Trace element solution (Vishniac & Santer, 1957) (from medium 69)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	22.00	g
$CaCl_2$ x 2 H_2O	5.54	g
$MnCl_2$ x 4 H_2O	5.06	g
$FeSO_4$ x 7 H_2O	5.00	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1.10	g
$CuSO_4$ x 5 H_2O	1.57	g
$CoCl_2$ x 6 H_2O	1.61	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water, adjust pH to 7 using 2 N KOH, then add remaining compounds. Adjust pH of final solution to 6.0 with KOH.

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142: THIOMICROSPIRA PELOPHILA MEDIUM

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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143: CLOSTRIDIUM SP. LA1 MEDIUM

Crotonic acid	6.00	g
NaOH	0.30	g
$(NH_4)_2HPO_4$	0.15	g
K_2HPO_4	0.10	g
$MgCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.04	g
NH_4Cl	0.05	g
$MgSO_4$ x 7 H_2O (0.1% w/v)	0.60	ml
$MnSO_4$ x 2 H_2O (0.1% w/v)	0.40	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	0.40	ml
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	10.00	mg
Biotin (0.1% w/v)	0.04	ml
p-Aminobenzoic acid (0.1% w/v)	0.80	ml
Tryptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
K_2CO_3	4.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except potassium carbonate) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. An anoxic stock solution of the potassium carbonate is autoclaved separately under 80% N_2 and 20% CO_2 gas mixture and thereafter added to the sterile medium. Adjust pH of the complete medium to 6.8 - 7.0. Prior to inoculation add 10 - 20 mg/l sodium dithionite from a 5% (w/v) stock solution that has been freshly prepared under 100% N_2 gas and sterilized by filtration to reduce the medium.

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144: THERMOANAEROBACTER BROCKII MEDIUM

NH_4Cl	0.90	g
NaCl	0.90	g
$MgCl_2$ x 6 H_2O	0.40	g
KH_2PO_4	0.75	g
K_2HPO_4	1.50	g
Trace element solution	9.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	3.00	ml
Yeast extract	3.00	g
Trypticase peptone (BD BBL)	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution	5.00	ml
D-Glucose	5.00	g
Na_2S x 9 H_2O	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, glucose and sulfide), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 7.2 - 7.4.

For DSM 3532: Add 10% NaCl; anaerobic

For DSM 12299: Omit D-glucose.

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	0.20	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.17	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve

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mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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146. HALOCOCCUS MEDIUM

Solution A:		
Skim milk (Difco)	50.0	g
Distilled water	500.0	ml
Solution B:		
$MgSO_4$ x 7 H_2O	10.0	g
KNO_3	2.0	g
NaCl	200.0	g
Fe-citrate	trace	-
Distilled water	100.0	ml
Solution C:		
Neopeptone	5.0	g
Glycerol	10.0	g
Agar	25.0	g
Distilled water	400.0	ml

Steam the three solutions separately, mix together solutions B and C and adjust pH to 8.4. Autoclave the skim milk at 112°C for 15 min., and the agar salt mixture at 121°C for 20 min. Add the hot mixture to the warm skim milk aseptically.

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147. RUMINOBACTER AMYLOPHILUS MEDIUM

K_2HPO_4	0.45	g
KH_2PO_4	0.45	g
$(NH_4)_2SO_4$	0.90	g
NaCl	0.90	g
$MgSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.12	g
Starch, soluble	5.00	g
Casitone (Difco)	10.00	g
Resazurin	1.00	mg
Cysteine-HCl x H_2O	0.50	g
$NaHCO_3$	6.00	g
Distilled water	1000.00	ml

Adjust pH to 7.0. Gas atmosphere: 100% CO_2.

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148: DESULFUROMONAS SP. MEDIUM

KH_2PO_4	1.00	g
NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na-acetate	0.50	g
Fumaric acid	1.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins and sulfide), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials. After autoclaving complete the medium by adding vitamins and sulfide from separately sterilized anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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149: MEGADESULFOVIBRIO MEDIUM

Solution A	970.00	ml
Solution B	20.00	ml
Solution C	1.00	ml
Solution D	10.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution D is autoclaved separately under 100% N_2 gas. To complete the medium appropriate amounts of solutions B to D are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0 - 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% inoculum.

Solution A

KH_2PO_4	1.00	g
NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
Na_2SO_4	2.00	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-6	1.00	ml
H_2SO_4 (2 N)	1.00	ml
Na-L-lactate	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	970.00	ml

Solution B

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

Na_2S x 9 H_2O	0.30	g

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Distilled water	10.00	ml

Trace element solution SL-6 (from medium 27)

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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150: ACIDIANUS BRIERLEYI MEDIUM

$(NH_4)_2SO_4$	3.00	g
K_2HPO_4 x 3 H_2O	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.10	g
$Ca(NO_3)_2$ x 4 H_2O	0.02	g
Yeast extract	0.20	g
Sulfur (powdered)	10.00	g
Distilled water	1000.00	ml

Dissolve ingredients except yeast extract and sulfur. Adjust pH with 6 N H_2SO_4 to 1.5 - 2.5. Yeast extract (10% w/v in distilled water) is autoclaved separately. For sterilization of the sulfur place it in screw-capped tubes or bottles, wet with a few drops of water and heat for 3 hours to 90 - 100°C in a water bath on each of three successive days. Before use, aseptically layer the sulfur onto the surface of the autoclaved liquid basal medium.

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152. NICOTINIC ACID MEDIUM

Nicotinic acid	5.0	g
Yeast extract	10.0	g
$CaCl_2$ x 2 H_2O	8.0	mg
$MgSO_4$ x 7 H_2O	8.0	mg
KH_2PO_4	40.0	mg
K_2HPO_4	40.0	mg
NaCl	80.0	mg
$NaHCO_3$	0.4	g
Cysteine-HCl x H_2O	0.5	g
Distilled water	1000.0	ml

Add nicotinic acid to the given amount of water, boil until dissolved and cool to room temperature. Add the other substances, adjust pH to 8.2 and distribute under 100% nitrogen atmosphere.

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153: THIOGLYCOLATE MEDIUM (MERCK 108190)

Trypticase (BBL)	15.00	g
L-Cystine	0.50	g
Glucose	2.00	g
Yeast extract	5.00	g
NaCl	2.50	g
Na-thioglycolate	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Agar, for solid medium (optional)	15.00	g
Distilled water	1000.00	ml

Dissolve ingredients, sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Adjust pH to 7.1 with Na_2CO_3 (5% w/v), if necessary. Then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

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155. BEGGIATOA MEDIUM

$CaSO_4$ (saturated solution)	20.000	ml
NH_4Cl	0.450	mg
K_2HPO_4	0.100	mg
$MgSO_4$ x 7 H_2O	0.200	mg
Trace element solution (see below)	5.000	ml
Na-acetate	0.500	g
Nutrient broth (Difco)	0.500	g
Agar (if necessary)	10.000	g
Catalase, filter-sterilized	15000 – 35000	units/1000 ml
Distilled water	1000.000	ml

Adjust pH to 7.4 using pH indicator paper before autoclaving. The catalase is added just before inoculation.

Trace element solution:

EDTA	0.200	g
$FeSO_4$ x 7 H_2O	0.700	g
$ZnSO_4$ x 7 H_2O	0.010	g
$MnSO_4$ x 4 H_2O	0.002	g
$CuSO_4$ x 5 H_2O	5.000	µg
H_3BO_3	10.000	mg
$Co(NO_3)_2$	1.000	mg
Na_2MoO_4 x 2 H_2O	1.000	mg
Distilled water	1000.000	ml

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156: ANAEROTIGNUM MEDIUM

L-Alanine	3.00	g
Peptone	3.00	g
Yeast extract	4.00	g
$MgSO_4$ x 7 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	18.00	mg
Potassium phosphate buffer (1 M, pH 7.1)	5.00	ml
$CaSO_4$ (saturated aq. solution)	2.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
L-Cysteine HCl x H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate and cysteine), adjust pH to 7.0, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and cysteine, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or seruim vials and autoclave. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

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157: WOLINELLA MEDIUM

$(NH_4)_2SO_4$	1.00	g
K_2HPO_4	5.00	g
Na_2-fumarate	4.00	g
Na-formate	3.00	g
Yeast extract	1.00	g
$MgCl_2$ x 6 H_2O	0.20	g
$FeSO_4$ x 7 H_2O	0.02	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine and sulfide), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and adjust pH of complete medium to 7.0 - 7.2, if necessary.

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158. THERMOPLASMA ACIDOPHILUM MEDIUM

$(NH_4)_2SO_4$	1.320	g
KH_2PO_4	0.372	g
$MgSO_4$ x 7 H_2O	0.247	g
$CaCl_2$ x 2 H_2O	0.074	g
Trace element solution (see below)	10.000	ml
Yeast extract (Oxoid)	2.000	g
Glucose	20.000	g
Distilled water (freshly distilled)	1000.000	ml

Adjust pH to 1.0 with 1 N H_2SO_4. Stock solutions of yeast extract (10% w/v) and glucose (50% w/v) in water are sterilized separately by autoclaving and thereafter added to the sterile mineral salt medium.

Trace element solution:

$FeCl_3$ x 6 H_2O	1.930	g
$MnCl_2$ x 4 H_2O	0.180	g
$Na_2B_4O_7$ x 10 H_2O	0.450	g
$ZnSO_4$ x 7 H_2O	22.000	mg
$CuCl_2$ x 2 H_2O	5.000	mg
Na_2MoO_4 x 2 H_2O	3.000	mg
$VOSO_4$ x 5 H_2O	3.800	mg
$CoSO_4$ x 7 H_2O	2.000	mg
Distilled water	1000.000	ml

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159: TREPONEMA MEDIUM

Clarified rumen fluid	300.00	ml
$CaCl_2$ x 2 H_2O	0.12	g
$MgSO_4$ x 7 H_2O	0.18	g
KH_2PO_4	0.45	g
K_2HPO_4	0.45	g
NaCl	0.90	g
$(NH_4)_2SO_4$	0.90	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	1.00	g
Na_2CO_3	1.50	g
D-Glucose	2.00	g
Distilled water	700.00	ml

Dissolve ingredients (except cysteine, carbonate and glucose) and sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH with KOH to 6.5. Distribute medium under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Therafter, add glucose from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Final pH of the medium should be 6.7 - 7.0.

For DSM 11370: Plus cellobiose (0.1%), maltose (0.1%) and soluble starch (0.1%); anaerobic

For DSM 11371: Plus cellobiose (0.1%), maltose (0.1%) and soluble starch (0.1%); anaerobic; grows within 12 hours, lyses quickly;

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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160. CYTOPHAGA HUTCHINSONII MEDIUM

1. To medium 67 add 0.5% cellobiose (filter-sterilized)

or

2. Distribute medium 67 (without agar) in 5 ml amounts in test tubes, add a strip of filter paper (7 cm long) to each and autoclave, or place sterile pieces of filter paper on the surface of agarized medium 67.

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161: METHANOFOLLIS MEDIUM

Final pH: 6.5
Final volume: 1001 ml

Clarified rumen fluid	300.00	ml
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$(NH_4)_2SO_4$	0.30	g
NaCl	0.60	g
$MgSO_4$ x 7 H_2O	0.13	g
$CaCl_2$ x 2 H_2O (0.1% w/v)	8.00	ml
Modified Wolin's mineral solution	10.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Yeast extract (DIFCO)	1.00	g
Trypticase (BBL)	1.00	g
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	660.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide) and sparge medium for 30 - 45 min with 80% H_2 and 20% CO_2 gas mixture to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.8, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas atmosphere. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.5. For incubation use sterile 80% H_2 and 20% CO_2 gas mixture at two atmospheres of pressure.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Modified Wolin's mineral solution (from medium 141)

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Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Fatty acid mixture (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml
Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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162. HYPHOMICROBIUM MEDIUM

KH_2PO_4	1.36	g
Na_2HPO_4	2.13	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	6.00	mg
$FeSO_4$ x 7 H_2O	3.00	mg
$MnSO_4$ x H_2O	1.00	mg
Na_2MoO_4 x 2 H_2O	1.50	mg
Methylamine hydrochloride	3.38	g
Yeast extract	0.10	g
Distilled water	1000.00	ml

Adjust pH to 7.4 with NaOH. Autoclave 20 min at 121°C; final pH: 7.2.

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163: HALODESULFOVIBRIO (POSTGATE) MEDIUM

Solution A	980.00	ml
Solution B	10.00	ml
Solution C	10.00	ml

Bring solution A to the boil, then cool to room temperature while sparging with 100% N_2 gas. Add solutions B and C, adjust pH to 7.8 with NaOH, and distribute under N_2 gas atmosphere into anoxic Hungate-type tubes. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave 15 min at 121°C. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

For DSM 10520: Adjust final pH of autoclaved medium to 7.5 using a sterile anoxic stock solution of Na_2CO_3 (5% w/v) prepared under 80% N_2 and 20% CO_2 gas atmosphere.

Solution A

NaCl	25.00	g
K_2HPO_4	0.50	g
NH_4Cl	1.00	g
Na_2SO_4	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	2.00	g
Na-DL-lactate	2.00	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	980.00	ml

Solution B

$FeSO_4$ x 7 H_2O	0.50	g
Distilled water	10.00	ml

Solution C

Na-thioglycolate	0.10	g
Ascorbic acid	0.10	g
Distilled water	10.00	ml

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165: ACETIVIBRIO CELLULOLYTICUS MEDIUM

Mineral solution 1	75.00	ml
Mineral solution 2	75.00	ml
Modified Wolin's mineral solution	10.00	ml
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Cellobiose	3.00	g
Cellulose (MN300) (optional)	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	820.00	ml

Dissolve ingredients except carbonate, cellobiose, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Add cellobiose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Cellobiose and vitamins have to be sterilized by filtration. Prior to use adjust pH of complete medium to 7.2 - 7.4. Cellulose (MN 300, Whatman CF II, Kleenex tissue paper, or HCl treated cotton) can be used as an alternative to cellobiose.

Mineral solution 1

K_2HPO_4	3.90	g
Distilled water	1000.00	ml

Mineral solution 2

KH_2PO_4	2.40	g
$(NH_4)_2SO_4$	6.00	g
NaCl	0.59	g
$MgSO_4$ x 7 H_2O	1.20	g
$CaCl_2$ x 2 H_2O	0.72	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g

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NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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166. HYPHOMICROBIUM STRAIN X MEDIUM

$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
NaH_2PO_4 x H_2O	0.50	g
K_2HPO_4	1.55	g
Trace element solution (see medium 69)	0.20	ml
Methylamine hydrochloride	3.40	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2. For liquid medium use only 0.7 g of methylamine hydrochloride per liter.

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167: SPOROCYTOPHAGA MEDIUM

$NaNO_3$	2.00	g
$MgSO_4$ x 7 H_2O	1.00	g
KCl	0.50	g
$FeSO_4$ x 7 H_2O	6.00	mg
KH_2PO_4	0.14	g
K_2HPO_4	1.20	g
Yeast extract	0.02	g
Distilled water	1000.00	ml
Agar (optional)	15.00	g/l

1. Adjust pH to 7.2.

2. Place a strip of sterile cellulose filter paper onto an agar plate. Alternatively, place a strip of sterile cellulose filter paper into a culture tube containing 5 ml of medium, thereby leaving 1 - 2 cm of the strip outside of the medium. Inoculate the strain onto the cellulose filter paper. The strain will grow on the cellulose paper only.

For DSM 11118: It is recommended to use the following modified medium for the reactivation of freeze-dried samples: Prepare agar plates of medium 167 by supplementing medium with 1.5% (w/v) agar. Place 4 pieces of sterile cellulose (not nitrocellulose!) filter paper on each agar plate. Suspend freeze-dried content of the inner vial of one ampoule with 0.5 ml of liquid medium 167 and inoculate each piece of the filter paper with 1 large drop of the cell suspension. Alternatively, the strain also grows with 0.1% glucose or 0.1% cellobiose as carbon source.

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169: TERETINEMA MEDIUM

Solution A	960.00	ml
Solution B	20.00	ml
Solution C	20.00	ml

1. Dissolve ingredients of solution A (except cysteine) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH 7.2 with KOH, then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere and solution C is autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Final pH of the complete medium should be 7.5.

2. For solid medium add 10.00 g/l agar (BD Bacto) to solution A.

Solution A

$CaCl_2$ x 2 H_2O	0.04	g
$MgSO_4$ x 7 H_2O	0.50	g
Yeast extract (OXOID)	4.00	g
D-Glucose	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Agar, for solid medium (optional)	10.00	g
Distilled water	960.00	ml

Solution B

$NaHCO_3$	1.00	g
Distilled water	20.00	ml

Solution C

K_2HPO_4 (0.5 M)	16.04	ml
KH_2PO_4 (0.5 M)	3.96	ml

Combine 16.04 ml of 0.5 M K_2HPO_4 with 3.96 ml of 0.5 M KH_2PO_4 stock solution to reach a pH of around 7.4.

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170. YEAST EXTRACT - SKIM MILK MEDIUM

Skim milk (Difco)	10.0	g
Yeast extract	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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171: THERMOANAEROBACTER KIVUI MEDIUM

K_2HPO_4	0.22	g
KH_2PO_4	0.22	g
$NaH_2PO_4 \times H_2O$	4.50	g
$Na_2HPO_4 \times 12\ H_2O$	6.10	g
NH_4Cl	0.31	g
$(NH_4)_2SO_4$	0.22	g
NaCl	0.45	g
$MgSO_4 \times 7\ H_2O$	0.09	g
$CaCl_2 \times 2\ H_2O$ (0.1% w/v)	6.00	ml
$FeSO_4 \times 7\ H_2O$ solution (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl $\times H_2O$	0.50	g
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except cysteine and sulfide, sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.5.

2. After inoculation pressurize cultivation vessels to one bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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172. CYTOPHAGA (marine) MEDIUM

Yeast extract (Difco)	1.0	g
Tryptone (Difco)	1.0	g
NaCl	24.7	g
KCl	0.7	g
$MgSO_4$ x 7 H_2O	6.3	g
$MgCl_2$ x 6 H_2O	4.6	g
$CaCl_2$ x 2 H_2O	1.2	g
$NaHCO_3$	0.2	g
Agar (Difco)	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2. Sodium bicarbonate and calcium chloride are autoclaved separately, each in a small volume of distilled water.

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173: OCEANISPIROCHAETA LITORALIS MEDIUM

D-Glucose	2.00	g
Trypticase peptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Tris-HCl buffer (1 M, pH 7.5)	50.00	ml
Sea water	750.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	200.00	ml

Dissolve ingredients (except cysteine) and sparge medium for 30 - 45 min with 100% N_2 gas to make it anoxic. Add cysteine and adjust pH to 7.2 - 7.4, then distribute medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Final pH of the medium should be about 7.2.

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174: ZUELZERA MEDIUM

D-Glucose	5.00	g
Yeast extract (OXOID)	2.00	g
Peptone (BD BACTO)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-thioglycolate	0.50	g
Agar, for stabs (optional)	10.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients, adjust pH to 7.6 with KOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

2. For stabs add 10.0 g/l agar.

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181. SELENOMONAS RUMINANTIUM MEDIUM

Glucose	1.0	g
Trypticase (BBL)	5.0	g
KH_2PO_4	1.0	g
Na-acetate	4.0	g
Yeast extract	2.0	g
n-Valeric acid	0.1	ml
Resazurin	1.0	mg
Na_2CO_3	4.0	g
Cysteine-HCl x H_2O	0.5	g
Distilled water	1000.0	ml

Adjust pH to 7.0. Gas atmosphere: 100% CO_2.

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182: SACCHAROLOBUS SOLFATARICUS MEDIUM

Yeast extract (OXOID)	1.00	g
Casamino acids (BD BBL)	1.00	g
KH_2PO_4	3.10	g
$(NH_4)_2SO_4$	2.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.25	g
Allen's trace element solution	10.00	ml
Distilled water	1000.00	ml

Dissolve all compounds except yeast extract and Casamino acids, adjust pH to 4.0 - 4.2 with 10 N H_2SO_4 at room temperature and autoclave. Prior to inoculation add yeast extract and Casamino acids from stock solutions sterilized by filtration.

For DSM 5354: Adjust pH of medium to 3.5.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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183. TRICHOCOCCUS MEDIUM

Tryptone (Difco)	10.0	g
Glucose	3.0	g
$MgCl_2$ x 6 H_2O	1.1	g
Na_2SO_4	4.0	g
KCl	0.7	g
Distilled water	1000.0	ml

Adjust pH to 7.3. Glucose is filter-sterilized.

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185. THERMOPROTEUS MEDIUM

$(NH_4)_2SO_4$	0.264	g
$FeSO_4$ x 7 H_2O	0.556	g
$MgSO_4$ x 7 H_2O	0.492	g
$CaSO_4$ x 2 H_2O	0.344	g
KH_2PO_4	0.014	g
Resazurin	1.000	mg
Trace elements solution (see below)	1.000	ml
Yeast extract	0.200	g
Soluble starch	5.000	g
Sulfur, powdered	10.000	g
Na_2S x 9 H_2O	0.500	g
Distilled water	1000.000	ml

Trace elements solution:

NaF	840.0	mg
$MnCl_2$ x 4 H_2O	180.0	mg
$Na_2B_4O_7$ x 10 H_2O	450.0	mg
$ZnSO_4$ x 7 H_2O	22.0	mg
$CuCl_2$ x 2 H_2O	5.0	mg
Na_2MoO_4 x 2 H_2O	3.0	mg
$CoSO_4$ x 7 H_2O	1.0	mg
Distilled water	1000.0	ml

Prepare medium (without starch, sodium sulfide, sulfur) anaerobically under N_2 gas atmosphere. Adjust pH to 5.5 with H_2SO_4 before sterilization. Distribute the medium into tubes containing the appropriate amount of sulfur powder. Sterilize medium by heating for 3 h at 90 - 100°C on three subsequent days. Before use, add to the medium starch and sodium sulfide.
Medium pH is 5.5.

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186. UNIVERSAL MEDIUM FOR YEASTS (YM)

Yeast extract	3.0	g
Malt extract	3.0	g
Peptone from soybeans	5.0	g
Glucose	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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187. MEDIUM FOR OSMOPHILIC FUNGI (M 40 Y)

Sucrose	400.0	g
Malt extract	20.0	g
Yeast extract	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml

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188. CHAETOMIUM MEDIUM

$NaNO_3$	2.00	g
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.50	g
$Fe_2(SO_4)_3$ x H_2O	0.01	g
KH_2PO_4	0.14	g
K_2HPO_4	1.20	g
Agar	15.00	g
Yeast extract	0.02	g
Distilled water	1000.00	ml

Adjust pH to 7.2. Place a strip of sterile filter paper on cooled agar and inoculate on filter strip.

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189. OAT FLAKE MEDIUM

Oat flakes	30.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Boil oat flakes in water for 10 min; fill up to 1 l and add agar. Autoclave 20 min at 121°C. Shake before pouring.

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190. YpSs MEDIUM

Yeast extract	4.0	g
Soluble starch	15.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.5	g
Agar	15.0	g
Distilled water	1000.0	ml

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191. CORN MEAL AGAR

Blend 50.0 g corn meal in 800 ml of distilled water. Leave overnight in the refrigerator. Heat at 60°C for one hour. Filter and make up volume to 1 l. Add 10.0 g agar and heat to dissolve, then autoclave.

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192. PILOBOLUS MEDIUM

Yeast extract	2.00	g
Na-acetate	10.00	g
$(NH_4)_2SO_4$	0.66	g
$MgSO_4$ x 7 H_2O	0.50	g
K_2HPO_4	1.00	g
Thiamine-HCl x 2 H_2O	10.00	mg
Haemin (dissolved in 37.5 ml 0.1 N NaOH)	10.00	mg
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 8.0.

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193: DESULFOBACTER MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

For DSM 1744, DSM 10631, DSM 16697: Omit Na-acetate and add after autoclaving 1.00 g/l yeast extract and 2.50 g/l Na-L-Lactate from sterile anoxic stock solutions.

For DSM 2059, DSM 27197: Omit Na-acetate and add after autoclaving 0.60 g/l Na -benzoate from an anoxic stock solution sterilized by filtration.

For DSM 4660: Omit Na-acetate and add after autoclaving 1.00 ml/l seven vitamins solution (see medium 503) and 0.10 g phenol from anoxic stock solutions sterilized by filtration.

For DSM 6637: Replace Na-acetate with 0.58 g/l acetone. Supplement medium with 3.40 g/l KNO_3 and omit solution E. Add after autoclaving 5.00 g/l Na-pyruvate from an anoxic stock solution sterilized by filtration.

For DSM 8775: Omit Na-acetate and add after autoclaving 2.50 g/l Na-pyruvate from an anoxic stock solution sterilized by filtration.

For DSM 10711: Omit Na-acetate and add after autoclaving 1.00 ml/l seven vitamins solution (see medium 503), 2.50 g/l Na-pyruvate and 0.50 g/l yeast extract from anoxic stock solutions sterilized by filtration.

For DSM 14956: Omit Na-acetate and add after autoclaving 1.60 g/l methanol from an anoxic stock solution sterilized by filtration.

For DSM 15102: Omit Na-acetate and add after autoclaving 1.00 g/l yeast extract and 3.50 g/l D-glucose from sterile anoxic stock solutions.

For DSM 18843: Omit Na-acetate and add after autoclaving 1.00 ml/l seven vitamins solution (see medium 503) and 0.62 g/l Na-stearate from anoxic stock solutions sterilized

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by filtration.

For DSM 25524: Omit Na-acetate and add after autoclaving 1.00 ml/l seven vitamins solution (see medium 503) and 0.33 g/l Na-octanoate from anoxic stock solution sterilized by filtration.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	7.00	g
$MgCl_2$ x 6 H_2O	1.30	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Na-acetate x 3 H_2O	2.50	g
Distilled water	20.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions B to E are added to the sterile, cooled solution A in appropriate amounts in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

For DSM 2055: Na-propionate is replaced with 0.70 g/l Na-butyrate, 0.30 g/l Na-caproate and 0.15 g/l Na-octanoate added after autoclaving from sterile anoxic stock solutions prepared under N_2. Adjust final pH of medium to 7.7.

For DSM 3852: Na-propionate is replaced with 0.70 g/l ethanol absolute added after autoclaving from a sterile anoxic stock solution prepared under N_2. In addition, the medium is supplemented with 0.10 g/l each of yeast extract and casamino acids.

For DSM 5092: Na-propionate is replaced with 0.90 g/l putrescine added after autoclaving from a sterile anoxic stock solution prepared under N_2.

For DSM 5193: Supplement medium with 0.50 g/l yeast extract and replace Na-propionate with 0.90 g/l Na-methoxyacetate added after autoclaving from a sterile anoxic stock solution prepared under N_2.

For DSM 5433: Na-propionate is replaced with 1.50 g/l 1,2-propanediol added after autoclaving from a sterile anoxic stock solution prepared under N_2.

For DSM 5651: Na-propionate is replaced with 1.50 g/l of Na-(D/L)-3-hydroxybutyrate as substrate.

For DSM 6283, DSM 12907: Na-propionate is replaced with 2.50 g/l Na-(DL)-lactate and 1.00 g/l yeast extract added after autoclaving from sterile anoxic stock solutions prepared under N_2. Final pH of the medium should be 6.8 - 7.0.

For DSM 7474, DSM 7475, DSM 7476: Na-propionate is replaced by 0.40 g/l 3,4,5-trimethoxybenzoic acid added after autoclaving from a sterile anoxic stock solution (2% w/v) prepared under N_2 and neutralized with NaOH. Stock solutions of 3,4,5-trimethoxybenzoate should be prepared freshly and sterilized by filtration.

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Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added from an anoxic stock solution sterilized by filtration.

For DSM 10291, DSM 24454: Na-propionate is replaced with 2.50 g/l Na-L-lactate added after autoclaving from a sterile anoxic stock solution prepared under N_2. Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added from an anoxic stock solution sterilized by filtration.

For DSM 12016: Na-propionate is replaced with 0.10 ml/l propanol added after autoclaving from a sterile anoxic stock solution prepared under N_2. Supplement medium with 1.00 g/l yeast extract. When growth has started, feed again same amount of propanol.

For DSM 13527, DSM 21556, DSM 24590: Na-propionate is replaced with 1.00 g/l Na -butyrate added after autoclaving from a sterile anoxic stock solution prepared under N_2.

For DSM 14880: Na-propionate is replaced with 0.50 g/l yeast extract and 2.20 g/l sodium pyruvate added after autoclaving from anoxic stock solutions sterilized by filtration.

For DSM 28570: Na-propionate is replaced with 0.15 g/l Na-benzoate added after autoclaving from a sterile, anoxic stock solution prepared under N_2.

For DSM 102358, DSM 102359, DSM 102360: Na-propionate is replaced with 1.50 g/ Na -benzoate and 1.00 g/l Na-acetate as substrates. Reduce amount of sulfide to 0.10 g/l. Dithionite is used to reduce medium completely.

For DSM 105758: Na-propionate is replaced with 0.70 g/l Na-butyrate, 0.30 g/l Na-caproate and 0.15 g/l Na-octanoate added after autoclaving from sterile anoxic stock solutions prepared under N_2. Adjust final pH of medium to 9.0.

For DSM 107641: Na-propionate is replaced with 1.50 g/ 1,2-propanediol added after autoclaving from a sterile, anoxic stock solution prepared under N_2.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g

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Distilled water	30.00	ml

Solution C

Na-propionate	1.50	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg

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194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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195: DESULFOBACTER MEDIUM

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclaved. Solutions C and E are autoclaved separately under 100% N_2 gas. Solution B is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

For DSM 28787: Replace acetate with 1.80 g/l glucose and supplement medium with 0.50 g/l yeast extract; anaerobic

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	21.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Na-acetate x 3 H_2O	2.50	g
Distilled water	10.00	ml

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Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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196: DESULFOCAPSA SULFEXIGENS MEDIUM

Solution A	961.00	ml
Solution B	30.00	ml
Solution C	10.00	ml

1. First dissolve 2.7 g $FeCl_3$ x 6 H_2O in distilled water and adjust the pH to 7.0 with 1 N NaOH (about 3 ml). Add and dissolve remaining salts of solution A and sparge medium with 80% N_2 and 20% CO_2 gas mixture for at least 30 min, then distribute under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is autoclaved separately under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be adjusted to 7.2.

2. Note: For transfers use 5 - 10% (v/v) inoculum.

Solution A

$FeCl_3$ x 6 H_2O	2.70	g
Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	21.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Distilled water	960.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

$Na_2S_2O_3$ x 5 H_2O	2.50	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g

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$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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198: DESULFOSARCINA MEDIUM (BRACKISH WATER)

Final pH: 7.1 - 7.4
Final volume: 1003 ml

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

For DSM 2650: Na-benzoate is replaced by 1.50 g/l Na-pyruvate and 1.00 g/l Na_2-malate added after autoclaving from sterile anoxic stock solutions prepared under N_2.

For DSM 9990: Na-benzoate is replaced by 6.20 g/l Na-acetate.

For DSM 103834: Na-benzoate is replaced by 2.5 g/l Na-lactate.

For DSM 113909: Supplement medium with 1.60 g Na_2-fumarate. Omit Na-benzoate. Adjust pH of final medium to 8.0 - 8.5.

For DSM 115219: Supplement medium with 0.35 g Na-formate and 0.16 g Na-acetate. Omit Na-benzoate.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	13.50	g
$MgCl_2$ x 6 H_2O	2.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Selenite-tungstate solution	1.00	ml
Trace element solution SL-10	1.00	ml

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Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Na-benzoate	0.60	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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203: METHANOTHERMUS MEDIUM

Mineral solution 1	37.50	ml
Mineral solution 2	37.50	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	1.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Na_2SO_4	3.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	920.00	ml

1. Dissolve ingredients except carbonate, yeast extract, Trypticase peptone, vitamins, sulfide and cysteine, then adjust pH to 6.0 with sulfuric acid. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum bottles to 30% of their volume and autoclave. Thereafter, add by injection yeast extract, Trypticase peptone, vitamins (sterilized by filtration), sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 6.5.

2. After inoculation pressurize bottles to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 3496: Omit yeast extract and Trypticase peptone from the medium.

Mineral solution 1 (from medium 203)

K_2HPO_4	6.00	g
Distilled water	1000.00	ml

Mineral solution 2 (from medium 203)

KH_2PO_4	6.00	g
$(NH_4)_2SO_4$	6.00	g
NaCl	12.00	g
$MgSO_4$ x 7 H_2O	2.40	g
$CaCl_2$ x 2 H_2O	1.60	g
Distilled water	1000.00	ml

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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204. ASPERGILLUS NIDULANS MINIMAL MEDIUM

Solution A:

$NaNO_3$	6.00	g
KH_2PO_4	1.52	g
KCl	1.52	g
Distilled water	500.00	ml

Adjust pH to 6.5 with 2 N NaOH.

Solution B:

$MgSO_4$ x 7 H_2O	0.52	g
$FeSO_4$ x 7 H_2O	trace	-
$ZnSO_4$ x 7 H_2O	trace	-
Agar	15.00	g
Distilled water	250.00	ml

Solution C:

Glucose	10.00	g
Distilled water	200.00	ml

Autoclave separately for 15 min. Mix A, B and C before pouring plates.

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206: THERMODESULFOBACTERIUM MEDIUM

Na_2SO_4	3.00	g
NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.20	g
KH_2PO_4	0.30	g
Na_2HPO_4 x 12 H_2O	2.00	g
Trace element solution	10.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	1.00	g
Na-L-lactate	4.00	g
Wolin's vitamin solution	5.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except yeast extract, lactate, vitamins and sulfide) and sparge medium for 30 - 45 min with 100% N_2 gas to make it anoxic. Distribute under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add yeast extract, lactate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Before use, neutralize the sodium sulfide solution by dropwise addition of 2 N H_2SO_4 (see medium 29). Adjust pH of complete medium to 6.8 - 7.0.

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	0.20	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.17	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

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Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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207. PROPIONISPIRA MEDIUM

KH_2PO_4	0.3	g
Na_2HPO_4 x 12 H_2O	2.1	g
NH_4Cl	1.0	g
$MgCl_2$ x 6 H_2O	0.2	g
Trace element solution (see medium 144)	10.0	ml
$FeSO_4$ x 7 H_2O	1.5	mg
Vitamin solution (see medium 141)	5.0	ml
Resazurin	1.0	mg
$NaHCO_3$	4.0	g
Yeast extract	2.0	g
Na-lactate	5.0	g
Na_2S x 9 H_2O	0.5	g
Distilled water	1000.0	ml

Adjust pH to 7.2. Gas atmosphere: 80% N_2 + 20% CO_2. Prepare a 5% (w/v) solution of sodium sulfide anoxically under nitrogen and autoclave separately. Before use, neutralize by dropwise addition of 1 N HCl.

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209. EUBACTERIUM LENTUM MEDIUM

To medium 78 add 0.5% (w/v) arginine.

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210. HALOANAEROBIUM MEDIUM

NaCl	130.0	g
$MgSO_4$ x 7 H_2O	8.8	g
KCl	1.0	g
Yeast extract	10.0	g
Trypticase (BBL)	10.0	g
Vitamin solution (see medium 141)	10.0	ml
Trace element solutionsee medium 144)	10.0	ml
Distilled water	1000.0	ml

Prepare the medium anaerobically under 100% N_2. After autoclaving add from sterile solutions:

Thioglycolate-ascorbate reducing agent (see below)	25.0	ml
Glucose 10% (w/v)	25.0	ml
2 N NaOH	10.0	ml

Final pH: 9.0.

Thioglycolate-ascorbate solution:

Na-thioglycolate	0.5	g
Na-ascorbate	0.5	g
Distilled water	25.0	ml

Filter sterilize.

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211: THERMOANAEROBACTERIUM MEDIUM

KH_2PO_4	0.30	g
Na_2HPO_4 x 12 H_2O	5.30	g
NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace element solution	10.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.50	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	5.00	g
Wolin's vitamin solution	5.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, vitamins and sulfide), adjust pH to 6.0, sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. After sterilization add glucose and sulfide from anoxic stock solutions autoclaved under 100% N_2 gas and vitamins from a filter-sterilized anoxic stock solution prepared under 100% N_2. Adjust pH of complete medium to 6.0 - 6.5, if necessary.

For DSM 8683, DSM 8684, DSM 16487: Adjust pH of complete medium to 5.5.

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	0.20	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.17	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

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Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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212: SYNTHROPHOMONAS MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

1. Add and dissolve ingredients of solution A and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Solution B is prepared under 80% N_2 and 20% CO_2 gas atmosphere and autoclaved. Solutions C, E and F are autoclaved under 100% N_2 gas atmosphere. Solution D is prepared under 100% N_2 gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.2, if necessary.

2. Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days . After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

For DSM 2805: Replace butyrate with 1.50 g/l Na-propionate and supplement medium with 1.00 g/l D-glucose added from a sterile anoxic stock solution sterilized by filtration.

For DSM 4212: Replace Na-butyrate in solution C with 0.61 g Na-stearate. Adjust the amount of distilled water in solution C to 20 ml. Dissolve stearate in water by heating in a water bath.

For DSM 16706: Replace butyrate with 1.50 g/l Na-propionate.

For DSM 26217: Omit butyrate and add to the complete medium 7.00 g/l sucrose from a sterile anoxic stock solution.

For DSM 102352: Replace butyrate with 2.00 g/l Na-benzoate.

Solution A

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml

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Selenite-tungstate solution	1.00	ml
Clarified rumen fluid	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
Na_2SO_4	2.80	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	890.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Na-butyrate	1.70	g
Distilled water	10.00	ml

Solution D (from medium 212)

Seven vitamins solution	1.00	ml

Solution E (from medium 212)

L-Cysteine HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution F (from medium 212)

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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213: SYNTHROPHOMONAS MEDIUM (SULFATE FREE)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

1. Add and dissolve ingredients of solution A, adjust pH to 7.2 and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Solution B is prepared under 80% N_2 and 20% CO_2 gas atmosphere and autoclaved. Solution C is prepared under 100% N_2 gas and sterilized by filtration. Solutions D, E and F are autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in in the sequence as indicated.

2. Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days . After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

For DSM 2909, DSM 2984: Replace butyric acid with 1.50 g/l acetoin.

For DSM 15682, DSM 16215: Replace butyric acid with 1.70 g/l crotonic acid.

For DSM 102353: Replace butyric acid with 2.00 g/l Na-benzoate.

Solution A

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Clarified rumen fluid	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	900.00	ml

Solution B

Na_2CO_3	1.50	g

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Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

Butyric acid	1.70	g
Distilled water	10.00	ml

Solution E

L-Cysteine HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution F

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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214. GYM+S MEDIUM

To medium 65 add 20 g starch.

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215. BHI MEDIUM

Brain Heart Infusion (Difco)	37.0	g
Distilled water	1000.0	ml

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216. BHI/1 MEDIUM

To medium 215 add 2.0 g yeast extract.

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217. BHI/2 MEDIUM

To medium 215 add 10.0 g casein hydrolysate, 5.0 g glucose and 5.0 g yeast extract.

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218. BHI/3 MEDIUM

To medium 215 add 10 g casein hydrolysate and 1 g starch.

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219. MYCOBACTERIUM MEDIUM

Yeast extract	2.0	g
Proteose peptone no. 3	2.0	g
Casein peptone, tryptic digested	2.0	g
Na_2HPO_4 x 12 H_2O	2.5	g
KH_2PO_4	1.0	g
Na-citrate	1.5	g
$MgSO_4$ x 7 H_2O	0.6	g
Glycerol	50.0	ml
Tween	0.5	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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220. CASO AGAR (Merck 105458)

Peptone from casein	15.0	g
Peptone from soymeal	5.0	g
NaCl	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.3. Medium is identical with Tryptone Soya Agar (Oxoid Cm131).

For strain DSM 21449 und DSM 21450

NaCl	10.0	g/L
$MnSO_4$	10.0	mg/L

For strain DSM 5746

Methanol	10.0	ml/L

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221. AZOSPIRILLUM MEDIUM

Yeast extract	0.05	g
K_2HPO_4	0.25	g
$FeSO_4$ x 7 H_2O	0.01	g
Na_2MoO_4 x 2 H_2O	1.00	mg
$MnSO_4$ x H_2O	2.00	mg
$MgSO_4$ x 7 H_2O	0.20	g
NaCl	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
$(NH_4)_2SO_4$	1.00	g
Biotin	0.10	mg
Distilled water	950.00	ml

Adjust pH of the medium to 7.1. Add 1.5% agar if needed. Autoclave for 15 min at 121°C. After sterilization add 25 ml each of filter-sterilized 20% glucose and 20% Na-malate.

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222. SP - MEDIUM

Raffinose	1.00	g
Sucrose	1.00	g
Galactose	1.00	g
Soluble starch	5.00	g
Casitone	2.50	g
$MgSO_4$ x 7 H_2O	0.50	g
K_2HPO_4	0.25	g
Agar (Difco)	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.4.

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225. SOUR DOUGH MEDIUM

Maltose	20.0	g
Yeast extract	3.0	g
Fresh yeast extract	15.0	ml
Tween 80	0.3	g
Casein peptone, tryptic digest	6.0	g
Distilled water up to	1000.0	ml

Adjust pH to 5.6 with 20% lactic acid or HCl. Fresh yeast extract is prepared by autoclaving a 20% suspension of commercial baker's yeast in distilled water for 30 minutes at 121°C, allowing the suspension to settle overnight at 2 to 8°C, decanting and further clarifying the supernatant by centrifugation. The extract prepared in this manner contains 1.5% solids and if not to be used within a few days, should be frozen or freeze-dried immediately.

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226. AUREOBACTERIUM TERREGENS MEDIUM

Casamino acids	2.0	g
Yeast extract	1.0	g
Glucose	1.0	g
(NH_4) citrate	1.0	g
K_2HPO_4	2.0	g
$MgSO_4$ x 7 H_2O	0.5	g
$FeCl_3$ x 6 H_2O	10.0	mg
Distilled water	1000.0	ml

Adjust pH to 7.0.

After sterilization, aseptically add 1 ml/l of a 10% solution of acetylacetone in ethanol.

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227. PEDIOCOCCUS HALOPHILUS MEDIUM

To medium 11 add 6.5% NaCl.

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231. PEDIOCOCCUS DAMNOSUS MEDIUM

Add 0.05% cysteine-hydrochloride to medium 11 and adjust to pH 5.2.
Medium should be filled up into crew capped tubes (Wheaton).

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232. MRS MEDIUM WITH CYSTEINE

Casein peptone, tryptic digest	10.00	g
Meat extract	10.00	g
Yeast extract	5.00	g
Glucose	20.00	g
Tween 80	1.00	g
K_2HPO_4	2.00	g
Na-acetate	5.00	g
$(NH_4)_2$ citrate	2.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$MnSO_4$ x H_2O	0.05	g
Distilled water	1000.00	ml

Adjust pH to 6.2 - 6.5.

Add 0.05% cysteine-hydrochloride.

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237. ENB MEDIUM

Nutrient Broth	8.0	g
Peptone	5.0	g
KH_2PO_4	1.5	g
K_2HPO_4	3.5	g
NaCl	5.0	g
Glucose	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 6.8 - 7.0. Sterilize glucose separately.

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238. STREPTOMYCIN NUTRIENT AGAR

To medium 1 add 40 mg/l streptomycin sulfate.

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239. DIETHYL PHOSPHONATE MEDIUM

KCl	0.20	g
$MgSO_4$ x 7 H_2O	0.20	g
NH_4Cl	0.20	g
Tris(hydroxymethyl)methylamine	6.00	g
p-Hydroxybenzoate, Na-salt	0.75	g
Agar	12.00	g
Distilled water	900.00	ml

Adjust pH to 7.4. After sterilization add 100 ml of a filter-sterilized solution of 1 mM diethyl phosphonate (Eastman Organic Chemical Div., Rochester, N.Y., USA) or of 20 mM phosphate buffer.

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240. CORYNEBACTERIUM MEDIUM WITH BLOOD

Sterilize medium 53 and cool to about 45°C. Aseptically add 5% defibrinated blood.

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242. BMM - MEDIUM

$(NH_4)_2SO_4$	3.0	g
KH_2PO_4	2.0	g
K_2HPO_4	7.0	g
NaCl	2.0	g
$MgSO_4$ x 7 H_2O	0.5	g
Yeast extract	0.1	g
Biotin	10.0	µg
Thiamine-HCl x 2 H_2O	100.0	µg
$MnSO_4$ x H_2O	6.2	mg
$FeSO_4$ x 7 H_2O	10.0	mg
Methanol	10.0	ml
Agar (if necessary)	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2 prior to autoclaving.

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243. SEAWATER YEAST PEPTONE AGAR

Peptone	5.0	g
Yeast extract	3.0	g
Agar	12.0	g
Distilled water	250.0	ml
Filtered, aged seawater	750.0	ml
(see below)		

Adjust pH to 7.8. Boil for 5 min, filter and readjust the pH to 7.3.

*Natural sea water is stored in the dark for at least three weeks to "age". If natural sea water is not available use artificial sea water.

Artificial sea water:

NaCl	28.13	g
KCl	0.77	g
$CaCl_2$ x 2 H_2O	1.60	g
$MgCl_2$ x 6 H_2O	4.80	g
$NaHCO_3$	0.11	g
$MgSO_4$ x 7 H_2O	3.50	g
Distilled water	1000.0	ml

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246. SEA WATER AGAR

Beef extract	10.0	g
Peptone	10.0	g
Agar	20.0	g
Tap water	250.0	ml
Sea water*	750.0	ml

Dissolve beef extract and peptone by heating in tap water, adjust pH to 7.8 and boil for 10 min. Readjust pH to 7.3. Add agar and autoclave at 121°C for 20 min. Directly after autoclaving, add warm (55°C) sterile sea water. Liquid medium without agar should be combined when cooled to room temperature.

*Natural sea water is stored in the dark for at least three weeks to "age". If natural sea water is not available use artificial sea water.

Artificial sea water:

NaCl	28.13	g
KCl	0.77	g
$CaCl_2 \times 2\ H_2O$	1.60	g
$MgCl_2 \times 6\ H_2O$	4.80	g
$NaHCO_3$	0.11	g
$MgSO_4 \times 7\ H_2O$	3.50	g
Distilled water	1000.0	ml

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248. OAT FLAKES AGAR WITH YEAST EXTRACT

Oat flakes	20.0	g
Trace element (see medium 252)	1.0	ml
Yeast extract	2.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

Boil oat flakes for 20 min, filter through cheese cloth and adjust to 1000 ml. Add trace elements and agar.

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249. FAECALICATENA OROTICA MEDIUM

Tryptone (BD BACTO)	5.00	g
Yeast extract	0.50	g
KH_2PO_4	1.36	g
K_2HPO_4	6.95	g
Na-orotate	2.50	g
Riboflavin	15.00	mg
Resazurin	1.00	mg
Na-thioglycolate	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients and adjust pH to 7.5. Sparge medium with 100% N_2 gas for 30 – 45 min to make it anoxic. Distribute medium under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave.

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250. PEPTONE MEAT EXTRACT GLYCEROL AGAR

Proteose peptone no. 3	5.0	g
Meat extract	3.0	g
Glycerol	20.0	ml
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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251. PEPTONE - MEAT EXTRACT - SOIL EXTRACT AGAR (PFE)

Proteose peptone no. 3	5.0	g
Meat extract	3.0	g
Glycerol	20.0	g
Soil extract (see medium 80)	150.0	ml
Agar	15.0	g
Distilled water	850.0	ml

Adjust pH to 7.0.

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252. STARCH - MINERAL SALT - AGAR (STMS)

Starch, soluble	10.0	g
$(NH_4)_2SO_4$	2.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
NaCl	1.0	g
$CaCO_3$	2.0	g
Trace element solution (see medium 84)	1.0	ml
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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253. MEDIUM FOR ECTOTHIORHODOSPIRA

KH_2PO_4	0.50	g
$CaCl_2$ x 2 H_2O	0.05	g
NH_4Cl	0.80	g
$MgCl_2$ x 6 H_2O	0.10	g
NaCl	180.00	g
Na_2SO_4	20.00	g
Na_2CO_3	6.00	g
Trace element solution (A)	1.00	ml
Yeast extract	0.50	g
Na-succinate	1.00	g
Distilled water	800.00	ml

Adjust pH to 8.5.
Bubble with nitrogen gas and fill in tubes with a rubber septum under a stream of nitrogen gas. Autoclave at 121°C for 15 min.
After sterilization add 1 ml/l filter-sterilized vitamin solution (B), 200 ml of filter-sterilized solution Bicarbonate solution (C) and 10 ml/l sterilised Na_2S x $9H_2O$ solution (D). pH 8.5

Trace element solution (A):

$FeCl_2$ x 4 H_2O	1.80	g
$CoCl_2$ x 6 H_2O	250.00	mg
$NiCl_2$ x 6 H_2O	10.00	mg
$CuCl_2$ x 2 H_2O	10.00	mg
$MnCl_2$ x 4 H_2O	70.00	mg
$ZnCl_2$	100.00	mg
H_3BO_3	500.00	mg
Na_2MoO_4 x 2 H_2O	30.00	mg
Na_2SeO_3 x 5 H2O	10.00	mg
Distilled water	1000.00	ml

For dissolving adjust pH to about 3 with 1 N HCl.

Vitamin solution (B):

Biotin	10.0	mg
Nicotinamide	35.0	mg
Thiamine-HCl x 2 H_2O	30.0	mg
p-Aminobenzoic acid	20.0	mg
Pyridoxal hydrochloride	10.0	mg
Ca-pantothenate	10.0	mg
Vitamin B_{12}	5.0	mg
Distilled water	100.0	ml

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Bicarbonate solution (C):

$NaHCO_3$	14.0	g
Distilled water	200.0	ml

Na_2S x $9H_2O$ solution (D)

Na_2S x $9H_2O$	10.0	g
Distilled water	100	ml

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254. ACETOBACTER PEROXYDANS MEDIUM

Malt extract	15.0	g
Yeast extract	5.0	g
Agar	15.0	g
Distilled water	940.0	ml

After sterilization add 60 ml ethanol (50% v/v), sterilized by filtration.

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255: CLOSTRIDIUM (GS) MEDIUM

KH_2PO_4	0.50	g
K_2HPO_4 x 3 H_2O	1.00	g
Urea	2.00	g
$MgCl_2$ x 6 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.05	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.25	ml
Morpholinopropane sulfonic acid	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	6.00	g
L-Cysteine HCl x H_2O	1.00	g
Cellobiose	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine and cellobiose and adjust pH to 7.2. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then add cysteine and readjust pH to 7.2, if necessary. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add cellobiose from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration.

For DSM 2360, DSM 103556, DSM 111537: Replace cellobiose with 5.00 g/l D-glucose added to the medium after autoclaving from an anoxic stock solution sterilized by filtration.

For DSM 29353: With 1% cellobiose; anaerobic

For DSM 101079: Add to the medium prior to autoclaving a stripe of lens cleaning tissue made of cellulose (e.g., from MACHEREY-NAGEL).

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256. THERMUS RUBER MEDIUM

Universal peptone (Merck)	5.0	g
Yeast extract	1.0	g
Starch, soluble	1.0	g
Agar	12.0	g
Distilled water	1000.0	ml

Adjust pH to 8.0.

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257: BDELLOVIBRIO (YP) MEDIUM

Yeast extract (BD Bacto)	3.00	g
Peptone (BD Bacto)	0.60	g
Tris-HCl buffer (0.05 M, pH 7.5)	1000.00	ml

1. Preparation of double layered agar plates:

Bottom layer: Dissolve 12.0 g Bacto agar in 900 ml distilled water and autoclave. After cooling to 60°C add 100 ml of sterile YP medium (autoclaved at 121°C for 15 min) and pour plates. Dry plates at room temperature for 2 - 3 days .

Top layer: Grow prey bacterium on agar slants using medium 54. Prepare 5 ml aliquots of 0.6% Bacto agar in distilled water and autoclave in test tubes. After cooling to 45°C in a water bath add 0.5 ml sterile YP medium (autoclaved at 121°C for 15 min) and 0.5 ml cell suspension of host organism, mix and pour top layer. For the top layer of one plate use one 24 h old agar slant culture and wash off grown cells in 0.5 ml sterile water.

2. Inoculate double layer plates the same day when prepared.

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258. NUTRIENT STARCH AGAR

Add 1% soluble starch to medium 1.

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259. YEAST EXTRACT MINERAL MEDIUM

Na_2HPO_4 x 12 H_2O	3.50	g
K_2HPO_4	1.00	g
$MgSO_4$ x 7 H_2O	0.03	g
NH_4Cl	0.50	g
Yeast extract	4.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.0 - 7.2.

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260. BACILLUS SCHLEGELII HETEROTROPHIC MEDIUM

Na_2HPO_4 x 2 H_2O	4.50	g
KH_2PO_4	1.50	g
NH_4Cl	1.00	g
$MnSO_4$ x H_2O	0.01	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.01	g
Ferric ammonium citrate	5.00	mg
Trace element solution SL-6 (see medium 27)	3.00	ml
Na-Pyruvate	1.50	g
Distilled water	1000.00	ml

Adjust pH to 7.1. Agar if necessary 15.0 g. Distribute in 30 - 50 ml amounts in Erlenmeyer flasks and sterilize for 15 minutes at 121°C. Incubate without agitation at 65°C.

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261. MEDIUM FOR CHEMOLITHOTROPHIC GROWTH OF BACILLUS SCHLEGELII

Use medium 260 without pyruvate. Incubate at 65°C without agitation under 5% (v/v) O_2, 10% CO_2, 45% H_2.

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262. J - AGAR

Tryptone	5.0	g
Yeast extract	15.0	g
Glucose	2.0	g
K_2HPO_4	3.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.3 - 7.5. Sterilize glucose separately and add after sterilization.

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263: TIBI MEDIUM

Sucrose	150.00	g
Fig (dried, quartered)	1.00	piece
Lemon (0.5 cm wedge-shaped segment)	1.00	piece
Tap water	1000.00	ml

Autoclave, cool to room temperature, and inoculate with about 50 ml Tibi grains. Set up the culture in a 1 l Erlenmeyer flask (cotton plugged).

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264. TOMATO JUICE MEDIUM

Casein peptone, tryptic digested	10.0	g
Yeast extract	10.0	g
Filtered tomato juice (pH 7.0)	200.0	ml
Tween 80	1.0	ml
Distilled water	800.0	ml

Adjust pH to 6.5.

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265. THERMOFILUM PENDENS MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
MgSO4 x 7 H2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
$MnCl_2$ x 4 H_2O	1.80	mg
$Na_2B_4O_7$ x 10 H_2O	4.50	mg
$ZnSO_4$ x 7 H_2O	0.22	mg
$CuCl_2$ x 2 H_2O	0.05	mg
Na_2MoO_4 x 2 H_2O	0.03	mg
$VOSO_4$ x 2 H_2O	0.03	mg
$CoSO_4$ x 7 H_2O	0.01	mg
Yeast extract	2.00	g
Sucrose	2.00	g
Sulfur, powdered	10.00	g
Polar lipid fraction prepared from *Thermoproteus tenax* (DSM 2078) or from any other archaebacterium, aqueous suspension	6 - 12.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Adjust final pH to 5.2.
Prepare the medium anaerobically under 100% nitrogen. The following constituents are prepared separately and added to the autoclaved mineral salt solution: Yeast extract (20 ml of 10% w/v solution)-boiled for few minutes, not autoclaved; sucrose (20 ml of 10% w/v solution)-filter-sterilized; sulfur (10 g)-sterilized by steaming for 3 h on each of three successive days; polar lipid fraction (6 - 12ml)-prepared as described by W. Zillig et al. (1983), Syst. Appl. Microbiol. 4: 79 - 87; Na_2S x 9 H_2O (10 ml of 3% w/v solution)-autoclaved under nitrogen atmosphere.

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266. THERMOCOCCUS CELER MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
$MnCl_2$ x 4 H_2O	1.80	mg
$Na_2B_4O_7$ x 10 H_2O	4.50	mg
$ZnSO_4$ x 7 H_2O	0.22	mg
$CuCl_2$ x 2 H_2O	0.05	mg
Na_2MoO_4 x 2 H_2O	0.03	mg
$VOSO_4$ x 2 H_2O	0.03	mg
$CoSO_4$ x 7 H_2O	0.01	mg
NaCl	40.00	g
Resazurin	1.00	mg
Yeast extract	2.00	g
Sulfur, powdered	5.00	g
Distilled water	1000.00	ml

Adjust final pH to 5.8.
Prepare the medium anaerobically under 100% nitrogen. The following constituents are prepared separately and added to the autoclaved mineral salt solution: Yeast extract (20 ml of 10% w/v solution)-boiled for a few minutes not autoclaved; sulfur (10 g)-sterilized by steaming for 3 h on each of three successive days; Na_2S x 9 H_2O (10 ml of 3% w/v solution)-autoclaved under nitrogen atmosphere.

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268: TEA FUNGUS MEDIUM

Tea (leaves, black)	5.00	g
Sucrose	50.00	g
Tap water	1000.00	ml

Add tea and sucrose to a 1 l flask, pour on freshly boiled tap water and allow to stand for 15 minutes. Filter and cool to room temperature. When starting with a small amount of tea fungus use a 50 ml beaker and 10 ml of the sweetened tea (freed of leaves) which has been acidified by addition of 0.05 ml of boiled vinegar (5%). Place a small disc of a cork stopper on the surface of the tea and place the inoculum onto it. Cover the beaker with aluminium foil and incubate. (Steam the cork disc for 15 min on two successive days).

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269: ACIDIPHILIUM MEDIUM

$(NH_4)_2SO_4$	2.00	g
KCl	0.10	g
K_2HPO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
Yeast extract	0.30	g
D-Glucose	1.00	g
Distilled water	1000.00	ml

Autoclave glucose and yeast extract separately as a 10% and 3%, resp., stock solution. Adjust pH of complete medium to 3.0 with 1 N H_2SO_4.

For DSM 11237, DSM 11244: Reduce amount of yeast extract to 0.1 g/l and adjust pH of medium to 3.5.

For DSM 11245: Replace yeast extract with 0.1 g/l Trypticase Soy Broth (BD BBL) added from a sterile stock solution and adjust pH of medium to 3.5.

For DSM 100189: Replace glucose with 1.8 g/l D-galactose and add 7.0 g/l $FeSO_4$ x 7 H_2O to the autoclaved medium from a 1 M stock solution (pH adjusted to 1.8 using 2 N H_2SO_4 and sterilized by filtration). Adjust pH of complete medium to 2.5.

For DSM 108313: Supplement medium with 1.0 g/l Trypticase Soy Broth (BD BBL) added from a sterile stock solution and adjust pH of medium to 4.0.

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271: ACIDITHIOBACILLUS (APH) MEDIUM

$(NH_4)_2SO_4$	2.00	g
K_2HPO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.10	g
$Ca(NO_3)_2$ x 4 H_2O	0.02	g
$FeSO_4$ x 7 H_2O	8.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients and adjust pH to 2.0 with 10 N H_2SO_4. Sterilize the medium by filtration. Alternatively, autoclave separately the basal medium (pH adjusted to 2.0) and the ferrous sulfate (8.00 g $FeSO_4$ x 7 H_2O in 50 ml 0.1 N H_2SO_4, pH not adjusted, in a sealed vessel under nitrogen gas atmosphere).

2. Note: Incubate statically without shaking.

For DSM 11478: Omit the ferrous sulfate from the medium and add 10.00 g/l elemental sulfur as substrate. For sterilization place the sulfur in screw-capped tubes, add 1-2 drops of distilled water and incubate on 3 successive days for 3 h at 90-100°C in a water bath. Before use, aseptically layer the sulfur onto the surface of autoclaved liquid basal medium. Adjust pH of complete medium to 2.0.

For DSM 24413: Omit the ferrous sulfate from the medium and add 10.00 g/l elemental sulfur as substrate. For sterilization place the sulfur in screw-capped tubes, add 1-2 drops of distilled water and incubate on 3 successive days for 3 h at 90-100°C in a water bath. Before use, aseptically layer the sulfur onto the surface of autoclaved liquid basal medium. Adjust pH of complete medium to 2.5.

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274: METHANOLACINIA MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	2.75	g
$MgSO_4$ x 7 H_2O	3.40	g
NH_4Cl	1.50	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	6.31	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
$NaHCO_3$	5.00	g
Na-acetate x 3 H_2O	4.15	g
Sodium resazurin (0.1% w/v)	0.50	ml
MOPS (3-(N-morpholino) propane-sulfonic acid)	20.93	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except MOPS, vitamins, cysteine and sulfide in a total volume of 890 ml. The MOPS buffer is dissolved separately in 90 ml of distilled water, the pH is adjusted to 7.0 with 2 M KOH, then add the buffer to the salt solution. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense medium in anoxic Hungate-type tubes under the same gas atmosphere to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 7.0, if necessary.

2. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g

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Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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275: TREPONEMA SUCCINIFACIENS MEDIUM

Final pH: 7.4
Final volume: 1000 ml

$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
KH_2PO_4	0.50	g
K_2HPO_4	0.50	g
NaCl	1.00	g
Yeast extract (Oxoid)	0.50	g
Peptone (BD Bacto)	0.50	g
$(NH_4)_2SO_4$	0.50	g
Clarified rumen fluid	300.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2CO_3	1.50	g
D-Glucose	10.00	g
Distilled water	700.00	ml

Dissolve ingredients except for cysteine, carbonate, and glucose, and sparge medium with 80% N_2 and 20% CO_2 gas atmosphere to make it anoxic. Add cysteine and adjust pH to 7.2 - 7.4 by adding and dissolving solid Na_2CO_3. Then distribute the medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose from a sterile anoxic stock solution prepared under a 100% N_2 gas atmosphere. The final pH of the medium should be at 7.4.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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276. HALOMONAS MEDIUM

NaCl	80.00	g
Casamino acids (with vitamins)	7.50	g
Proteose peptone No. 3	5.00	g
Yeast extract	1.00	g
Na_3-citrate	3.00	g
$MgSO_4$ x 7 H_2O	20.00	g
K_2HPO_4	0.50	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.05	g
Agar, if necessary	15.00	g
Distilled water	1000.00	ml

Before autoclaving adjust pH to 7.5 with NaOH

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277. CHERRY AGAR (CBS formula)

Add 1 l water to 200.0 g pulp of sour stone cherries.
Heat to boiling and simmer gently for 2 h. Strain through cloth and sterilize at 110°C for 30 minutes. Dissolve 20.0 g agar in 800 ml water and sterilize. Add 200 ml cherry extract, distribute in presterilized tubes and sterilize for 5 min at 120°C.
Final pH 3.8 - 4.6.

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281. HYPHOMONAS MEDIUM

Casitone (Difco)	2.0	g
Yeast extract (Difco)	1.0	g
$MgCl_2$ x 6 H_2O	2.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5 (use indicator paper).

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282: METHANOCALDOCOCCUS MEDIUM

K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.10	g
KCl	0.33	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	0.50	ml
NaCl	30.00	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.01	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, vitamins, cysteine and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium into anoxic tubes to 30% of their volume under 80% H_2 and 20% CO_2 gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of completed medium and adjust to 6.0, if necessary.

2. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.

For DSM 5666, DSM 111364: Adjust pH of complete medium to 6.5.

For DSM 16983, DSM 27223: Adjust pH of complete medium to 7.0 - 7.2.

For DSM 26965: Supplement medium with 3.00 g/l yeast extract and add 2.50 g/l maltose to the autoclaved medium from a sterile anoxic stock solution prepared under 100% N_2 gas. Adjust pH of complete medium to 6.5 and use without overpressure of 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g

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$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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283: PYRODICTIUM MEDIUM

Final pH: 5.5
Final volume: 1025 ml

NaCl	13.85	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
$(NH_4)_2SO_4$	10.00	mg
Citric acid (0.1% w/v)	5.00	ml
KI (0.01% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.75	g
KH_2PO_4	0.50	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sulfur (powdered)	30.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract (OXOID)	2.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Prepare the medium without sulfur, yeast extract and sodium sulfide, adjust pH to 5.0 - 5.5 with 10 N sulfuric acid, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% H_2 and CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. For sterilization of medium heat vessels for at least 1 hour to 90 - 100°C on each of 3 successive days. Do not autoclave! Complete the medium by adding yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 5.5, if necessary.

2. After inoculation pressurize the vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 2709: Reduce amount of yeast extract to 0.20 g/l.

For DSM 6158, DSM 112164: Reduce amount of yeast extract to 0.50 g/l and omit citric acid.

For DSM 113394: Adjust the amount of yeast extract to 0.5 g/l.

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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284: KOSMOTOGA MEDIUM

K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.10	g
KCl	0.33	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	0.50	ml
NaCl	30.00	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.01	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Yeast extract	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, yeast extract, vitamins, cysteine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), yeast extract, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use check pH of completed medium and adjust to 7.0 - 7.2, if necessary.

2. After inoculation add sterile 80% N_2 and 20% CO_2 gas mixture to 1 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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286: CLOSTRIDIUM AMINOBUTYRICUM MEDIUM

KH_2PO_4	1.30	g
K_2HPO_4	7.00	g
$MgCl_2$ x 6 H_2O	0.20	g
$FeCl_3$ x 6 H_2O	0.01	g
$CaCl_2$ x 2 H_2O	0.01	g
Na_2MoO_4 x 2 H_2O (0.1% w/v)	1.00	ml
Yeast extract	3.00	g
gamma-Aminobutyric acid	5.00	g
Agar	1.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfide), adjust pH to 7.2, bring medium to the boil, then cool to room temperature under 100% N_2 gas. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere.

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287: METHANOCULLEUS CHIKUGOENSIS MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$(NH_4)_2SO_4$	0.30	g
NaCl	0.61	g
$CaCl_2 \times H_2O$	0.14	g
$MgSO_4 \times 7\ H_2O$	0.13	g
Modified Wolin's mineral solution	10.00	ml
NH_4Cl	2.70	g
Na-acetate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl $\times H_2O$	0.30	g
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide) and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 6.8 and then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum bottles and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0. For incubation use sterile 80% H_2 and 20% CO_2 gas mixture at one atmospheres of pressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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288: METHANOCOCCUS MARIPALUDIS MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1011 ml

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$(NH_4)_2SO_4$	0.30	g
NaCl	35.00	g
$MgCl_2$ x 6 H_2O	4.00	g
$CaCl_2$ x H_2O	0.14	g
Modified Wolin's mineral solution	10.00	ml
NH_4Cl	2.70	g
Na-acetate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide) and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 6.8 and then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum bottles to 30% of their volume and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of final medium to 6.8 - 7.0. For incubation use sterile 80% H_2 and 20% CO_2 gas mixture at one atmospheres of pressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g

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Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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289: RUMINICLOSTRIDIUM PAPYROSOLVENS MEDIUM

Sea water (filtered)	200.00	ml
Mineral salt solution	150.00	ml
NH_4Cl	1.00	g
Yeast extract	0.60	g
K_2HPO_4	1.65	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Cellobiose	5.00	g
Cellulose, Lens tissue or MN 301 (optional)	5.00	g
Distilled water	650.00	ml

1. Dissolve ingredients (except cysteine and cellobiose) and sparge medium with 100% N_2 gas for 30 - 45 m in to make it anoxic. Add cysteine, adjust pH to 7.2 and dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cellobiose from an anoxic stock solution sterilized by filtration.

2. Note: Some strains can be adapted to cellulose as substrate using 5.0 g/l cellulose (Lens tissue, OXOID; or cellulose powder MN 301, MACHEREY-NAGEL). If necessary adjust pH of completed medium to 7.2.

Mineral salt solution

$(NH_4)_2SO_4$	6.00	g
NaCl	6.00	g
$MgSO_4$ x 7 H_2O	1.20	g
$CaCl_2$ x 2 H_2O	0.80	g
Distilled water	1000.00	ml

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291. PYROBACULUM NEUTROPHILUM MEDIUM

Prepare medium 88 without yeast extract, then add per liter:

Sulfur, powdered	8.0	g
Resazurin	0.4	mg

Adjust pH to 6.5 with NaOH, bring to boil for 5 min, then cool to room temperature under H_2 + CO_2 (80% + 20%) gas mixture. Add 0.85 g $NaHCO_3$ per liter and equilibrate the pH to c. 6.5 by further gassing.

Dispense in serum bottles (10 ml/30 ml bottle), seal and heat the bottles for one hour at 85°C on each of three successive days.

Before inoculation, add from anaerobic stock solutions 0.02% yeast extract (for mixotrophic growth only) and 0.01 ml Na-dithionite solution (250 mg Na-dithionite/10 ml dist. water, filter-sterilized). After inoculation, pressurize to 1 bar H_2 + CO_2 overpressure.

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292: ACIDAMINOBACTER MEDIUM

Glycine	1.50	g
Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.40	g
NaCl	1.20	g
$CaCl_2$ x 2 H_2O	0.15	g
KCl	0.40	g
Yeast extract	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide), adjust pH to 6.5, then sparge with 100% N_2 gas for 30 - 45 min to make it anoxic and dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials. After autoclaving complete the medium by adding vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.4 - 7.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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293: PROPIONIGENIUM MODESTUM MEDIUM (MARINE)

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.25	g
Disodium succinate	3.25	g
Na_2S x 9 H_2O	0.36	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, succinate and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Add succinate (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.2 - 7.5.

2. Note: Dispense medium into anoxic Hungate-type tubes or serum vials only to 30% of their volume to allow a large head space.

For DSM 2376: Supplement medium with 0.10% yeast extract (separately sterilized).

For DSM 2377, DSM 2378: Replace Na_2-succinate with 0.68 g/l of gallic acid.

For DSM 2380: Replace Na_2-succinate with 0.90 g/l of 2,3-butanediol.

For DSM 2382: Replace Na_2-succinate with 2.00 g/l of sodium L-tartrate.

For DSM 2394: Replace Na_2-succinate with 1.00 g/l of polyethylene glycol (molecular weight 106 - 20000).

For DSM 3247: Replace Na_2-succinate with 1.00 g/l acetoin.

For DSM 6831: Supplement medium with 1.00 ml/l of a Wolin's vitamin solution 10x (see medium 120) and replace Na_2-succinate with 1.00 g/l of quinic acid added from a neutralized anoxic stock solution.

For DSM 105538: Replace Na_2-succinate with 2.00 g/l of D-glucose and supplement medium with 1.00 g/l yeast extract and 1.00 ml/l of a Wolin's vitamin solution 10x (see medium 120) added from sterile anoxic stock solutions.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml

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293: PROPIONIGENIUM MODESTUM MEDIUM (MARINE)

$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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294: SYNTROPHUS HQGö1 MEDIUM

Final pH: 7.2
Final volume: 1003 ml

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Seven vitamins solution	1.00	ml
Gentisic acid (2,5-Dihydroxybenzoic acid), sodium salt	0.30	g
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, gentisic acid and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere in culture vessels and autoclave. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Add gentisic acid (2,5-dihydroxybenzoic acid) from a sterile and neutralized 200 mM stock solution prepared under 100% N_2 gas atmosphere. If necessary, adjust the final pH of the medium to 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum. Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days. After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg

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$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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295: OPITUTUS MEDIUM

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$NaNO_3$	0.80	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
D-Glucose	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, glucose, vitamins, and cysteine, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration), and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.

2. Note: For transfers use 5 - 10% (v/v) inoculum.

For DSM 11249: Replace glucose with 1.00 g/l cellobiose added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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298: PELOBACTER PROPIONICUS MEDIUM (FRESHWATER)

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
2,3-butanediol	0.90	g
Na_2S x 9 H_2O	0.36	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, 2,3-butanediol and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium in anoxic Hungate-type tubes or serum vials under the same gas atmosphere and autoclave. Add 2,3-butanediol and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.2 - 7.5.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning.

For DSM 2395: Replace 2,3-butanediol with 1.00 g/l of polyethylene glycol (molecular weight 106 - 20000).

For DSM 3246: Replace 2,3-butanediol with 1.00 g/l acetoin.

For DSM 6652: Replace 2,3-butanediol with 2.60 g/l Na-glutarate and add 20.00 ml/l rumen fluid, clarified (see medium 1310) to the autoclaved medium.

For DSM 6832: Supplement medium with 10.00 ml/l of a Wolin's vitamin solution (see medium 141) and replace 2,3-butanediol by 1.00 g/l of quinic acid added from a neutralized anoxic stock solution.

For DSM 8385: Supplement medium with 1.00 g/l yeast extract and replace 2,3-butanediol by 2.00 g/l D-galactose added from an anoxic stock solution sterilized by filtration.

For DSM 8909: Supplement medium with 1.00 ml/l of seven vitamins solution (see medium 503) and replace 2,3-butanediol by 1.40 g/l triethanolamine hydrochloride added from a 1 M stock solution prepared and autoclaved under 100% N_2 gas).

For DSM 11264: Supplement medium with 0.20 g/l yeast extract and 10.00 ml/l of a Wolin's vitamin solution (see medium 141). Replace 2,3-butanediol by 1.24 g/l ethylene glycol added from a separately sterilized stock solution.

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For DSM 17541: Supplement medium with 10.00 ml/l of a Wolin's vitamin solution (see medium 141) and 1.00 ml/l of selenite-tungstate solution (see medium 385).

For DSM 29698: Replace 2,3-butanediol with 2.20 g/l Na-gluconate.

For DSM 102354: Supplement medium with 1.00 ml/l of seven vitamins solution (see medium 503) and replace 2,3-butanediol by 0.86 g/l crotonic acid added after autoclaving from an anoxic sterile stock solution neutralized with NaOH.

For DSM 102355: Supplement medium with 1.00 ml/l of seven vitamins solution (see medium 503) and replace 2,3-butanediol by 1.25 g/l sodium pyruvate added after autoclaving from an anoxic stock solution sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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300. RUMINOCOCCUS PASTEURII MEDIUM

Use medium 298, but replace butanediol by 2.0 g/l of sodium L-tartrate. Add 0.4 mg/l biotin or 1.0 g/l of yeast extract.

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301. FALCIVIBRIO MEDIUM

Wilkins-Chalgren-Anaerobe-Broth (Oxoid) is supplemented with 0.3 g/l of L-cysteine and 1.0 mg/l of resazurin. Prepare the medium under 100% nitrogen gas. To the autoclaved medium add 50 ml/l of sterile serum (horse, bovine or lamb).

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302. NUTRIENT BROTH WITH 10% HORSE SERUM

To autoclaved medium 1 add 100 ml/l of sterile horse serum.

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303. SIMONSIELLA MEDIUM

Trypticase (BBL)	17.0	g
Phytone (BBL)	3.0	g
NaCl	5.0	g
K_2HPO_4	2.5	g
Yeast extract	4.0	g
Horse serum	100.0	ml
Agar, if necessary	15.0	g
Distilled water	1000.0	ml

Prepare the medium without horse serum. After autoclaving cool to 50°C, then add 100 ml/l of sterile horse serum. Transfer cultures when actively growing. Cultures die rapidly in stationary growth phase!

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305. BACILLUS THERMOGLUCOSIDASIUS MEDIUM

Soluble starch	10.0	g
Peptone	5.0	g
Meat extract	3.0	g
Yeast extract	3.0	g
KH_2PO_4	3.0	g
Agar	30.0	g
Distilled water	1000.0	ml

Adjust final pH to 7.0.

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306. NY-AGAR

To medium 1 add 0.5% yeast extract.

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308. VIBRIO MEDIUM

Tryptone	10.0	g
NaCl	10.0	g
$MgCl_2$ x 6 H_2O	4.0	g
KCl	1.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5.

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309. NEOMYCIN AGAR

Beef extract	1.5	g
Yeast extract	3.0	g
Peptone	6.0	g
Glucose	1.0	g
Casitone	4.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.
Aseptically add neomycin to a final concentration of 1.0 mg/ml of medium.

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310. V-8 JUICE AGAR

*V-8 Juice	200.0	ml
$CaCO_3$	3.0	g
Agar	15.0	g

Fill up with distilled water to 1000.0 ml. Adjust pH to 7.2.

* May be replaced by tomato mash if not available.

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311: SPOROMUSA MEDIUM (BETAINE)

NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
NaCl	2.25	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	2.00	g
Casitone	2.00	g
Betaine x H_2O	6.70	g
Sodium resazurin (0.1% w/v)	0.50	ml
K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphates, carbonate, vitamins, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add phosphates, vitamins (sterilized by filtration), cysteine and sulfide to the medium after autoclaving from sterile stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to pH 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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312. SCY - MEDIUM

Sucrose	1.00	g
Casitone	0.75	g
Yeast extract	0.25	g
Trypticase soy broth without dextrose (BBL)	0.17	g
Thiamine-HCl x 2 H_2O	0.40	mg
Vitamin B_{12}	0.01	mg
Distilled water	1000.00	ml

Adjust pH to 7.1.
Sterilize vitamins separately by filtration.

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314: ILYOBACTER POLYTROPUS MEDIUM

NaCl	21.00	g
$MgCl_2$ x 6 H_2O	3.10	g
KCl	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.27	g
KH_2PO_4	0.20	g
Na_2SO_4	2.84	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na-3-hydroxybutyrate	1.40	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Prior to use check pH of complete medium and adjust to 7.2 - 7.4, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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315. BACTEROIDES CELLULOSOLVENS MEDIUM

NH_4Cl	0.68	g
K_2HPO_4	0.30	g
KH_2PO_4	0.18	g
$(NH_4)_2SO_4$	0.15	g
$MgSO_4$ x 7 H_2O	0.12	g
$CaCl_2$ x 2 H_2O	0.06	g
$FeSO_4$ x 7 H_2O	0.02	g
Trace element solution (see below)	10.00	ml
Vitamin solution (see below)	10.00	ml
Cellobiose	5.00	g
or Cellulose (i.e. MN 300)	5.00	g
Resazurin	1.00	mg
$NaHCO_3$	2.00	g
Cysteine-HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Adjust pH to 7.0. Gas atmosphere: 80% N_2 + 20% CO_2.
Filter sterilize cellobiose separately.

Trace element solution:

Nitrilotriacetic acid	1.500	g
$MgSO_4$ x 7 H_2O	3.000	g
$MnSO_4$ x H_2O	0.500	g
NaCl	1.000	g
$FeSO_4$ x 7 H_2O	0.100	g
$CoSO_4$ x 7 H_2O	0.180	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnSO_4$ x 7 H_2O	0.180	g
$CuSO_4$ x 5 H_2O	0.010	g
$KAl(SO_4)_2$ x 12 H_2O	0.020	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.010	g
$NiCl_2$ x 6 H_2O	0.025	g
Na_2SeO_3 x 5 H_2O	0.300	mg
Distilled water	1000.000	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

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Vitamin solution:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine-HCl	10.000	mg
Thiamine-HCl x 2 H_2O	5.000	mg
Riboflavin	5.000	mg
Nicotinic acid	5.000	mg
D-Ca-pantothenate	5.000	mg
Vitamin B_{12}	0.100	mg
p-Aminobenzoic acid	5.000	mg
Lipoic acid	5.000	mg
Distilled water	1000.000	ml

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316: MOORELLA THERMOACETICA (TYE-CO) MEDIUM

NH_4Cl	1.00	g
KH_2PO_4	0.30	g
Na_2HPO_4 x 12 H_2O	2.80	g
$MgCl_2$ x 6 H_2O	0.20	g
Trypticase peptone (BD BBL)	10.00	g
Yeast extract (OXOID)	3.00	g
Modified Wolin's mineral solution	10.00	ml
Wolin's vitamin solution	5.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2S x 9 H_2O	0.60	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfide), adjust pH to 7.0, bring medium to the boil, then cool to room temperature under 100% carbon monoxide gas. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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317: METHANOSARCINA THERMOPHILA MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.40	g
$MgCl_2$ x 6 H_2O	0.20	g
Minerals solution	50.00	ml
Modified Wolin's mineral solution	10.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Methanol	5.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.25	g
Distilled water	950.00	ml

Dissolve ingredients except carbonate, methanol, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vilas and autoclave. Add methanol, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 6.8 - 7.0, if necessary.

Minerals solution (from medium 317)

KH_2PO_4	6.00	g
$(NH_4)_2SO_4$	6.00	g
NaCl	12.00	g
$MgSO_4$ x 7 H_2O	2.60	g
$CaCl_2$ x 2 H_2O	0.16	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g

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H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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318: METHANOSARCINA THERMOPHILA (BCYT) MEDIUM

KH_2PO_4	0.30	g
NaCl	0.60	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.08	g
Trace element solution	10.00	ml
NH_4Cl	1.00	g
Yeast extract (OXOID)	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$KHCO_3$	2.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, vitamins, methanol, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), methanol (50% v/v solution), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 6.8, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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319: SPOROHALOBACTER LORTETII MEDIUM

Casamino acids (BD Bacto)	2.00	g
Nutrient broth (BD Difco)	2.00	g
Yeast extract	2.00	g
L-Glutamic acid	4.00	g
NaCl	105.00	g
KCl	0.75	g
Modified Wolin's mineral solution	10.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
$MgCl_2$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	3.70	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, magnesium chloride, calcium chloride and vitamins), bring medium to the boil, then cool to room temperature under 100% N_2 gas atmosphere. Thereafter, add and dissolve cysteine, adjust pH to 6.5 and dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add magnesium chloride and calcium chloride from sterile anoxic stock solutions autoclaved under 100% N_2 gas and vitamins from a filter-sterilized stock solution prepared under N_2 gas.

2. Note: For agar medium add 20.00 g/l agar, 5.00 g/l $CaCO_3$, and 2.00 g/l soluble starch.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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320: CLOSTRIDIUM CELLULOVORANS MEDIUM

K_2HPO_4 x 3 H_2O	1.00	g
NH_4Cl	1.00	g
KCl	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.50	g
Clarified rumen fluid	20.00	ml
Sludge fluid, alternative (optional)	20.00	ml
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.15	g
Na_2CO_3	1.00	g
Cellobiose	5.00	g
Cellulose, MN 301 (optional)	10.00	g
Na_2S x 9 H_2O	0.15	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, carbonate, cellobiose and sulfide), bring medium to the boil, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture and add cysteine. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Sterilize cellobiose separately by filtration under 100% N_2 gas. Adjust pH of the complete medium to 7.0, if necessary.

2. Note: Some strains can be adapted to cellulose as substrate using 10.0 g/l cellulose powder MN 301 (MACHEREY-NAGEL).

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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321: METHANOCORPUSCULUM AGGREGANS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4 x 3 H_2O	0.40	g
$MgCl_2$ x 6 H_2O	0.40	g
Minerals solution	50.00	ml
Na-formate	5.00	g
Na-acetate	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sludge fluid	5.00	ml
Clarified rumen fluid, alternative (optional)	5.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
L-Cysteine HCl x H_2O	0.20	g
Na_2S x 9 H_2O	0.20	g
Distilled water	935.00	ml

1. Dissolve ingredients except carbonate, cysteine and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.

Minerals solution (from medium 317)

KH_2PO_4	6.00	g
$(NH_4)_2SO_4$	6.00	g
NaCl	12.00	g
$MgSO_4$ x 7 H_2O	2.60	g
$CaCl_2$ x 2 H_2O	0.16	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g

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$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

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322: METHANOSPHAERA MEDIUM

Clarified rumen fluid	100.00	ml
Trypticase peptone (BD BBL)	2.00	g
Yeast extract	2.00	g
Na-acetate	0.50	g
Na-formate	0.50	g
Modified Wolin's mineral solution	10.00	ml
Na_2SeO_4 (0.1% w/v)	1.90	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	0.70	ml
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	3.00	ml
K_2HPO_4	0.60	g
KH_2PO_4	2.80	g
$(NH_4)_2SO_4$	0.30	g
NH_4Cl	1.00	g
NaCl	0.60	g
$MgSO_4$ x 7 H_2O	0.15	g
$CaCl_2$ x 2 H_2O	0.08	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	2.00	ml
DL-Dithiothreitol	0.50	g
Distilled water	900.00	ml

1. Dissolve ingredients except bicarbonate, methanol, and dithiothreitol (DTT), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH of medium to 6.8 - 7.0, then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add methanol (50% v/v solution), vitamins, and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins and DTT should be sterilized by filtration. Prior to use check pH of complete medium and adjust to 6.7 - 6.9, if necessary.

2. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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323: TREPONEMA SACCHAROPHILUM MEDIUM

$CaCl_2$ x 2 H_2O	0.12	g
$MgSO_4$ x 7 H_2O	0.19	g
KH_2PO_4	0.45	g
K_2HPO_4	0.45	g
NaCl	0.90	g
$(NH_4)_2SO_4$	0.90	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
n-Butyric acid	0.40	ml
iso-Butyric acid	0.40	ml
DL-2-Methylbutyric acid	0.20	ml
n-Valeric acid	0.20	ml
iso-Valeric acid	0.20	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	1.00	g
Na_2CO_3	2.50	g
D-Glucose	2.00	g
Agar (BD Bacto), for solid medium	12.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, carbonate and glucose), adjust pH to 7.0 and sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add the cysteine and carbonate, then equilibrate the medium with the CO_2 gas to pH 7.0. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

2. For solid medium add 12.00 g/l agar (BD Bacto).

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325: SYNTROPHOSPORA MEDIUM

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	21.00	g
$MgCl_2$ x 6 H_2O	3.10	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-caproate	1.40	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, caproate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add caproate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.2 - 7.4, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

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325: SYNTROPHOSPORA MEDIUM

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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326: THERMOCLOSTRIDIUM (A-GÖ) MEDIUM

KH_2PO_4	3.30	g
$MgCl_2$ x 6 H_2O	0.16	g
$CoCl_2$ x 6 H_2O	12.00	mg
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Sucrose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, sucrose, vitamins, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add sucrose, cysteine and sulfide to the medium from sterile stock solutions autoclaved under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to pH 6.8 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg

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Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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328. PYG MEDIUM WITH VOLATILE FATTY ACIDS

Beef extract	5.00	g
Trypticase (BBL)	5.00	g
Yeast extract	10.00	g
Mineral solution (see below)	40.00	ml
$(NH_4)_2SO_4$	0.50	g
Fatty acid mixture (see below)	3.10	ml
Glucose	5.00	g
Resazurin	1.00	mg
Haemin (dissolved in 0.5 ml 1 N NaOH)	5.00	mg
Vitamin K_1 (0.5% sol. in ethanol)	0.20	ml
Cysteine-HCl x H_2O	0.50	g
Distilled water	960.00	ml

Adjust pH to 6.9.
Dissolve ingredients except cysteine-HCl in water, boil for 5 min, cool to room temperature while gassing with 100% CO_2, add cysteine-HCl and adjust pH to 6.9 with 8 N NaOH while gassing is continued. Switch to 100% N_2 gas after pH has reached the desired value, distribute in anaerobic tubes under nitrogen, and autoclave.

Mineral solution:

$CaCl_2$ x 2 H_2O	0.30	g
$MgSO_4$ x 7 H_2O	0.48	g
K_2HPO_4	1.00	g
KH_2PO_4	1.00	g
$NaHCO_3$	10.00	g
NaCl	2.00	g
Distilled water	1000.00	ml

Fatty acid mixture:

Acetic acid	17.0	ml
Propionic acid	6.0	ml
n-Butyric acid	4.0	ml
n-Valeric acid	1.0	ml
iso-Valeric acid	1.0	ml
iso-Butyric acid	1.0	ml
DL-2-Methylbutyric acid	1.0	ml

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329: METHANOHALOPHILUS HALOPHILUS MEDIUM

NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
K_2SO_4	0.01	g
NaCl	70.00	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	0.05	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	5.00	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Trimethylamine-HCl	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, magnesium chloride, calcium chloride, vitamins, trimethylamine, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate and equilibrate pH to 6.9 - 7.0, then distribute under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add magnesium chloride, calcium chloride, vitamins (sterilized by filtration), trimethylamine, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use adjust pH of complete medium to 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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330: RUMEN BACTERIA MEDIUM

Mineral solution	38.00	ml
K_2HPO_4	0.30	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	0.50	g
Volatile fatty acid mixture	3.10	ml
Haemin solution (0.05% w/v)	2.00	ml
Glycerol	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
D-Glucose	0.50	g
Maltose	0.50	g
Cellobiose	0.50	g
Starch, soluble	0.50	g
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	960.00	ml

Dissolve ingredients (except carbonate, glucose, maltose, cellobiose, soluble starch, cysteine and sulfide), then sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add the carbonate and equilibrate the medium with the CO_2 gas to pH 6.8. Distribute under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose, maltose, cellobiose, soluble starch, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

For DSM 17630: Plus rumen fluid (30ml/l)

Mineral solution (from medium 330)

KH_2PO_4	6.00	g
NaCl	12.00	g
$(NH_4)_2SO_4$	6.00	g
$CaCl_2$ x 2 H_2O	1.60	g
$MgSO_4$ x 7 H_2O	2.50	g
Distilled water	1000.00	ml

Volatile fatty acid mixture (from medium 330)

Acetic acid	548.50	ml
Propionic acid	193.50	ml
Butyric acid	129.00	ml
n-Valeric acid	32.25	ml
iso-Butyric acid	32.25	ml

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DL-2-Methylbutyric acid	32.25	ml
iso-Valeric acid	32.25	ml

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml
Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

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331. ACETOBACTEROIDES GLYCINOPHILUS MEDIUM

$CaCl_2$ x 2 H_2O	0.13	g
NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace elements (see medium 144)	10.00	ml
Vitamin solution (see medium 141)	5.00	ml
Resazurin	1.00	mg
Yeast extract	0.50	g
Glycine	1.50	g
KH_2PO_4	3.00	g
Na_2HPO_4	5.80	g
$NaHCO_3$	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Adjust pH to 7.2 - 7.4 with NaOH.
Prepare the medium anaerobically under 100% nitrogen. Prepare concentrated solutions each of yeast extract, glycine, phosphates, $NaHCO_3$ and Na_2S separately and sterilize under nitrogen by autoclaving.

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333: THERMITHIOBACILLUS MEDIUM

NH_4Cl	0.40	g
$MgSO_4$ x 7 H_2O	0.80	g
Trace element solution	10.00	ml
KH_2PO_4	4.00	g
K_2HPO_4	4.00	g
$K_2S_4O_6$	3.00	g
Bromocresol purple (saturated aq. solution)	2.00	ml
Agar (BD Bacto), for solid medium	10.00	g
Distilled water	1000.00	ml

Dissolve ingredients except phosphates and tetrathionate. The phosphates are sterilized separately in 1/10 of medium volume and mixed with the other salts when cool. Tetrathionate is sterilized by filtration and added to the autoclaved medium. Adjust pH of complete medium to 6.9. For agar medium autoclave the phosphates (in 100 ml), other salts (in 300 ml), and agar (in 600 ml) separately.

Trace element solution (from medium 333)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
NaOH	11.00	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds. Adjust pH of final solution to 6.0 with NaOH.

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334: METHANOTHRIX MEDIUM

Solution A	951.00	ml
Solution B	25.00	ml
Solution C	10.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, dispense under same gas atmosphere into anoxic serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Prior to inoculation complete the medium by adding appropriate amounts of sterile solutions C, D, E and F prepared under 100% N_2 gas and solution B prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins are sterilized by filtration. Adjust the pH of the complete medium to 7.2.

2. Note: Use 20% (v/v) inoculum.

For DSM 17206: Supplement medium with 0.50 g/l yeast extract (OXOID) and 0.50 g/l Trypticase peptone (BD BBL) added from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere.

Solution A

KH_2PO_4	0.22	g
Na_2HPO_4 x 2 H_2O	0.86	g
Na-acetate	6.80	g
Na_2-EDTA (0.1% w/v)	1.00	ml
Trace elements solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Solution B

Na_2CO_3	1.25	g
Distilled water	25.00	ml

Solution C

NaCl	0.30	g
$CaCl_2$ x 2 H_2O	0.11	g
$MgCl_2$ x 6 H_2O	0.10	g
Distilled water	10.00	ml

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Solution D

NH_4Cl	0.30	g
Distilled water	10.00	ml

Solution E

Wolin's vitamin solution (10x)	1.00	ml

Solution F

Na_2S x 9 H_2O	0.50	g
Distilled water	10.00	ml

Trace elements solution (from medium 334)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_3$ x 6 H_2O	1.35	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.03	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.12	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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335. EUBACTERIUM OXIDOREDUCENS MEDIUM

Solution A:

Mineral solution (see below)	50.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract	2.00	g
Crotonic acid	5.00	g
Vitamin solution (see medium 503)	1.00	ml
Resazurin	1.00	mg
Distilled water	840.00	ml

Adjust pH to 6.9 before autoclaving.

Solution B:

$NaHCO_3$	5.00	g
Distilled water	100.00	ml

Solution C:

L-Cysteine-HCl x H_2O	0.24	g
Distilled water	10.00	ml

Solution D:

Na_2S x 9 H_2O	78.00	mg
Distilled water	1.00	ml

Prepare solution A under 80% N_2 and 20% CO_2 gas mixture, distribute 8.9 ml per Hungate-type tube under same gas atmosphere and autoclave. Filter sterilize $NaHCO_3$ solution and gas for 20 min with 80% N_2 and 20% CO_2 gas mixture. Autoclave cysteine and sulfide under 100% N_2 gas. Complete medium by adding the solutions B, C, and D with a syringe to solution A. Adjust final pH of medium to 7.0 - 7.5.

Mineral solution:

KH_2PO_4	10.0	g
$MgCl_2$ x 6 H_2O	6.6	g
NaCl	8.0	g
NH_4Cl	8.0	g
$CaCl_2$ x 2 H_2O	1.0	g
Distilled water	1000.0	ml

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336: OXOBACTER PFENNIGII MEDIUM

Solution A	972.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	3.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere 8.9 ml medium per Hungate-type tube and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Autoclave solutions D and E under 100% N_2 gas. To complete the medium add appropriate amounts of the solutions B to E to the sterile solution A. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Solution A

Mineral solution	50.00	ml
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Na-vanillate	2.00	g
Clarified rumen fluid	300.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	620.00	ml

Adjust pH to 6.9 before autoclaving.

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

L-Cysteine HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution E

Na_2S x 9 H_2O	90.00	mg

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Distilled water	3.00	ml

Mineral solution (from medium 335)

KH_2PO_4	10.00	g
$MgCl_2$ x 6 H_2O	6.60	g
NaCl	8.00	g
NH_4Cl	8.00	g
$CaCl_2$ x 2 H_2O	1.00	g
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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337: SYNTROPHOCOCCUS SUCROMUTANS MEDIUM

Solution A	921.00	ml
Solution B	50.00	ml
Solution C	1.00	ml
Solution D	25.00	ml
Solution E	10.00	ml
Solution F	1.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.4 and dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions D, E and F are autoclaved separately under 100% N_2 gas. To complete the medium add appropriate amounts of solutions B - F to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 6.4 - 6.8, if necessary.

2. Note: Rumen fluid may be replaced by supplementing the medium with 200 µg/ml of crude egg yolk phosphatidylcholine (SIGMA, type IX-E).

Solution A

Mineral solution	50.00	ml
Trace element solution SL-10	1.00	ml
Clarified rumen fluid	300.00	ml
Trypticase peptone (BD Bacto/BD BBL)	5.00	g
Na-formate	0.60	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	570.00	ml

Solution B

$NaHCO_3$	2.50	g
Distilled water	50.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

Lactose	5.00	g
Distilled water	25.00	ml

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Solution E

L-Cysteine HCl x H_2O	0.24	g
Distilled water	10.00	ml

Solution F

Na_2S x 9 H_2O	78.00	mg
Distilled water	1.00	ml

Mineral solution (from medium 335)

KH_2PO_4	10.00	g
$MgCl_2$ x 6 H_2O	6.60	g
NaCl	8.00	g
NH_4Cl	8.00	g
$CaCl_2$ x 2 H_2O	1.00	g
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg

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337: SYNTROPHOCOCCUS SUCROMUTANS MEDIUM

D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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338. CAMPYLOBACTER RECTUS MEDIUM

Beef extract (Difco)	3.00	g
Trypticase (BBL)	9.00	g
Yeast extract	11.00	g
NaCl	2.00	g
Na_2HPO_4	0.40	g
Na_2CO_3	0.25	g
Haemin	5.00	mg
Na-formate	2.00	g
Na-fumarate	3.00	g
Resazurin	1.00	mg
Distilled water	1000.00	ml

Adjust pH to 7.5. Prepare the medium anaerobically under 100% N_2.
Autoclave solutions of haemin (dissolved in 0.1 ml 1N NaOH, then diluted with water to 10 ml and neutralized), formate, and fumarate separately under nitrogen.

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339. WILKINS-CHALGREN ANAEROBE BROTH

Dissolve 33.0 g/l dehydrated Wilkins-Chalgren medium (Oxoid CM0643) in distilled water and add 0.5 ml/l Na-resazurin solution (0.1% w/v), bring to the boil and cool to room temperature while sparging with 100% N_2 gas. Add L-cysteine-HCL x H_2O (0.3 g/l), dispense the medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving check pH and adjust to pH 6.8 – 7.0, if necessary.
Note: In case resazurin is not colourless addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) can be used to completely reduce the medium.

For DSM 5676 supplement medium after autoclaving with 10.0 g/l D-glucose added from a sterile stock solution.

For DSM 12679 and DSM 12858 use medium at half-strength (16.5 g/l).

For DSM 14204 supplement medium after autoclaving with 5.0 g/l D-glucose and 2.0 g/l $NaHCO_3$ added from sterile stock solutions prepared under 100% N_2 or 80% N_2 and 20% CO_2 gas atmosphere, respectively. Adjust pH to 7.0 - 7.2, if necessary.

For DSM 100423 adjust pH of complete medium to 7.4.

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340. CAPNOCYTOPHAGA MEDIUM

Trypticase (BBL)	17.0	g
Yeast extract	3.0	g
Glucose	3.0	g
NaCl	3.0	g
KNO_3	3.0	g
Haemin	3.0	mg
Distilled water	1000.0	ml

Adjust pH to 7.0.
Sterilize glucose separately. Incubate in oxygen-free, 5 - 10% CO_2 containing atmosphere.

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342: METHANOBACTERIUM ALCALIPHILUM MEDIUM

Solution A	792.00	ml
Solution B	210.00	ml
Solution C	10.00	ml

1. Dissolve ingredients of solution A (except cysteine), bring to the boil, then cool to room temperature under 100% H_2 gas. Add cysteine and adjust pH to 7.0, if necessary. Dispense medium under 100% H_2 gas atmosphere into anoxic Hungate-type tubes or serum vials vials to 30% of their volume and then autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is prepared under 100% N_2 gas atmosphere. Complete the medium by adding appropriate amounts of solutions B and C to the sterile solution A. Adjust pH of complete medium to 8.3 - 8.4, if necessary.

2. After inoculation add sterile H_2 gas to 1 - 2 bar overpressure.

Solution A

NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
K_2HPO_4	0.40	g
Na_2SeO_3 x 5 H_2O (0.01% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	780.00	ml

Solution B

$NaHCO_3$	10.00	g
Na_2CO_3	0.50	g
Distilled water	210.00	ml

Solution C

Tris-HCl buffer (pH 8.4, 2 M)	10.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g

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$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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343: THERMOTOGA NEAPOLITANA MEDIUM

Final pH: 6.5
Final volume: 1007 ml

Starch (soluble)	5.00	g
KH_2PO_4	0.50	g
Modified Wolin's mineral solution	15.00	ml
Artificial sea water	250.00	ml
Yeast extract (OXOID)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	740.00	ml

Dissolve ingredients (except vitamins, sulfide, and cysteine) and adjust pH to 6.5. Boil medium for 1 min, then cool to room temperature under 100% N_2 gas atmosphere. Dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of volume and autoclave. Add vitamins, sulfide, and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Sterilize vitamins by filtration. Adjust the pH of the complete medium to 6.5, if necessary.

Artificial sea water (from medium 343)

NaCl	27.70	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_2O	5.50	g
KCl	0.65	g
NaBr	0.10	g
H_3BO_3	30.00	mg
SrCl2 x 6 H_2O	15.00	mg
Citric acid	10.00	mg
KI	0.05	mg
$CaCl_2$ x 2 H_2O	2.25	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g

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$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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344. FLEXIBACTER MEDIUM

Na-glutamate	5.0	g
$MgSO_4$ x 7 H_2O	1.0	g
Yeast extract (Difco)	1.0	g
Glucose (sterilized separately)	2.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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346: DESULFALLAS MEDIUM

Solution A	966.00	ml
Solution B	20.00	ml
Solution C	11.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	1.00	ml
Solution G	1.00	ml
Solution H	1.70	ml

Sparge solution A with 100% N_2 gas for 30 - 45 min to make it anoxic. Thereafter, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Anoxic stock solutions C, D, E, F, and H are prepared under 100% N_2 gas atmosphere and solution G under 80% N_2 and 20% CO_2 gas atmosphere. Filter sterilize solutions D, F and G. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 7.2 - 7.5, if necessary.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
$MgCl_2$ x 6 H_2O	0.40	g
NaCl	1.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	965.00	ml

Solution B

$NaHCO_3$	1.00	g
Distilled water	20.00	ml

Solution C

Ethanol	1.00	ml
Distilled water	10.00	ml

Solution D

Na-benzoate	0.70	g

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Distilled water	10.00	ml

Solution E

Clarified rumen fluid	1.00	ml

Solution F

Wolin's vitamin solution (10x)	1.00	ml

Solution G

Na-dithionite solution (5% w/v)	1.00	ml

Solution H

Na_2S x 9 H_2O	50.00	mg
Distilled water	1.70	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg

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Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Na-dithionite solution (5% w/v) (from medium 829)

$NaHCO_3$	50.00	g
$Na_2S_2O_4$	50.00	g
Distilled water	1000.00	ml

Dissolve $NaHCO_3$ in water and make the solution anoxic by sparging with 80% N_2 and 20% CO_2 gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.

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347: SYNTROPHOMONAS SAPOVORANS MEDIUM

Solution A	921.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	25.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml

Dissolve ingredients of solution A, adjust pH to 7.0, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is filter-sterilized and then equilibrated with 80% N_2 and 20% CO_2 gas mixture for at least 15 min. Solution C is prepared under 100% N_2 gas and sterilized by filtration. Solutions D, E, F and G are autoclaved under 100% N_2 gas. To complete the medium, appropriate amounts of the solutions B to G are added to solution A in the sequence indicated. Adjust pH of complete medium to 7.2, if necessary.

Solution A

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Clarified rumen fluid	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
PIPES (SIGMA)	15.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	870.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

Na-laurate	2.78	g

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Distilled water	25.00	ml

Solution E

$CaCl_2$ x 2 H_2O	1.84	g
Distilled water	10.00	ml

Solution F

L-Cysteine HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution G

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg

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Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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348: METHANOHALOPHILUS PORTUCALENSIS MEDIUM

NaCl	120.00	g
$MgCl_2$ x 6 H_2O	7.00	g
$MgSO_4$ x 7 H_2O	6.00	g
$CaCl_2$ x 2 H_2O	0.50	g
K_2HPO_4 x 3 H_2O	0.40	g
NH_4Cl	1.00	g
KCl	3.80	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2CO_3	1.50	g
Trimethylamine-HCl	1.90	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, carbonate, trimethylamine and sulfide, bring medium to the boil, then cool to room temperature while sparging with 80% N_2 and 20% CO_2 gas mixture. Add cysteine and adjust pH to 6.5, then dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add trimethylamine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.3 - 7.4, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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350. CELLULOMONAS FERMENTANS MEDIUM

K_2HPO_4	2.21	g
KH_2PO_4	1.50	g
$(NH_4)_2SO_4$	1.30	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
$FeSO_4$ x 7 H_2O	1.25	mg
Yeast extract	5.00	g
Cellobiose	5.00	g
or		
Cellulose (MN 300)	5.00	g
$NaHCO_3$	0.80	g
Resazurin	1.00	mg
Cysteine-HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Adjust pH to 7.4 with 8 M NaOH.
Prepare the medium anaerobically under 100% nitrogen. Cellobiose, $NaHCO_3$, and cysteine are sterilized separately by autoclaving under N_2. For aerobic growth omit resazurin and cysteine.

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351. RHODOBACTER ADRIATICUS MEDIUM

Na acetate	0.82	g
Yeast extract	0.1	g
Na_2S x $9H_2O$	0.06	d
K_2HPO_4	1.0	g
$CaCl_2$ x 2 H_2O	0.1	g
$MgCl_2$ x 6 H_2O	0.5	g
NH_4Cl	1.0	g
$NaHCO_3$	3.0	g
NaCl	25.0	g
Na-ascorbate	0.5	g
Trace element solution (SLA)	1.0	ml
Distilled water	1000.0	ml

Adjust pH to 7.0.
The medium may be prepared without the addition of Na acetate, yeast extract and Na_2S x $9H_2O$ and may be added to the sterile medium from sterile stock solutions. Prepare the medium under an atmosphere of N_2. Dispense into screw capped or crimp top bottles fitted with a rubber septum. Sterilize at 121°C for 15 minutes in screw-caped bottles. After sterilization add 1.0 ml/l filter-sterilized vitamin solution (VA, see below). Incubate at 30°C at a light intensity of 1500 lux under anaerobic conditions.

Trace element solution (SLA):

$FeCl_2$ x 4 H_2O	1.8	g
$CoCl_2$ x 6 H_2O	250.0	mg
$NiCl_2$ x 6 H_2O	10.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
$MnCl_2$ x 4 H_2O	70.0	mg
$ZnCl_2$	100.0	mg
H_3BO_3	500.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
Na_2SeO_3 x 5 H_2O	10.0	mg
Distilled water	1000.0	ml

Adjust pH to 2.0 - 3.0.

Vitamin solution (VA):

Biotin	10.0	mg
Nicotinic acid amide	35.0	mg
Thiamine-HCl x 2 H_2O	30.0	mg
p-Aminobenzoic acid	20.0	mg
Pyridoxal hydrochloride	10.0	mg
Ca-pantothenate	10.0	mg
Vitamin B_{12}	5.0	mg
Distilled water	100.0	ml

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352. AZOSPIRILLUM AMAZONENSE MEDIUM

K_2HPO_4	0.200	g
KH_2PO_4	0.600	g
$CaCl_2$ x 2 H_2O	0.020	g
$MgSO_4$ x 7 H_2O	0.200	g
Na_2MoO_4 x 2 H_2O	0.002	g
$FeCl_3$	0.010	g
Bromothymol blue (0.5% in 0.2N KOH)	5.000	ml
Sucrose	5.000	g
Agar	1.750	g
Distilled water	1000.000	ml

Adjust pH to 6.0.

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354. LÖWENSTEIN-JENSEN MEDIUM

KH_2PO_4	2.50	g
$MgSO_4$	0.24	g
Mg-citrate	0.60	g
L-Asparagin	3.60	g
Potato flour	30.00	g
Malachite green	0.40	g
Distilled water	600.00	ml

Addition:

Glycerol	12.00	ml
*Fresh egg mixture (yolk and whites)	1000.00	ml

pH 7.0

Sterilize medium for 30 min at 121°C, cool to 30°C and add fresh egg mixture carefully to the medium.

* You need for 1 l of egg mixture 20 - 25 fresh eggs (not older then 1 week). Clean the eggs, wipe away with 70% alcohol, open the eggs and homogenize the egg mixture carefully.

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357. FLEXIBACTER CANADENSIS MEDIUM

$MgSO_4$ x 7 H_2O	0.1	g
KNO_3	0.1	g
$CaCl_2$ x 2 H_2O	0.1	g
Na-glycerophosphate	0.1	g
Trace elements SL-10 (see medium 320)	1.0	ml
Tris	1.0	g
Thiamine-HCl x 2 H_2O	1.0	mg
Casamino acids	1.0	g
Glucose (sterilized separately)	1.0	g
Vitamin B_{12}	1.0	µg
Agar	10.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5.

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358: ACIDIANUS MEDIUM (AEROBIC)

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Yeast extract (OXOID)	1.00	g
Sulfur (powder)	5.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients, except sulfur, bring medium to the boil, then cool to room temperature and adjust pH to 2.5 using 10 N H_2SO_4. Dispense medium under air atmosphere enriched with 1 - 10% CO_2 in culture vessels suitable for anaerobic cultivation (e.g., 20 ml medium in 100 ml serum bottles) containing already the appropriate amount of sulfur. For sterilization sealed bottles with medium are heated in a boiling water bath for 2 - 3 h on each of 3 successive days.

2. Note: Inoculate with 5% (w/v) culture. Incubate without shaking.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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359. GFY MEDIUM

Glucose	5.000	g
Fructose	5.000	g
Yeast extract	5.000	g
$CaCO_3$	3.000	g
Vitamin B_{12}	0.002	g
Agar	15.000	g
Distilled water	1000.000	ml

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360. YPM MEDIUM

Yeast extract	5.0	g
Peptone	3.0	g
Mannitol	25.0	g
Distilled water	1000.0	ml

pH not adjusted.

For solid media add agar at a concentration of 12.0 g/l

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362. FLAVOBACTERIUM TIRRENICUM MEDIUM

To medium 1 add 2 g/l of ethanolamine and 10 g/l NaCl and adjust pH to 7.5.

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363. PHENYLOBACTERIUM MEDIUM

Antipyrine	1.00	g
KH_2PO_4	0.30	g
Na_2HPO_4 x 12 H_2O	0.70	g
$(NH_4)_2HPO_4$	0.70	g
$NH_4H_2PO_4$	0.30	g
$(NH_4)_2SO_4$	0.10	g
$CaCl_2$ x 6 H_2O	0.05	g
$MgSO_4$ x 7 H_2O	0.25	g
H_3BO_3	0.50	mg
$CuSO_4$ x 5 H_2O	0.04	mg
KI	0.10	mg
$FeCl_3$ x 6 H_2O	0.20	mg
$MnSO_4$ x 4 H_2O	0.40	mg
$ZnSO_4$ x 7 H_2O	0.40	mg
$(NH_4)_2MoO_4$	0.20	mg
Biotin	0.10	mg
Vitamin B_{12}	0.03	mg
Distilled water	1000.00	ml

For DSM 2118, replace antipyrin by 1 g/l L-phenylalanine.

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364. PYEA MEDIUM

Peptone	10.0	g
Yeast extract	10.0	g
NaCl	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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365. PSEUDOMONAS INDIGOFERA MEDIUM

Yeast extract	10.0	g
Glucose	5.0	g
Na-acetate	0.5	g
Agar	12.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 - 7.2. Rehydrate and grow lyophilized cells in liquid broth and on agar.

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368. KUNKEE MEDIUM

Tryptone	20.0	g
Peptone	5.0	g
Yeast extract	5.0	g
Glucose	5.0	g
Tween 80	0.5	ml
Filtered tomato juice	250.0	ml
Distilled water	750.0	ml

Adjust pH to 5.5.
After sterilization aseptically add 10% (v/v) ethanol

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369. BACILLUS TUSCIAE MEDIUM

Adjust medium 261 to pH 4.0.
Incubate at 50°C without agitation under 5% O_2, 10% CO_2, 45% H_2 (v/v).

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370. HELIOBACTERIUM CHLORUM MEDIUM

K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
Yeast extract	10.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.
Boil the medium for few minutes under nitrogen gas. Add sodium ascorbate (0.5 g) and continue to bubble with nitrogen gas. Adjust the pH to 6.8. Fill immediately about 45 ml medium into 50 ml screw-capped bottles with rubber septum and screw tighten. Autoclave at 121°C for 15 min. Incubate in low light.

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371. NATRONOBACTERIA MEDIUM

KH_2PO_4	1.00	g
KCl	1.00	g
NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.24	g
$CaSO_4$ x 2 H_2O	0.17	g
Trace element solution SL-10		
(see medium 320)	1.00	ml
Agar, if necessary	20.00	g
NaCl	200.00	g
Na_2-glutamate	1.00	g
Yeast extract (Fisher BioReagents BP1422)	5.00	g
Casamino acids	5.00	g
Na_2CO_3	5.00	g

Add distilled water to give a final volume of 1000 ml.
Adjust pH to 6.5 before autoclaving!
Sterilize Na_2CO_3 separate from medium, add after cooling.
Check final pH to be 9.0 - 9.5.
If agar medium is prepared, heat and dissolve agar before adding sodium chloride.

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372. HALOBACTERIA MEDIUM

Yeast extract (Fisher BioReagents BP1422)	5.00	g
Casamino acids	5.00	g
Na-glutamate	1.00	g
KCl	2.00	g
Na_3-citrate	3.00	g
$MgSO_4$ x 7 H_2O	20.00	g
NaCl	200.00	g
$FeCl_2$ x 4 H_2O	36.00	mg
$MnCl_2$ x 4 H_2O	0.36	mg
Agar	20.00	g

Add distilled water to give a final volume of 1000 ml.
Adjust pH to 7.0 - 7.2

For **DSM 102807** **DSM 102808** **DSM 102809** add 20g/l soluble starch.

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373: METHANOGENIUM CV MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Na-acetate	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	5.00	g
Isopropyl alcohol	7.50	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	980.00	ml

Dissolve ingredients (except bicarbonate, isopropyl alcohol, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add isopropyl alcohol and cysteine from sterile anoxic stock solutions autoclaved under 100% N_2 gas atmosphere. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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374: METHANOSPIRILLUM SK MEDIUM

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Isopropyl alcohol (25% v/v)	30.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	970.00	ml

Dissolve ingredients except carbonate, isopropyl alcohol and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add isopropyl alcohol and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 7.0 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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375: METHANOHALOBIUM MEDIUM

NaCl	250.00	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$MgSO_4$ x 7 H_2O	4.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	0.05	g
Trimethylamine-HCl	5.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, trace elements, yeast extract, trimethylamine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trace elements, yeast extract, trimethylamine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.4 with an anoxic sterile solution of 5% w/v Na_2CO_3, if necessary.

2. Note: A white precipitate that forms after autoclaving of the mineral base will dissolve again after storage at room temperature for 2 - 3 days.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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376. AN1 MEDIUM

Trypticase (BBL)	10.0	g
K_2HPO_4	1.5	g
NaCl	2.5	g
Na-thioglycolate	1.0	g
Sulfur, powdered	8.0	g
Resazurin	1.0	mg
Distilled water	1000.0	ml

Adjust pH to 7.3.
Prepare the medium anaerobically under 100% nitrogen. Sterilize thioglycolate and sulfur separately. Sterilize the sulfur by steaming for 3 h on each of three successive days.

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377: PYROCOCCUS MEDIUM

NaCl	13.85	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
$(NH_4)_2SO_4$	10.00	mg
Citric acid (0.1% w/v)	5.00	ml
KI (0.01% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.75	g
KH_2PO_4	0.50	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	30.00	g
Peptone (BD Bacto)	5.00	g
Yeast extract (OXOID)	1.00	g
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	960.00	ml

Dissolve ingredients except for sulfur, peptone, yeast extract, and sulfide, adjust the pH to 6.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that already contain the appropriate amount of sulfur. Sterilize the medium by autoclaving at **110°C** for 20 min. After sterilization, add peptone and yeast extract from sterile stock solutions prepared under 100% N_2 gas atmosphere and sulfide from a neutralized stock solution.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g

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H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.

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378. ARTHROBACTER MEDIUM

K_2HPO_4	1.770	g
KH_2PO_4	0.680	g
NaCl	0.140	g
$CaCl_2$	0.132	g
$MgSO_4$ x 7 H_2O	0.200	g
Mannitol	10.000	g
Yeast extract	0.080	g
$FeSO_4$ x 7 H_2O	2.500	mg
H_3BO_3	2.900	mg
$CoSO_4$ x 7 H_2O	1.200	mg
$CuSO_4$ x 5 H_2O	0.100	mg
$MnCl_2$ x 4 H_2O	0.090	mg
Na_2MoO_4 x 2 H_2O	2.500	mg
$ZnSO_4$ x 7 H_2O	1.200	mg
Distilled water	1000.000	ml

Adjust pH to 7.0. Incubate under 5% O_2 + 95% N_2.

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380: MAGNETOSPIRILLUM MEDIUM

Final pH: 6.7
Final volume: 1008 ml

KH_2PO_4	0.68	g
$NaNO_3$	0.12	g
L(+)-Tartaric acid	0.37	g
Succinic acid	0.37	g
Na-acetate	0.05	g
Yeast extract	0.10	g
Modified Wolin's mineral solution	5.00	ml
Fe(III) quinate solution 0.01 M	2.00	ml
Agar (BD Bacto), for semi-solid medium (optional)	1.30	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-thioglycolate	0.05	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except thioglycolate and vitamins) in the order given and adjust pH to 6.75 with NaOH.

2. Preparation of liquid medium: Sparge medium with 100% N_2 gas for 30 -45 min and dispense under the same gas atmosphere into anoxic Hungate-type tubes to 50% of their volume. Seal vials with screw caps and gas tight butyl rubber closures. Autoclave at 121°C for 15 min. Before inoculation add thioglycolate and vitamins from stock solutions prepared under 100% N_2 gas and filter-sterilized. Then add sterile air (with hypodermic syringe through the rubber closure) to a concentration of ca. 1% (v/v) O_2 in the vial (e.g., add 1 ml air to a Hungate-type tube of 16 ml total volume).

3. Preparation of semi solid medium: Supplement medium with agar, bring medium to the boil and cool under 100% N_2 gas atmosphere. Dispense under same gas atmosphere aliquots of 10 ml semi-solid medium into Hungate-type tubes. Prior to inoculation add thioglycolate from a 0.5% (w/v) stock solution, freshly prepared under 100% N_2 gas and filter-sterilized. Then add sterile air (with hypodermic syringe through the rubber closure) to a concentration of ca. 1% (v/v) in the vial.

4. Note: Prior to inoculation media should be slightly pink in color. Strongly reduced conditions will not support growth of microaerophilic Magnetospirillum species. Use as inoculum 10% (v/v). Incubate tubes with medium without agitation in an inclined position. During growth O_2 will be consumed and the pH will increase. For cultivation of magnetic cells we recommend preparation of liquid medium, while semi-solid medium is more suitable for demonstration of microaerophilic band formation and storage.

For DSM 6361: Increase the amount of added O_2 to a concentration of 5% (v/v) in the vial (e.g., add 4 ml sterile air to a Hungate-type tube of 16 ml total volume).

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For DSM 29233: Supplement medium with 0.25 g/l sodium thiosulfate.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Fe(III) quinate solution 0.01 M (from medium 380)

$FeCl_3$ x 6 H_2O	4.50	g
(-)-Quinic acid	1.90	g
Distilled water	1000.00	ml

Sterilize by filtration under 100% N_2 gas atmosphere.

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381. LB (Luria-Bertani) MEDIUM

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	10.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

For **DSM 26804** add 100g NaCl (10%) for 1000 ml medium.

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382: MINERAL MEDIUM M9 for E. coli JM strains

10 x M9 salts (per l)	100.00	ml
$MgSO_4$ (1 M)	1.00	ml
$CaCl_2$ (0.1 M)	1.00	ml
Thiamine-HCl x 2 H_2O (filter-sterilized, 1 M)	1.00	ml
Glucose (20%)	10.00	ml
Distilled water	900.00	ml

1. The above solutions should be sterilized separately by filtration (thiamine, glucose) or autoclaving.

2. Adjust pH to 7.4.

10 x M9 salts (per l)

Na_2HPO_4	60.00	g
KH_2PO_4	30.00	g
NH_4Cl	10.00	g
NaCl	5.00	g

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383: DESULFOBACTERIUM MEDIUM

Solution A	952.00	ml
Solution B	20.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed in anoxic cultivation vials and autoclaved under the same gas atmosphere. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas and filter-sterilized. Solution E is autoclaved under 100% N_2 gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0 – 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% inoculum. Incubate all strains in the dark.

For DSM 2056: Replace pyruvate with 0.70 g/l Na-butyrate, 0.30 g/l Na-caproate, and 0.15 g/l sodium octanoate.

For DSM 3383: Replace pyruvate with indole as substrate. Dissolve 0.30 g of indole in 90 ml water by heating and shaking in a closed bottle under 100% N_2 gas atmosphere, autoclave, then add 7.00 ml of a sterile anoxic stock solution of NaCl (30% w/v) and 0.70 ml of a sterile anoxic stock solution of $MgCl_2$ x 6 H_2O (40% w/v). Store the indole-salt solution in the dark. Reheat and shake before use. Add to sterile medium 30.00 ml/l of the indole-salt solution in the beginning, and 2 x 30.00 ml/l during growth.

For DSM 3384: The vitamin solution of medium 120 is replaced with 1.00 ml/l of seven vitamins solution of medium 503. Replace pyruvate with 0.40 g/l Na-benzoate and add 10.00 ml/l clarified rumen fluid (see medium 1310) as growth supplement. Initiation of growth requires addition of freshly prepared sodium dithionite.

For DSM 4661: Replace pyurvate with resorcinol. During growth the culture is fed once with the same amount of resorcinol.

For DSM 5091, DSM 9788: Replace pyruvate with 2.00 g/l malonic acid.

For DSM 7044, DSM 7120, DSM 7467, DSM 12567, DSM 13228, DSM 23484: Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503). Replace pyruvate with 0.40 g/l benzoate and 0.10 g/l yeast extract sterilized separately by filtration and add to the autoclaved medium from anoxic stock solutions.

For DSM 8540: Replace pyruvate with 0.30 g/l p-hydroxybenzoate.

For DSM 9705: Replace pyruvate with 1.00 g/l Na-glycolate sterilized by filtration.

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For DSM 12861, DSM 12883: Replace pyruvate with 1.00 g/l valeric acid.

For DSM 12888: Replace pyruvate with 1.10 g/l Na-butyrate and 1.10 g/l caproic acid.

For DSM 13036: Replace pyruvate with 1.00 g/l betaine.

For DSM 14454: Naphthalene dissolved in heptamethylnonane can be used as substrate instead of pyruvate.

For DSM 15576, DSM 16219: Replace pyruvate with 1.00 g/l Na-caprylate and supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

For DSM 17291: Replace pyruvate with 3.00 g/l Casamino acids.

For DSM 17477: Replace pyruvate with 1.90 g/l sodium glutamate monohydrate and 1.00 g/l yeast extract.

For DSM 21856: Replace pyruvate with 0.90 ml/l 1-butanol.

For DSM 28890: Replace pyruvate with 0.50 g/l yeast extract and 0.68 g/l sodium formate.

For DSM 100305, DSM 105015: Change amount of pyruvate to 2.20 g/l and add 0.10 g/l yeast extract.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	21.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution C

Na-pyruvate	2.50	g
Distilled water	20.00	ml

Solution D

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Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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385: DESULFOCASTAENA MEDIUM

Solution A	952.00	ml
Solution B	20.00	ml
Solution C	10.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and F are autoclaved under 100% N_2 gas. Solutions D and E are prepared under 100% N_2 gas and sterilized by filtration. The pyrocatechol stock solution has to be prepared always freshly prior to use. To complete the medium appropriate amounts of the solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 6.9 - 7.1.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 10 - 20% inoculum.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	7.00	g
$MgCl_2$ x 6 H_2O	1.30	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution C

Na-benzoate	0.40	g

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Distilled water	10.00	ml

Solution D

Pyrocatechol	0.06	g
HCl (1 N)	0.06	ml
Distilled water	10.00	ml

Solution E

Wolin's vitamin solution (10x)	1.00	ml

Solution F

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg

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Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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386: DESULFOLUTIVIBRIO MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	13.00	ml

1. Solution A is sparged 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic, then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas mixture. Solutions C and F are autoclaved under 100% N_2 gas. Solutions D and E are prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of the solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.1 - 7.4.

2. Note: When growth has started feed culture again with same amount of pyrosulfite. After a further two days repeat feeding once more.

For DSM 7269: Replace pyrosulfite with 2.50 g/l $Na_2S_2O_3$ x 5 H_2O added to the medium after autoclaving from a sterile anoxic stock solution.

Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Na-acetate x 3 H_2O	0.30	g
Distilled water	10.00	ml

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Solution D

Seven vitamins solution	1.00	ml

Solution E

$Na_2S_2O_5$ (sodium pyrosulfite)	0.95	g
Distilled water	10.00	ml

Adjust pH to 7.5-8.0 with NaOH.

Solution F

Na_2S x 9 H_2O	0.40	g
Distilled water	13.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg

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Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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388: DICTYOGLOMUS MEDIUM

KH_2PO_4	1.50	g
Na_2HPO_4 x 12 H_2O	4.20	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	0.38	g
$CaCl_2$ x 2 H_2O	0.06	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.04	g
$CoCl_2$ x 6 H_2O (0.1% w/v)	2.90	ml
Na_2MoO_4 x 2 H_2O (0.1% w/v)	2.40	ml
Na_2SeO_3 x 5 H_2O (0.01% w/v)	1.70	ml
$MnCl_2$ x 4 H_2O (0.1% w/v)	2.00	ml
$ZnSO_4$ (0.1% w/v)	2.80	ml
Yeast extract	2.00	g
Polypeptone	2.00	g
Starch (soluble)	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
L-Cysteine HCl x H_2O	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, cysteine and vitamins), bring medium to the boil, then cool to room temperature under 100% N_2 gas atmosphere. Add and dissolve carbonate and cysteine, adjust pH to 7.2, then dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.2, if necessary.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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389: ANNWOODIA MEDIUM

Solution A	100.00	ml
Solution B	870.00	ml
Solution C	10.00	ml
Solution D	20.00	ml

Autoclave solutions A, B and C and filter sterilize solution D. To complete the medium combine sterile solutions when cool.

Solution A

Na_2HPO_4 x 2 H_2O	7.90	g
KH_2PO_4	1.50	g
Distilled water	100.00	ml

Adjust to pH 7.6.

Solution B

NH_4Cl	0.40	g
$MgSO_4$ x 7 H_2O	0.10	g
Agar, for solid medium	15.00	g
Distilled water	870.00	ml

Solution C

Trace element solution	10.00	ml

Solution D

$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	20.00	ml

Trace element solution (from medium 333)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g

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NaOH	11.00	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds. Adjust pH of final solution to 6.0 with NaOH.

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390: PYROBACULUM MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Trypticase peptone	0.50	g
Yeast extract	0.20	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except Trypticase peptone, yeast extract, sodium thiosulfate and sulfide), adjust pH to 6.0 and sparge medium with 100% N_2 gas for at least 30 min to remove dissolved oxygen. After autoclaving add peptone, yeast extract, thiosulfate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of substrates can be autoclaved with the exception of sodium thiosulfate which should be sterilized by filtration. Prior to inoculation adjust pH of final medium to 6.0.

For DSM 4185: Replace thiosulfate with sulfur. Sulfur is sterilized by steaming for 3 hours on each of 3 succesive days and added aseptically to the sterile medium while retaining anoxic conditions.

For DSM 13380: Omit Trypicase peptone. Increase amount of yeast extract to 1 g/l and lower amount of thiosulfate to 1 g/l. Adjust pH of final medium to 7.0.

For DSM 13514, DSM 103086: Adjust pH of final medium to 6.8.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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391: METHANOCOCCUS VOLTAE MEDIUM

Mineral solution 2xW	500.00	ml
Modified Wolin's mineral solution	10.00	ml
LIP-solution	50.00	ml
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
TYC-solution	50.00	ml
Na_2CO_3	1.50	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	400.00	ml

Dissolve ingredients except TYC solution, carbonate, cystein and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add TYC solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.2, if necessary.

For DSM 4254: Supplement medium with 80.00 mg/l L-histidine added from a sterile anoxic stock solution sterilized by filtration.

For DSM 4310: Supplement medium with 32.00 mg/l of hypoxanthine and 1.00 ml/l of seven vitamins solution (see medium 503) added from anoxic stock solutions sterilized by filtration.

Mineral solution 2xW

NaCl	40.00	g
$MgCl_2$ x 6 H_2O	5.60	g
$MgSO_4$ x 7 H_2O	0.70	g
KCl	0.68	g
NH_4Cl	0.50	g
K_2HPO_4	0.28	g
$CaCl_2$ x 2 H_2O	0.28	g
Distilled water	1000.00	ml

LIP-solution

L-Leucine	5.00	g
L-Isoleucine	10.00	g
Pantothenate	0.10	g
Distilled water	1000.00	ml

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May be stored frozen without sterilization.

TYC-solution

Casamino acids (BD Bacto)	100.00	g
Yeast extract (OXOID)	50.00	g
L-Tryptophan	1.00	g
Distilled water	1000.00	ml

Prepare and autoclave under 100% N_2 gas.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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392. BAF Medium

Glucose	30.0	g
Peptone	2.0	g
Yeast extract	0.2	g
KH_2PO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.5	g
$FeCl_3$ x 6 H_2O	10.0	mg
$ZnSO_4$ x 7 H_2O	1.0	mg
$MnSO_4$	5.0	mg
$CaCl_2$ x 2 H_2O	100.0	mg
Thiamin HCl	50.0	µg
Biotin	1.0	µg
Folic acid	100.0	µg
Inositol	50.0	µg
Agar	15.0	g
Distilled water	1000.0	ml

pH 5.8 - 6.3.

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393. YPD MEDIUM

Yeast extract	10.0	g
Peptone	20.0	g
Glucose	20.0	g
Distilled water	1000.0	ml

Adjust pH to 6.5.

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394. CHI 1776 MEDIUM

Tryptone	25.0	g
Yeast extract	7.5	g
1M Tris-HCl (pH 7.5)	20.0	ml
Distilled water	1000.0	ml

Autoclave, cool, and then add:

1M $MgCl_2$	5.0	ml
1% Diaminopimelic acid	10.0	ml
0.4% Thymidine	10.0	ml
20% Glucose	25.0	ml

Magnesium chloride should be sterilized separately by autoclaving, and the other ingredients should be sterilized separately by filtration.

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395: DESULFUROCOCCUS MEDIUM

NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$CaCl_2$ x 2 H_2O	0.44	g
$MgCl_2$ x 6 H_2O	0.70	g
NaCl	0.50	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
D-Glucose	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, glucose, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4 and dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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396: METHANOSALSUM ZHILINAE MEDIUM

Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
NaCl	40.00	g
NH_4Cl	1.00	g
$MgCl_2 \times 6\ H_2O$	3.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
KCl	1.00	g
K_2HPO_4	0.40	g
Modified Wolin's mineral solution	5.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
$NaHCO_3$	3.00	g
Na_2CO_3	2.00	g
Trimethylamine-HCl	2.00	g
$Na_2S \times 9\ H_2O$	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, bicarbonate, carbonate, trimethylamine and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add cysteine and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trimethylamine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas, whereas bicarbonate and carbonate are added from sterile anoxic stock solutions prepared under 80% N_2 and 20% CO_2 gas atmosphere. Adjust pH of complete medium to 9.2 - 9.4.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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397. FERULATE MEDIUM

Use medium 212 without Na_2SO_4 and butyrate. Add sodium ferulate to 10 mM final concentration from filter-sterilized anaerobic stock solution.

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398. THERMOPLASMA VOLCANIUM MEDIUM

KH_2PO_4	3.00	g
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.25	g
$(NH_4)_2$ SO_4	0.20	g
Yeast extract	1.00	g
Glucose	5.00	g
Sulfur (for anaerobic media only)	4.00	g
Distilled water	1000.00	ml

Adjust pH of the mineral base to about 2 with sulfuric acid and autoclave. Add glucose and yeast extract from filter-sterilized stock solutions. For DSM 4301 add 0.5 g/l meat extract (Merck); aerobic growth of that strain is sometimes difficult to achieve.
Check pH to be 2 to 3.

For anaerobic growth, the acidified mineral base containing sulfur is filled into serum bottles (20 ml per 100 ml bottle) under N_2 + CO_2 (80 + 20) gas mixture. Then, the bottles are sterilized by tyndallisation. Yeast extract, glucose, and meat extract (for DSM 4301 only) are added from separately sterilized stock solutions.

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399: ARCHAEOGLOBUS MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
Yeast extract (OXOID)	0.50	g
Na-L-lactate	1.50	g
$Fe(NH_4)_2(SO_4)_2$ x 7 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	3.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate and adjust pH to 6.9, then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into serum vials (e.g., 20 ml per 100 ml bottle), seal and autoclave. Before use reduce the medium with sodium sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas.

2. After inoculation, add 2 bar overpressure of sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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399: ARCHAEOGLOBUS MEDIUM

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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402. ALICYCLOBACILLUS MEDIUM

Solution A:

$CaCl_2$ x 2 H_2O	0.25	g
$MgSO_4$ x 7 H_2O	0.50	g
$(NH_4)_2$ SO_4	0.20	g
Yeast extract	2.00	g
Glucose	5.00	g
KH_2PO_4	3.00	g
Distilled water (for liquid medium)	1000.00	ml
Distilled water (for solid medium)	500.00	ml

Adjust pH to 4.0

Solution B:

Trace element sol. SL-6 (see medium 27)	1.00	ml

Solution C:

Agar	15.00	g
Distilled water	500.00	ml

Sterilize separately. For liquid medium combine solution A (with 1000.0 ml distilled water) and solution B. For solid medium combine solution A (with 500 ml distilled water), solution B and solution C. For strains of *A. cycloheptanicus* add 5 g/l of yeast extract instead of 2 g/l.

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403. BSK-MEDIUM

Solution A:

CMRL 1066/10 x Glutamin (GIBCO 042-1545)	100.0	ml
Proteose peptone No 2 (DIFCO 0121-01-3)	5.0	g
Proteose tryptone (DIFCO 0123-01)	1.0	g
Proteose yeastolate (TC) (DIFCO 5577-15)	1.0	g
HEPES buffer (acid) (SIGMA H3375)	6.0	g
Na-citrate	0.7	g
Glucose (α-, D+)	3.0	g
Na-pyruvate	0.8	g
N-Acetylglucosamine	0.4	g
$MgCl_2$ x 6 H_2O	0.3	g
L-Glutamine	0.1	g
Aqua bidest. filter-sterilized	900.0	ml

Stir slowly at 4 - 10°C for 3 hours, adjust pH to 7.6 with 5 M NaOH. Filter sterilize.

Solution B:
Dissolve 14.0 g gelatine in 200 ml bidest. water, autoclave 15 minutes at 115°C.

Solution C:
Dissolve 50.05 g bovine serum albumine, fract. V (important: Sigma No A9647) in 143 ml bidest. water, or use BSA-solution Sigma A7409.

Solution D:

Rabbit serum, inactivated (GIBCO 037-06120M)	86.0	ml

Combine the warm (37°C) solutions A, B, C, D, and filter-sterilize the warm medium. Solution B can also be added after filtration.

Alternatively, buy supplemented or un-supplemented BSK-H medium (Fa. Bio&SELL or Sigma) and supplement the medium according to manufacturer's advice. Sterilize the mixed and warm (37°C) medium by filtration.

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404: METHANOSPHAERA CUNICULI MEDIUM

Mineral solution 1	40.00	ml
Minerals solution	40.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 7 H_2O (0.1% w/v)	2.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	0.20	ml
Modified Wolin's mineral solution	10.00	ml
Na-acetate x 3 H_2O	3.00	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	3.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	900.00	ml

1. Dissolve ingredients (except bicarbonate, methanol, vitamins, cysteine and sulfide), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then distribute under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add methanol (50% v/v), vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2.

2. After inoculation, pressurize culture bottles with sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.

Mineral solution 1 (from medium 203)

K_2HPO_4	6.00	g
Distilled water	1000.00	ml

Minerals solution (from medium 317)

KH_2PO_4	6.00	g
$(NH_4)_2SO_4$	6.00	g
NaCl	12.00	g
$MgSO_4$ x 7 H_2O	2.60	g
$CaCl_2$ x 2 H_2O	0.16	g
Distilled water	1000.00	ml

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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405. SP2 MEDIUM

Casitone (Difco)	1.00	g
Yeast extract (Difco)	0.20	g
Na-acetate	0.02	g
Agar (Difco), if necessary	15.00	g
Sea water-natural or artificial	1000.00	ml

Adjust pH to 7.2.
If natural sea water is used, the pH need not be adjusted.

Artificial sea water:

NaCl	24.70	g
KCl	0.70	g
$MgSO_4$ x 7 H_2O	6.30	g
$MgCl_2$ x 6 H_2O	4.60	g
$CaCl_2$ x 2 H_2O	1.30	g
$NaHCO_3$	0.20	g
Distilled water	1000.00	ml

To prevent precipitation, $CaCl_2$ and $NaHCO_3$ are autoclaved each as separate stock solutions in 10 ml distilled water.

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408: PSYCHRILYOBACTER SP. MEDIUM

Peptone (BD Bacto)	10.00	g
Yeast extract (OXOID)	3.00	g
Casamino acids (BD Bacto)	10.00	g
L-Glutamic acid	20.00	g
NH_4Cl	2.00	g
K_2HPO_4 x 3 H_2O	2.00	g
NaCl	25.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine), bring medium to the boil, then cool to room temperature under 100% N_2 gas atmosphere. Add and dissolve cysteine, adjust pH to 7.0 and dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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410: BRACKISH WATER DESULFOVIBRIO (POSTGATE) MEDIUM

Solution A	980.00	ml
Solution B	10.00	ml
Solution C	10.00	ml

Dissolve ingredients of solution A, bring to the boil, then cool to room temperature while sparging with 100% N_2 gas. Add solutions B and C, adjust pH to 7.8 with NaOH, and distribute under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials. During distribution continuously swirl the medium to keep the grey precipitate suspended. Autoclave 15 min at 121°C.

Solution A

K_2HPO_4	0.50	g
NH_4Cl	1.00	g
Na_2SO_4	1.00	g
NaCl	10.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	2.00	g
Na-DL-lactate	2.00	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	980.00	ml

Solution B

$FeSO_4$ x 7 H_2O	0.50	g
Distilled water	10.00	ml

Solution C

Na-thioglycolate	0.10	g
Ascorbic acid	0.10	g
Distilled water	10.00	ml

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411: CLOSTRIDIUM ACETOBUTYLICUM MEDIUM

Potato (fresh, washed, peeled and sliced)	200.00	g
$CaCO_3$	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	6.00	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Mix potatoes and $CaCO_3$ then autoclave 30 min at 121°C. Filter through cheese cloth, add glucose and cysteine, distribute under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 20 min. Adjust pH of complete medium to 6.8.

2. Note: For the reactivation of freeze-dried strains it is recommended to use the following simple milk medium:

Milk Medium

Milk (fresh)	100.00	ml
Sodium resazurin (0.1% w/v)	0.10	ml

Adjust pH to 7.1. Tube and autoclave under 100% N_2 gas atmosphere for 12 min at 121°C.

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412. ACETOMICROBIUM FAECALIS MEDIUM

Trypticase peptone	2.000	g
Yeast extract	2.000	g
Glucose	4.000	g
K_2HPO_4	0.225	g
KH_2PO_4	0.255	g
$(NH_4)_2SO_4$	0.255	g
NaCl	0.500	g
$MgSO_4$ x 7 H_2O	0.100	g
$CaCl_2$ x 2 H_2O	0.070	g
Na-acetate	5.000	g
Vitamin solution (see medium 141)	10.000	ml
Trace element solution (see medium 141)	10.000	ml
Resazurin	1.000	mg
$NaHCO_3$	6.000	g
Cysteine-HCl x H_2O	0.500	g
Distilled water	1000.000	ml

Autoclave under 2 bar N_2:CO_2 (80:20).

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413. BTU MEDIUM

Medium 78 + Formiate/Fumarate:

78. Chopped Meat Medium

Ground beef (fat free)	500.0	g
Distilled water	1000.0	ml
NaOH 1 N	25.0	ml

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1000 ml, and then add:

Casitone	30.0	g
Yeast extract	5.0	g
K_2HPO_4	5.0	g
Resazurin	1.0	mg

Boil, cool under nitrogen atmosphere, add 0.5 g/l cysteine and adjust pH to 7.0. Dispense anoxically 7 ml medium into Hungate tubes containing meat particles (use 1 part meat particles to 4 or 5 parts fluid). Autoclave at 121°C for 30 min. For agar slants use 15 g agar per 1000.0 ml medium.

In some cases (as indicated in the catalogue) the addition of Haemin and Vitamin K_1 or Vitamin K_3 is necessary. Add to 1000 ml of medium after autoclaving:

Haemin solution (see below)	10.0	ml
Vitamin K_1 or Vitamin K_3 solution (see below)	0.2	ml

Haemin solution:
Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K_1 solution:
Dissolve 0.1 ml of vitamin K_1/K_3 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K_3 solution:
Dissolve 5 mg/ml of vitamin K_3 in 10 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

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Formiate/Fumarate Solution

Na-formiate	6.0	g
Na-fumarate	6.0	g
Distilled water	100.0	ml

Filter sterilize. Add 30 µl per ml of medium 78 before inoculation.

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414. ACIDAMINOCOCCUS FERMENTANS MEDIUM

Trypticase peptone	5.0	g
Casamino acids	10.0	g
Yeast extract	5.0	g
Glucose	5.0	g
Na-glutamate	4.0	g
Arginine	1.0	g
Glycine	1.0	g
DL-Tryptophan	0.1	g
KH_2PO_4	2.0	g
Tween 80	0.5	ml
Cysteine-HCl x H_2O	0.5	g
Distilled water	1000.0	ml

pH 7.0 - 7.2.

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419: OXALOBACTER MEDIUM

K_2HPO_4	0.25	g
KH_2PO_4	0.25	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	25.00	mg
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na-oxalate	5.00	g
Na-acetate	0.82	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate and cysteine, adjust pH to 6.8, bring medium to the boil and then cool to room temperature under 100% CO_2 gas atmosphere. Add solid carbonate and cysteine, then equilibrate pH to 6.8. Dispense under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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420. BLOOD AGAR I

Sterilize blood agar No 2 (Oxoid). After autoclaving cool to 50°C, then add 40 ml/l of sterile horse blood.
Rehydrate pellets of freeze-dried ampoules in liquid medium 220 or 1. For rehydration of *Helicobacter* and *Campylobacter* spp., use medium 215 (brain heart infusion).

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421. CHROMATIUM SALEXIGENS MEDIUM

Supplement medium 28 with NaCl (10%), $MgCl_2$ x 6 H_2O (0.3%) Na-acetate (0.05%) and sodium thiosulfate (0.05%). Incubate at 500 to 1000 Lux light intensity.

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422. AMOEBOBACTER MEDIUM

Supplement medium 28 with 2 mmol acetate and 1 mmol thiosulfate.

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423. XENORHABDUS MEDIUM

Peptone	10.0	g
Yeast extract	5.0	g
NaCl	5.0	g
Distilled water	1000.0	ml

Dissolve and adjust pH to 7.2. Add 1.5% agar if required. Sterilize at 121°C for 20 min.

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425. OATMEAL AGAR

Oat flakes	10.0	g
Oatmeal	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 - 7.2.

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426. ORGANIC MEDIUM 79

Dextrose	10.0	g
Peptone	10.0	g
Casein peptone	2.0	g
Yeast extract	2.0	g
NaCl	6.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.8.

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427. MYKORRHIZA MEDIUM

Malt extract	8.0	g
Glucose	7.0	g
Asparagine	0.5	g
Casein hydrolysate	1.0	g
KH_2PO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.5	g
Yeast extract	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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428. HETEROTROPHIC MEDIUM H3P

Solution A:		
KH_2PO_4	2.300	g
Na_2HPO_4 x 2 H_2O	2.900	g
Distilled water	50.000	ml
Solution B:		
NH_4Cl	1.000	g
$MgSO_4$ x 7 H_2O	0.500	g
$CaCl_2$ x 2 H_2O	0.010	g
$MnCl_2$ x 4 H_2O	0.005	g
$NaVO_3$ x H_2O	0.005	g
Trace element sol. SL-6 (see medium 27)	5.000	ml
Distilled water	850.000	ml
Agar (if necessary)	20.000	g
Solution C:		
Ferric ammonium citrate	0.050	g
Distilled water	20.000	ml
Solution D:		
Yeast extract	1.000	g
Na-acetate	1.000	g
Na_2-succinate	1.000	g
DL-Malate	1.000	g
Distilled water	30.000	ml
pH 7.0		
Solution E:		
Na-lactate	1.000	g
Na-pyruvate	1.000	g
D-Mannitol	1.000	g
D-Glucose	2.000	g
Distilled water	50.000	ml
pH 7.0		
filter-sterilized		

Standard vitamin solution: *See next page*

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Standard vitamin solution:

Riboflavin	10.000	mg
Thiamine-HCl x 2 H_2O	50.000	mg
Nicotinic acid	50.000	mg
Pyridoxine-HCl	50.000	mg
Ca-pantothenate	50.000	mg
Biotin	0.100	mg
Folic acid	0.200	mg
Vitamin B_{12}	1.000	mg
Distilled water	100.000	ml

H_3P is a heterotrophic medium for growth, purity checking and isolation of a broad spectrum of aerobic bacteria (Ref. 3367).

Solutions A, B, C and D are autoclaved separately for 15 min at 121°C, cooled down to 50°C and then mixed aseptically with filter-sterilized solution E (at 50°C) and 5.0 ml of standard vitamin solution. The final pH of this medium should be 6.8 without adjustment.

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429. COLUMBIA BLOOD AGAR

Columbia agar base supplemented with 4% horse blood. Rehydrate pellets of freeze-dried ampoules in liquid medium 545, 215 or 1 (see below). For rehydration of *Helicobacter* and *Campylobacter* spp., use medium 215 (brain heart infusion).

545. TRYPTONE SOYA BROTH (TSB)

Peptone from casein	17.0	g
Peptone from soymeal	3.0	g
D(+)-Glucose	2.5	g
NaCl	5.0	g
K_2HPO_4	2.5	g
Distilled water	1000.0	ml

Adjust pH to 7.3.

The same as Caso Bouillon (Merck or Oxoid, Germany).

215. BHI MEDIUM

Brain Heart Infusion (Difco)	37.0	g
Distilled water	1000.0	ml

1. NUTRIENT BROTH

Peptone	5.0	g
Meat extract	3.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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430. ECTOTHIORHODOSPIRA ABDELMALEKII MEDIUM

KH_2PO_4	0.80	g
NH_4Cl	0.80	g
$CaCl_2$ x 2 H_2O	0.05	g
$MgCl_2$ x 6 H_2O	0.10	g
NaCl	120.00	g
Na_2SO_4	15.00	g
$NaHCO_3$	10.00	g
Na_2CO_3	4 - 5.00	g
Vitamin solution VA *	1.00	ml
Trace element solution SLA *	1.00	ml
Na_2S x 9 H_2O	0.50	g
Na-acetate	1.00	g
Distilled water	1000.00	ml

Adjust pH to 8.5, filter-sterilize and add appropriate amount of a sterile Na_2S solution.

*see medium 351

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431. ECTOTHIORHODOSPIRA VACUOLATA MEDIUM

KH_2PO_4	1.00	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
$NaHCO_3$	3.00	g
NaCl	30.00	g
Trace element solution SLA*	1.00	ml
Vitamin solution VA*	1.00	ml
Na_2S x 9 H_2O	0.05	g
$Na_2S_2O_3$ x 5 H_2O	0.50	g
Na-malate	1.00	g
Distilled water	1000.00	ml

Adjust pH to 8.7, filter sterilize and add appropriate amount of Na_2S solution.

* see medium 351

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432. HALOBACTEROIDES ACETOETHYLICUS MEDIUM

Use medium no. 144 supplemented with 100 g/l of NaCl.

Sterilize glucose, yeast extract and sodium sulfide separately (autoclave under nitrogen atmosphere).

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434. MARINOCOCCUS ALBUS - MEDIUM

NaCl	81.000	g
$MgCl_2$ x 6 H_2O	7.000	g
$MgSO_4$ x 7 H_2O	9.600	g
$CaCl_2$	0.360	g
KCl	2.000	g
$NaHCO_3$	0.060	g
NaBr	0.026	g
Proteose Peptone No. 3	5.000	g
Yeast extract	10.000	g
Glucose	1.000	g
Distilled water	1000.000	ml

Adjust pH to 7.2.

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435. KDM-2 MEDIUM

Peptone	10.0	g
Yeast extract	0.5	g
Cysteine-HCl x H_2O	1.0	g
Agar	15.0	g
Calf serum	200.0	ml
Distilled water	800.0	ml

Adjust pH to 6.5.

Dissolve peptone, yeast extract and cysteine in 800 ml of distilled water. Adjust pH to 6.5 with NaOH. Add agar and dissolve by heating.

Add 2 g/l activated charcoal to the agar base solution before autoclaving. After autoclaving, cool to 45°C and add serum.

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436. RUMINOCOCCUS ALBUS MEDIUM

Tryptone	5.0	g
Yeast extract	2.0	g
Glucose	3.0	g
Cellobiose	2.0	g
Mineral solution 1	40.0	ml
Mineral solution 2	40.0	ml
Resazurin	1.0	mg
Distilled water	920.0	ml

Boil and cool under CO_2. Under a gentle stream of CO_2 add:

Na_2CO_3	4.0	g
Fatty acid mixture	1.0	ml
Cysteine-HCl x H_2O	500.0	mg

Adjust pH to 7.0, distribute under N_2 into appropriate vessels and sterilize.

Mineral solution 1:

K_2HPO_4	0.6	%

Mineral solution 2:

KH_2PO_4	0.6	%
$(NH_4)_2SO_4$	2.0	%
NaCl	1.2	%
$MgSO_4$ x 7 H_2O	0.25	%
$CaCl_2$ x 7 H_2O	0.16	%

Fatty acid mixture:

Isobutyric acid	10.0	ml
Isovaleric acid	10.0	ml
2-Methylbutyric acid	10.0	ml
Distilled water	70.0	ml

Inoculate under CO_2 and incubate at 37°C.

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438. BORDET-GENGOU-MEDIUM (DIFCO)

Bordet-Gengou-Agar-Base supplemented with 15% horse blood.
Rehydrate pellets of freeze-dried ampoules in liquid medium 220 or 1.

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441. DIAZOTROPHIC MEDIUM (RBA)

Solution A:

KH_2PO_4	0.100	g
K_2HPO_4	0.900	g
NaCl	0.100	g
$CaCl_2$ x 2 H_2O	0.100	g
$MgSO_4$ x 7 H_2O	0.100	g
Na_2MoO_4 x 2 H_2O	0.005	g
$NaVO_3$ x H_2O	0.005	g
$MnSO_4$ x H_2O	0.005	g
$FeSO_4$ x 7 H_2O	0.010	g
Yeast extract	0.050	g
Trace element sol. SL-6 (see medium 27)	3.000	ml
Distilled water	950.000	ml
Agar (if necessary)	15.000	g

Adjust pH to 7.3.

Solution B:

Na_2-succinate	1.000	g
DL-Malate	2.000	g
Na-pyruvate	1.000	g
D-Mannitol	2.000	g
D-Glucose	2.000	g
Distilled water	50.000	ml

Adjust pH to 7.3.

Sterilize solution A separately at 121°C for 15 min., cool to 50°C and then mix aseptically with filter-sterilized solution B and 5.0 ml of filter-sterilized standard vitamin solution (see medium 428).

RBA is an ammonium-free medium which has successfully been used for the isolation, growth and purity check of a broad spectrum of nitrogen fixing bacteria (Ref. 3363). For microaerophilic nitrogen-fixing bacteria use semisolid medium with 0.3% end concentration of agar and incubate the liquid cultures under 10% (v/v) air and 90% (v/v) N_2.

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442. MINERAL MEDIUM WITH TOLUENE

Na_2HPO_4 x 12 H_2O	9.0	g
KH_2PO_4	1.5	g
NH_4Cl	2.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Ferric ammonium citrate	5.0	mg
$MnSO_4$ x H_2O	3.0	mg
$ZnSO_4$ x 7 H_2O	0.2	mg
$CoSO_4$	10.0	µg
Nitrilotriacetic acid (NTA)	10.0	mg
Distilled water	1000.0	ml

Adjust pH to 7.0.
One drop of toluene is evaporated in a desiccator so that the atmosphere is saturated.
0.1% succinate may be added to the medium as additional carbon source.

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444. MINERAL MEDIUM WITH NAPHTHALENE

Use medium 442, but few pea-size crumbs of naphthalene instead of toluene are evaporated in a desiccator so that the atmosphere is saturated.

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445. MINERAL MEDIUM WITH SALICYLATE

To medium 442 add 0.05% salicylate as carbon source instead of toluene, sterilized by filtration.

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447. MINERAL MEDIUM WITH CAMPHOR

Use medium 442, but crumbs of camphor instead of toluene are evaporated in a desiccator.

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448. MINERAL MEDIUM WITH NICOTINIC ACID

To medium 442 add 0.05% nicotinic acid as carbon source instead of toluene, sterilized by filtration.

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449. M17 MEDIUM FOR LACTIC STREPTOCOCCI

Peptone from casein	5.00	g
Soya peptone	5.00	g
Peptone bacteriological	5.00	g
Yeast extract	2.50	g
Ascorbic acid	0.50	g
$MgSO_4$ x 7 H_2O	0.25	g
Na_2-ß-glycerophosphate	19.00	g
Lactose	5.00	g
Distilled water	1000.00	ml

Adjust pH to 6.9 ± 0.2.
Lactose should be sterilized by filtration.

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450. SUPPLEMENTED (ARGININE) M9 MEDIUM

To medium 382 add 20 mg/l arginine.

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452. GLUCOSE MEDIUM (NAKAYAMA)

Glucose	10.00	g
Peptone	10.00	g
Yeast extract	15.00	g
Agar	10.00	g
Distilled water	980.00	ml
Solution A	10.00	ml
Solution B	10.00	ml
Solution C	1.00	ml

Solution A:

KH_2PO_4	0.50	g
K_2HPO_4	0.50	g
Distilled water	100.00	ml

Solution B:

$MgSO_4$ x 7 H_2O	3.00	g
NaCl	0.10	g
$MnSO_4$ x 5 H_2O	0.10	g
$CuSO_4$ x 5 H_2O	0.01	g
Distilled water	100.00	ml

Solution C:

$FeSO_4$ x 7 H_2O	0.10	g
Na_3-Citrate	2.00	g
Distilled water	100.00	ml

Autoclave all solutions separately, final pH is 6.8.

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453. STANDARD I MEDIUM

Peptone from meat	7.8	g
Peptone from caseine	7.8	g
Yeast extract	2.8	g
NaCl	5.6	g
D(+)-Glucose	1.0	g
Distilled water	1000.0	ml

Autoclave for 15 min at 121°C. pH of the final medium is 7.5.

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454. EBA MEDIUM

KH_2PO_4	0.20	g
NaCl	1.00	g
KCl	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.25	g
Trace element sol. SL-10 (see medium 320)	1.00	ml
Resazurin	1.00	mg
Na_2SeO_3 x 5 H_2O (0.26 g/ 1000 ml 10 mM NaOH)	1.00	ml
Glycine	2.00	g
Distilled water	950.00	ml

pH 7.2 to 7.4

Before inoculation add per 100 ml of medium:

Biotin (10 mg/l) (autoclave for 10 minutes under N_2)	0.20	ml
$NaHCO_3$ (84 g/l) (autoclaved under CO_2)	3.00	ml
Na_2S (60 g/l) (autoclaved under N_2)	1.00	ml

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455. AMB MEDIUM

Trypticase peptone	4.00	g
Yeast extract	5.00	g
Starch, soluble	1.00	g
Meat infusion	25.00	g
Glucose	5.00	g
Cysteine	1.00	g
$CaCl_2$	0.01	g
$MgSO_4$	0.20	g
$(NH_4)_2SO_4$	1.00	g
K_2HPO_4	15.00	g
Distilled water	1000.00	ml

pH: 6.9.

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456. KPL MEDIUM

Lactic acid whey (see below)	1000.0	ml
White table wine (see below)	140.0	ml
Glucose	10.0	g
Galactose	10.0	g
Tween 80	1.0	ml
Agar	15.0	g

Adjust pH to 5.5

Lactic acid whey:
Adjust 10% skim milk to pH 5.5 with lactic acid and heat at 100°C for 30 minutes. Remove precipitate by filtration. For preparing broth medium, adjust filtrate to pH 7.0 with 2 N NaOH and heat at 100°C for 30 minutes. Remove precipitate by filtration. Adjust filtrate to pH 5.5 with 2 N HCl.

White table wine:
Filter-sterilize and add to autoclaved basal medium.

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457. MINERAL MEDIUM (BRUNNER)

Na_2HPO_4	2.44	g
KH_2PO_4	1.52	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-4 (see below)	10.00	ml
Distilled water	1000.00	ml

Adjust pH to 6.9.
Prepare a separate solution of the phosphates and autoclave separately. Combine the two solutions after cooling.

Rehydrate and cultivate lyophilized cells in complex medium (e.g. medium 1, 220 or 535). After this reactivation, cultivate in mineral medium 457 with the appropriate carbon source.

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6 (see below)	100.00	ml
Distilled water	900.00	ml

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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458. MEDIUM FOR BACILLUS STEAROTHERMOPHILUS - PHENOL DEGRADING

K_2HPO_4	0.50	g
NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.02	g
Yeast extract	0.20	g
Casamino acids	0.10	g
Distilled water	900.00	ml

pH 7.4

Autoclave for 15 min. Add 1 ml of sterile SL-4 (see medium 14) and 100 ml of filter-sterilized phenol-solution (4.7g/l).

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459. SUCROSE-PEPTONE-MEDIUM

Peptone	20.0	g
Sucrose	20.0	g
Distilled water	1000.0	ml

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460. NITROGEN-FREE MEDIUM FOR PSEUDOMONAS STUTZERI

K_2HPO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.2	g
NaCl	0.1	g
Yeast extract	0.2	g
$FeCl_3$ x 6 H_2O	15.0	mg
DL-Na-malate	6.6	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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461. MINERAL MEDIUM (NAGEL AND ANDREESEN)

Distilled water	1000.00	ml
Na_2HPO_4 x 2 H_2O	1.45	g
KH_2PO_4	0.25	g
$CaCl_2$	0.01	g
$MgSO_4$ x 7 H_2O	0.50	g
$MnSO_4$	0.01	g
NH_4Cl	0.30	g
NaCl	0.05	g
Vitamin Solution	5.00	ml
Trace element sol. SL-10	1.00	ml

Dissolve and autoclave the phosphates separately. Add the vitamin solution and trace element solution after autoclaving. After mixing, pH should be 7.5.

Rehydrate and cultivate the lyophilized cells in complex medium recommended for the specific strain (e.g. medium 1, 220 or 535) first. After this reactivation, cultivate on mineral medium 461 with the appropriate carbon source.

Vitamin solution:

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

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462. MINERAL MEDIUM (BRUNNER) WITH VITAMINS

Na_2HPO_4	2.44	g
KH_2PO_4	1.52	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element sol. SL-4 (see below)	10.00	ml
Vitamin solution (Schlegel, see below)	2.50	ml
Distilled water	1000.00	ml

Adjust pH to 6.9.
Rehydrate and cultivate lyophilized cells in the complex medium recommended for the specific strain (e.g. medium 1, 220 or 535). After this reactivation, cultivate in mineral medium 462 with the appropriate carbon source.

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6 (see below)	100.00	ml
Distilled water	900.00	ml

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

Vitamin solution:

p-Aminobenzoate	1.0	mg
Biotin	0.2	mg
Nicotinic acid	2.0	mg
Thiamine-HCl x 2 H_2O	1.0	mg
Ca-pantothenate	0.5	mg
Pyridoxamine	5.0	mg
Vitamin B_{12}	2.0	mg
Distilled water	100.0	ml

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463. NITRILOTRIACETATE MEDIUM

Na_2HPO_4 x 2 H_2O	0.41	g
KH_2PO_4	0.26	g
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.20	g
Trace element solution	1.00	ml
Vitamin solution	1.00	ml
Nitrilotriacetate	1.00	g
Distilled water	1000.00	ml

pH 6.5

Trace element solution:

$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	68.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	62.00	mg
$CoCl_2$ x 6 H_2O	120.00	mg
$CuCl_2$ x 2 H_2O	17.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	24.00	mg
HCl, 0.05 molar	1000.00	ml

Vitamin solution:

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

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464. PLATE COUNT AGAR

Tryptone	5.0	g
Yeast extract	2.5	g
Dextrose	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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465. MINERAL MEDIUM PH 7.25

Na_2HPO_4 x 2 H_2O	3.50	g
KH_2PO_4	1.00	g
$(NH_4)_2SO_4$	0.50	g
$MgCl_2$ x 6 H_2O	0.10	g
$Ca(NO_3)_2$ x 4 H_2O	0.05	g
Trace element solution SL-4 (see below)	1.00	ml
Distilled water	1000.00	ml

pH 7.25

Rehydrate and cultivate lyophilized cells in the complex medium recommended for the specific strain (e.g. medium 1, 220, 535 or 830). After this reactivation, cultivate on mineral medium with the appropriate carbon source.

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6 (see below)	100.00	ml
Distilled water	900.00	ml

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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466. TSBY SALT MEDIUM

Trypticase soy broth	30.00	g
Yeast extract	3.00	g
NaCl	13.00	g
KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
Distilled water	1000.00	ml

Adjust pH to 7.2

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467. OTTOW MEDIUM

Glucose	1.0	g
Peptone	7.5	g
Meat extract	5.0	g
Yeast extract	2.5	g
Casamino acids	2.5	g
NaCl	5.0	g
Tap water	1000.0	ml

Adjust pH to 8.5.

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468. EXIGUOBACTERIUM MEDIUM

Nutrient broth No. 2 (Oxoid)	25.0	g
Yeast extract	3.0	g
Glucose	5.0	g
Distilled water	1000.0	ml

Adjust pH to 8.0.

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469. YM-CATALASE MEDIUM

Yeast extract	5.0	g
Malt extract	5.0	g
K_2HPO_4	5.7	g
$MgSO_4$ x 7 H_2O	0.2	g
$NH_4H_2PO_4$	1.2	g
Catalase (Sigma C-10)	60.0	mg
Distilled water	1000.0	ml

Adjust pH to 7.0. Catalase and $MgSO_4$ are sterilized separately by filtration.

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470. PSEUDOMONAS HALOPHILA MEDIUM

Solution A:

NaCl	46.8	g
$MgSO_4$ x 7 H_2O	39.4	g
NH_4Cl	1.0	g
Glycerol	5.0	g
Trace element sol. SL-10 (see medium 320)	1.0	ml
Vitamin solution (see medium 131)	10.0	ml
Distilled water	890.0	ml

Solution B:

KH_2PO_4	1.0	g
Distilled water	100.0	ml

Vitamin solution is filter-sterilized and added after autoclaving. Solution A and B are autoclaved separately and combined after cooling.
Adjust pH to 7.0 with 6 M NaOH or HCl.

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471. ALCALIGENES XYLOSOXYDANS MEDIUM WITH BENZOATE

Solution A:

K_2HPO_4	5.12	g
KH_2PO_4	1.50	g
Distilled water	500.00	ml

Adjust pH to 9.0 with KOH.

Solution B:

NH_4Cl	0.30	g
$CaCl_2$	0.01	g
$MgSO_4$ x 7 H_2O	0.20	g
KNO_3	2.00	g
Na-benzoate	0.92	g
Distilled water	500.00	ml

Solution C:

Trace element solution SL-4	2.00	ml

Autoclave solutions A, B and C separately, combine, adjust pH to 8.2.

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6	100.00	ml
Distilled water	900.00	ml

First dissolve EDTA in distilled water by adjusting the pH to 7.0 - 8.0 using a 2 M solution of NaOH; then add ferrous sulfate and the trace element solution SL−6.

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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473. MEDIUM WITH EDTA AS CARBON SOURCE

K_2HPO_4	1.400	g
KH_2PO_4	0.272	g
$Ca(NO_3)_2$ x 4 H_2O	0.020	g
$MgSO_4$ x 7 H_2O	0.490	g
Ferric ammonium citrate	0.080	g
Na_2-EDTA	0.200	g
Agar	15.000	g
Trace element solution SL-6		
(see medium 27)	5.000	ml
Distilled water	1000.000	ml

Autoclave phosphate buffer separately. pH should be 7.5 - 7.6. Add 5.0 ml sterile stock solution (see medium 462). For DSM 6780, add 5.0 ml sterile vitamin solution as follows.

Vitamin solution:

Biotin	0.080	g
Folic acid	0.080	g
Thiamin-HCl	0.080	g
Distilled water	100.000	ml

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474. MEDIUM WITH POLYHYDROXYBUTYRIC ACID AS CARBON SOURCE

Prepare a suspension of 3.0 g poly-ß-hydroxybutyric acid (PHB) in 100 ml distilled water, stir over night and sonicate until a white homogenous suspension is obtained. Autoclave for 5 minutes at 121°C. Prepare double layer agar plates. First layer: medium 457 with 16.0 g agar per l. Second layer: medium 457 with agar, supplemented with 6.6 ml sterile PHB-suspension per 100 ml.

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477: ACIDIHALOBACTER PROSPERUS MEDIUM

KCl	0.33	g
$MgCl_2$ x 6 H_2O	2.75	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	1.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
KH_2PO_4	0.14	g
NaCl	0.50	g
Modified Wolin's mineral solution	10.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
$FeSO_4$ x 7 H_2O (40% w/v)	50.00	ml
Distilled water	940.00	ml

Dissolve ingredients except ferrous sulfate and adjust pH to 2.5 with 4 N H_2SO_4. Sterilize medium by autoclaving for 15 min at 121°C. Prior to inoculation add $FeSO_4$ x 7 H_2O from a 40% (w/v) stock solution prepared with 0.2 N H_2SO_4 and filter-sterilized under 100% N_2 gas atmosphere.

For DSM 14174: Supplement medium prior to inoculation with 0.3 g/l $K_2S_4O_6$ added from a stock solution sterilized by filtration.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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479: METHANOHALOPHILUS MEDIUM

NaCl	87.00	g
KCl	1.50	g
$MgCl_2 \times 6\ H_2O$	6.00	g
$CaCl_2 \times 2\ H_2O$	0.40	g
NH_4Cl	1.00	g
$K_2HPO_4 \times 3\ H_2O$	0.40	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Trimethylamine-HCl	2.00	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.20	g
$Na_2S \times 9\ H_2O$	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, trimethylamine, coenzyme M and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trimethylamine, coenzyme M and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

For DSM 5219: Supplement medium with 0.50 g/l Casamino acids (DIFCO), 10.00 ml/l of fatty acid mixture (see medium 119) and 0.25 g/l L-cysteine-HCl x H_2O from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 7.4 - 7.5 with a sterile anoxic stock solution of NaOH.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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480: DESULFURELLA MEDIUM

NH_4Cl	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
Trace element solution SL-10	1.00	ml
Yeast extract	0.10	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powder)	10.00	g
Na-acetate	5.00	g
$NaHCO_3$	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
DL-Dithiothreitol	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, acetate, bicarbonate, vitamins, and dithiothreitol) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 5.9, dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and sterilize by heating for 2-3 hours in a boiling water bath on each of 3 successive days. Add acetate, vitamins, and dithiothreitol from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of vitamins and dithiothreitol are sterilized by filtration. Prior to using adjust the pH of the complete medium to 6.8 - 7.0, if necessary.

For DSM 8415: Replace acetate with 5.00 g/l Na-butyrate.

For DSM 29984: Adjust pH of complete medium to 6.0 - 6.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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481: COPROTHERMOBACTER PROTEOLYTICUS MEDIUM

K_2HPO_4	0.40	g
NH_4Cl	1.00	g
Yeast extract	2.00	g
Trypticase	2.00	g
$MgCl_2$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.40	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate after autoclaving from an anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmospher and sulfide from a sterile anoxic stock solution autoclaved under 100% N_2 gas. Adjust pH of the complete medium to pH 7.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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482: ANAEROBACTER MEDIUM

KH_2PO_4	0.33	g
NH_4Cl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
KCl	0.33	g
Trace element solution SL-10	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.50	g
Sucrose	2.00	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, sucrose, cysteine and sulfide), adjust pH to 7.0, and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add sucrose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to pH 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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483: DEFLUVIITALEA MEDIUM

KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
NH_4Cl	1.00	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2CO_3	1.00	g
D-Glucose	4.00	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, carbonate, glucose and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add cysteine and adjust pH to 6.5, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add glucose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to pH 7.2 -7.4, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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483: DEFLUVIITALEA MEDIUM

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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484: THIOMICROSPIRA THYASIRAE MEDIUM

Solution A	100.00	ml
Solution B	860.00	ml
Solution C	10.00	ml
Solution D	20.00	ml
Solution E	10.00	ml

Autoclave solutions A, B and C separately and mix when cool. After autoclaving, add thiosulfate, trace elements and vitamins from sterile stock solutions to complete the medium. Adjust pH of complete medium to 7.3 - 7.6.

Solution A

Na_2HPO_4 x 2 H_2O	7.90	g
KH_2PO_4	1.50	g
Distilled water	100.00	ml

Adjust to pH 7.6.

Solution B

NH_4Cl	0.40	g
$MgSO_4$ x 7 H_2O	0.10	g
NaCl	25.00	g
Agar, for solid medium	15.00	g
Distilled water	860.00	ml

Solution C

Trace element solution	10.00	ml

Solution D

$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	20.00	ml

Solution E

Vitamin solution	10.00	ml

Vitamin solution (from medium 484)

Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinic acid	20.00	mg

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Pyridoxine hydrochloride	20.00	mg
p-Aminobenzoic acid	10.00	mg
Riboflavin	20.00	mg
Calcium pantothenate	20.00	mg
Biotin	1.00	mg
Vitamin B_{12}	1.00	mg
Distilled water	1000.00	ml

Adjust pH to 7.0.

Trace element solution (from medium 333)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
NaOH	11.00	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds. Adjust pH of final solution to 6.0 with NaOH.

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486: THIOBACILLUS THIOPARUS (TK-m) MEDIUM

KH_2PO_4	2.00	g
K_2HPO_4	2.00	g
NH_4Cl	0.40	g
Na_2CO_3	0.40	g
$MgCl_2$ x 6 H_2O	0.20	g
Vitamin solution	3.00	ml
Trace element solution	1.00	ml
Bromocresol purple (saturated aq. solution)	2.00	ml
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Sterilize the phosphates separately in 1/10 of medium volume and mix with the other salts when cool. Add filter-sterilized vitamin solution after autoclaving to sterile medium. Adjust pH of complete medium to 7.1, if necessary.

Vitamin solution (from medium 484)

Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinic acid	20.00	mg
Pyridoxine hydrochloride	20.00	mg
p-Aminobenzoic acid	10.00	mg
Riboflavin	20.00	mg
Calcium pantothenate	20.00	mg
Biotin	1.00	mg
Vitamin B_{12}	1.00	mg
Distilled water	1000.00	ml

Adjust pH to 7.0.

Trace element solution (from medium 333)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
NaOH	11.00	g
Distilled water	1000.00	ml

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486: THIOBACILLUS THIOPARUS (TK-m) MEDIUM

Dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds. Adjust pH of final solution to 6.0 with NaOH.

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487: PEPTOCLOSTRIDIUM MEDIUM (BETAINE)

Betaine x H_2O	6.00	g
L-Alanine	2.20	g
NaCl	10.00	g
$MgSO_4$ x 7 H_2O	0.50	g
NH_4Cl	1.00	g
$CaCl_2$ x 2 H_2O	0.03	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	5.00	g
K_2HPO_4	0.36	g
KH_2PO_4	0.23	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, phosphates, vitamins and sulfide), bring medium to the boil, then cool to room temperature under 100% N_2 gas atmosphere. Add and dissolve bicarbonate, adjust pH to 7.5, dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add phosphates, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.3 - 7.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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489: LACRIMISPORA MEDIUM

K_2HPO_4	0.22	g
KH_2PO_4	0.22	g
NaCl	0.45	g
$(NH_4)_2SO_4$	0.45	g
$CaCl_2$ x 2 H_2O	0.06	g
$MgSO_4$ x 7 H_2O	0.09	g
Yeast extract	5.00	g
Xylan	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.15	g
Na_2S x 9 H_2O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine and sulfide), adjust pH to 7.0, bring medium to the boil, then cool to room temperature under 100% N_2 gas. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas.

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490: METHANOLOBUS OREGONENSIS MEDIUM

Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
NaCl	29.00	g
KCl	1.50	g
NH_4Cl	1.00	g
K_2HPO_4 x 3 H_2O	0.40	g
$MgCl_2$ x 6 H_2O	1.70	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.50	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.50	g
Trimethylamine-HCl	2.00	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, trimethylamine and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add trimethylamine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 8.5 with a sterile anoxic stock solution of Na_2CO_3 (5% w/v).

For DSM 9005: Adjust pH of complete medium to 8.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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491: HYPERTHERMUS BUTYLICUS MEDIUM

NaCl	17.00	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
$(NH_4)_2SO_4$	10.00	mg
NH_4Cl	0.50	g
Citric acid (0.1% w/v)	5.00	ml
KI (0.1% w/v)	2.50	ml
$CaCl_2$ x 2 H_2O	0.75	g
KH_2PO_4	0.50	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Tryptone (BD BBL)	6.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powder)	6.00	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur and sulfide and adjust pH to 6.0 with sulfuric acid. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Sterilize medium by heating for 2 - 3 hours in a boiling water bath on each of 3 successive days. Before use reduce the medium by adding sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere. Check pH and adjust to pH 6.5 - 7.0, if necessary.

2. After inoculation pressurize culture vessels to 1 bar overpressure with a sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g

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$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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492: CLOSTRIDIUM METHYLPENTOSUM MEDIUM

Solution A	954.00	ml
Solution B	20.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	20.00	ml

Sparge solution A with 100% N_2 gas for at least 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution E is autoclaved separately under 100% N_2 gas. Solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 6.8 - 7.0.

Solution A

NH_4Cl	0.90	g
KH_2PO_4	0.90	g
NaCl	0.90	g
$CaCl_2$ x 2 H_2O	20.00	mg
$MnCl_2$ x 4 H_2O	20.00	mg
$MgSO_4$ x 7 H_2O	20.00	mg
$CoCl_2$ x 6 H_2O (0.1% w/v)	5.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	5.00	ml
$ZnSO_4$ x 7 H_2O (0.1% w/v)	2.00	ml
$CuSO_4$ x H_2O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Adjust pH to 6.5 with KOH.

Solution B

$NaHCO_3$	1.00	g
Distilled water	20.00	ml

Solution C

L-Rhamnose	2.00	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

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Solution E

L-Cysteine HCl x H_2O	1.00	g
Distilled water	20.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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493: THIOMONAS (HÖ5) MEDIUM

KCl	0.33	g
$MgCl_2$ x 6 H_2O	2.75	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	1.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
KH_2PO_4	0.14	g
NaCl	0.50	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except yeast extract and adjust pH to 3.5 with sulfuric acid. After autoclaving add yeast extract from a sterile stock solution.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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494: ACETOHALOBIUM MEDIUM

NaCl	150.00	g
KCl	0.33	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$MgCl_2$ x 6 H_2O	4.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.00	g
Yeast extract	0.05	g
Trimethylamine-HCl	2.40	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, yeast extract, trimethylamine, vitamins and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, trimethylamine, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Prior to use check pH of complete medium and adjust to 7.6 - 8.0 with a sterile anoxic stock solution of Na_2CO_3 (5% w/v), if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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496: PSEUDODESULFOVIBRIO MEDIUM

Solution A	962.00	ml
Solution B	20.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then dispensed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is filter-sterilized and gassed with 100% N_2 gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 6.8 – 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	70.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	960.00	ml

Solution B

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution C

Na-L-lactate	2.50	g
Distilled water	10.00	ml

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Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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497: CLOSTRIDIUM KN MEDIUM (HYDROXYBENZOATE)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution E is autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days. After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

For DSM 5672: Replace 2,4-dihydroxybenzoic acid with 0.15 g/l salicylic acid (2-hydroxybenzoic acid). The concentration of salicylic acid in the medium should not exceed 1.5 mmol per liter; feed substrate several times in order to reach a reasonable cell density.

For DSM 5673: Replace 2,4-dihydroxybenzoic acid with 0.75 g/l 3-hydroxybenzoic acid.

Solution A

Na_2SO_4	1.50	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

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Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

Solution D

2,4-Dihydroxybenzoic acid	0.40	g
Distilled water	10.00	ml

Solution E

$Na_2S \times 9\ H_2O$	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
$Na_2SeO_3 \times 5\ H_2O$	3.00	mg
$Na_2WO_4 \times 2\ H_2O$	4.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg

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Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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498: PSEUDOTHERMOTOGA MEDIUM

Yeast extract (BD Bacto)	0.50	g
Modified Wolin's mineral solution	15.00	ml
NaCl	3.46	g
Na_2-EDTA	0.77	g
$MgSO_4$ x 7 H_2O	0.88	g
$MgCl_2$ x 6 H_2O	0.69	g
$CaCl_2$ x 2 H_2O	0.14	g
KCl	0.08	g
NaBr	12.50	mg
H_3BO_3 (0.1% w/v)	4.00	ml
$(NH_4)_2Ni(SO_4)_2$ (0.1% w/v)	3.00	ml
SrCl2 x 6 H_2O (0.1% w/v)	1.90	ml
KI (0.01% w/v)	0.10	ml
Sodium resazurin (0.1% w/v)	0.50	ml
KH_2PO_4	0.50	g
Starch (soluble)	5.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except hydrogenphosphate, starch and sulfide) and adjust pH to 7.0. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% volume and autoclave. Add hydrogenphosphate, starch and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Check pH of the complete medium and adjust to 7.0 - 7.2, if necessary.

For DSM 28089: Omit starch and supplement medium after autoclaving with 5.00 g/l yeast extract added from an anoxic sterile stock solution prepared under a 100% N_2 gas atmosphere.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g

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H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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499: DESULFOHALOBIUM MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	20.00	g
NaCl	100.00	g
$CaCl_2$ x 2 H_2O	2.70	g
KCl	4.00	g
Na_2SO_4	3.00	g
Na-acetate	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Na-(L)-lactate	2.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Adjust pH to 6.8 - 7.0, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 7.0, if necessary. A small amount of white precipitate forms after autoclaving, but this has no negative effect on growth.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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500: CLOSTRIDIUM (GV) MEDIUM

K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
NH_4Cl	0.50	g
NaCl	2.25	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	3.00	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, magnesium sulfate, calcium chloride, glucose, vitamins, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 7.0, dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add magnesium sulfate, calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

For DSM 5704: Replace glucose with 2.00 g/l of sodium crotonate.

For DSM 6222: Replace glucose with 6.00 g/l of trisodium citrate.

For DSM 9179: Adjust pH of complete medium to 6.5 - 6.7.

For DSM 9187, DSM 10612: Reduce amount of glucose to 2.00 g/l.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg

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$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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501: FERVIDOBACTERIUM ISLANDICUM MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Yeast extract (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	2.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose and sulfide), adjust pH at room temperature to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to inoculation check pH and adjust to 7.0, if necessary.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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502: PSEUDOCLOSTRIDIUM MEDIUM

NaCl	1.20	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.27	g
KH_2PO_4	0.21	g
Na_2SO_4	0.10	g
Trace element solution SL-10	1.00	ml
Inulin	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	0.03	g
Casamino acids (BD Bacto)	0.03	g
Na_2CO_3	1.50	g
Na_2HPO_4	2.66	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except yeast extract, Casamino acids, vitamins, carbonate, hydrogenphosphate and sulfide), bring medium to the boil, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add yeast extract, Casamino acids, hydrogenphosphate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas atmosphere and strerilized by filtration. Adjust pH of complete medium to 7.0, if necessay.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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503: ANAEROBIC FRESHWATER (FWM) MEDIUM

Final pH: 7.2 - 7.4
Final volume: 1003 ml

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C and D are prepared under 100% N_2 gas and sterilized by filtration. Solution E is autoclaved under 100% N_2 gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.2 - 7.4, if necessary.

For DSM 5847: Replace D-glucose with 1.50 g/l of Na (D/L) 3 hydroxybutyrate or 2.00 g/l D-fructose as substrate.

For DSM 5849: Replace D-glucose with 2.50 g/l of Na_2 succinate as substrate.

For DSM 5885: Replace D-glucose with 1.60 g/l Na_2-maleate as substrate added from an anoxic stock solution sterilzed by filtration. Adjust pH of complete medium to 6.7 - 6.8.

For DSM 6779: Replace D-glucose with 2.00 g/l of xylose or xylan as substrate.

For DSM 10092: Omit glucose. Add 0.70 g/l Na_2SO_4 to solution A and use 1.50 g/l Na -propionate as substrate. Reduce the amount of sulfide to 0.10 g/l and use 25 - 50 mg sodium dithionite per liter (e.g. from a freshly prepared filter-sterilized 5% w/v solution) for reduction of the medium prior to inoculation.

For DSM 11045: Omit solution C. Use as final concentration: 10 mM taurine, 60 mM Na -formate, and 200 µM 1,4-naphthochinone as substrates added from anoxic stock solutions sterilized by filtration

For DSM 11046: Add 1.70 g/l $NaNO_3$ to solution A and replace D-glucose with 1.25 g/l taurine as substrate.

For DSM 11261: Replace D-glucose with 1.00 g/l yeast extract and 2.00 g/l Na glycolate as substrates.

For DSM 11262: Replace D-glucose with 1.00 g/l yeast extract and 2.50 g/l Na (D/L)-3 hydroxybutyrate as substrates.

For DSM 11263, DSM 11489: Replace D-glucose with 0.82 g/l Na acetate and 3.20 g/l Na_2 fumarate as substrates added from anoxic stock solutions sterilized by filtration.

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For DSM 11270: Replace D-glucose with 2.50 g/l taurine as substrate.

For DSM 11480, DSM 12595: Reduce amount of D-glucose to 1.80 g/l.

For DSM 12018: Replace D-glucose with 0.20 g/l yeast extract and 2.66 g/l L-aspartic acid as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 13305: Replace D-glucose with 1.00 g/l yeast extract and 5.00 g/l D-fructose as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 14424, DSM 28450, DSM 100378, DSM 103557, DSM 105239: Supplement medium with 2.00 g/l yeast extract added from an anoxic stock solution sterilized by filtration.

For DSM 15206: Replace D-glucose with 2.00 g/l yeast extract and 2.00 g/l Trypticase peptone (BD BBL) as substrates.

For DSM 15978, DSM 27305: Supplement medium with 20 ml/l $FeSO_4$ x 7 H_2O solution (0.1% w/v), 1 ml/l Na-dithionite solution (5% w/v), 0.8 ml/l Methanol, and 2 g/l Trimethylamine-HCl. Omit D-Glucose.

For DSM 19598: Reduce amount of glucose to 2 mM (0.36 g/l); defined coculture with Methanospirillum hungatei. Incubate in the dark. Anaerobic

For DSM 24856: Omit glucose. Add 0.85 g/l $NaNO_3$ to solution A. Use 0.10 g/l yeast extract and 1.10 g/l Na-pyruvate as substrates. Na-pyruvate is added to the autoclaved medium from a sterile anoxic stock solution sterilized by filtration.

For DSM 25964: Replace D-glucose with 0.50 g/l Na-pyruvate as substrate added from an anoxic stock solution sterilized by filtration. Supplement solution A with 2.0 g/l yeast extract.

For DSM 27520: Omit sodium carbonate, reduce amount of D-glucose to 2.00 g/l and add 1.00 g/l yeast extract as substrate. Adjust pH of complete medium to 5.5.

For DSM 27582: Supplement medium after autoclaving with 0.1 g/l yeast extract, 2.0 g/l D-mannose, and 1 ml/l Wolin's vitamin solution (10x) added from anoxic stock solutions sterilized by filtration. Omit D-Glucose.

For DSM 100618: Reduce glucose to 3g/l. Add 1g/l yeast extract.

For DSM 101182: Supplement medium after autoclaving with 0.50 g/l yeast extract and 0.40 g/l L-cysteine HCl x H_2O added from sterile anoxic stock solutions.

For DSM 103526: Reduce amount of D-glucose to 0.40 g/l and add 1.00 g/l yeast extract as substrate.

For DSM 111565: Replace D-glucose with 0.50 g/l yeast extract and 1.00 g/l L-rhamnose as substrates added from anoxic stock solutions sterilized by filtration .

For DSM 112739: Omit glucose and sodium resazurin. Supplement medium with 2.20 g/l sodium phosphite hydrate.

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Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

D-Glucose	5.00	g
Distilled water	20.00	ml

Solution D

Seven vitamins solution	1.00	ml

Solution E

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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504: ANAEROBIC SEAWATER (SWM) MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas and sterilized by filtration. Solution E is autoclaved under 100% N_2 gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.2 7.4, if necessary.

For DSM 5848, DSM 9537: Replace glucose with 2.50 g/l of Na_2-succinate and 0.50 g/l yeast extract as substrates.

For DSM 6233: Replace glucose with 0.50 g/l of pyrogallol as the substrate. When growth appears feed once more with 0.50 g/l pyrogallol.

For DSM 12881, DSM 19012, DSM 25728: Supplement medium with 0.50 g/l yeast extract.

For DSM 15285, DSM 19306: Replace glucose with 10.00 g/l Proteose peptone (BD Difco) as substrate. Adjust pH of complete medium to 6.5 - 7.0.

For DSM 19335, DSM 17953: Replace glucose with 2.50 g/l maltose and 2.00 g/l yeast extract as substrates.

For DSM 106009: Use 0.30 g/l L-Cysteine-HCl x H_2O and 1.50 g/l acetaldehyde as substrates. Acetaldehyde should be added in aliquots of 0.20 g/l at regular intervals until full growth is achieved.

Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

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Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

D-Glucose	2.00	g
Distilled water	20.00	ml

Solution D

Seven vitamins solution	1.00	ml

Solution E

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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505: FORMIVIBRIO MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is prepared under 100% N_2 gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.5 - 7.8 with a sterile anoxic stock solution of Na_2CO_3, if necessary.

For DSM 6139: Use instead of citrate 10 mM sodium cinnamate (or 0.2% sucrose or 0.2% sodium pyruvate) as substrate and supplement the medium with 0.05 g/l of yeast extract. Sterilize substrates separately. Adjust pH of the complete medium to 7.7 - 7.9.

Solution A

KH_2PO_4	1.40	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
Sodium resazurin (0.1% w/v)	0.50	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Distilled water	940.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Trisodium citrate x 2 H_2O	3.00	g
Distilled water	20.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

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Solution E

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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506: METHANOBACTERIUM ESPANOLAE MEDIUM

Na-acetate x 3 H_2O	2.50	g
$(NH_4)_2SO_4$	0.45	g
K_2HPO_4	0.29	g
KH_2PO_4	0.18	g
$MgSO_4$ x 7 H_2O	0.12	g
$CaCl_2$ x 2 H_2O	0.06	g
NaCl	0.05	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve carbonate, then dispense medium into anoxic Hungate-type tubes or serum vials to 30% of their volume under 80% H_2 and 20% CO_2 gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 5.8 - 6.0, if necessary.

2. Note: After growth has started and the culture is becoming turbid add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 - 1 bar overpressure.

For DSM 19849: Adjust pH of complete medium to 7.0 - 7.2 with a sterile anoxic stock solution of Na_2CO_3 prepared under 80% N_2 and 20% CO_2 gas mixture. After inoculation tubes are pressurized with sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g

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H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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507: CARBOXYDOTHERMUS MEDIUM

KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.52	g
$CaCl_2$ x 2 H_2O	0.29	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Yeast extract	0.05	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	20.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, yeast extract, vitamins and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Neutralize the sodium sulfide with sterile H_2SO_4 (see medium 29). After completing the medium adjust pH to 6.8-7.0.

2. Inoculated vessels are pressurized with sterile carbon monoxide gas to 2 bar overpressure.

For DSM 12326: Supplement medium with 1.00 ml/l selenite-tungstate solution (see medium 385).

For DSM 19011: Supplement medium with 0.20 g/l Na-lactate. Change gas phase to 100% carbon monoxide prior to inoculation, but do not use excess pressure.

For DSM 21830: Adjust pH of complete medium to 6.0.

For DSM 22663: Supplement medium with 10.00 g/l sulfur and increase amount of yeast extract to 1.00 g/l. Sulfur is sterilized separately by steaming for 3 h on each of three successive days.

For DSM 23698: Supplement medium with 2.00 g/l Na-pyruvate and omit sulfide. Adjust final pH of medium to 6.2. Change gas phase to 100% carbon monoxide prior to inoculation, but do not use excess pressure.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g

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$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.

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508: CARBOXYDOTHERMUS MEDIUM (HETEROTROPH)

KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.52	g
$CaCl_2$ x 2 H_2O	0.29	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
Trace element solution SL-4	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na-pyruvate	2.50	g
Yeast extract	0.05	g
Seven vitamins solution	1.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, pyruvate, yeast extract, vitamins and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 -45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of pyruvate and vitamins are sterilized by filtration. After completing the medium adjust pH to 6.8-7.0 with a sterile anoxic stock solution of Na_2CO_3 (5% w/v), if necessary.

For DSM 18923: Replace pyruvate with 2.00 g/l D-glucose added to the medium from a sterile anoxic stock solution.

For DSM 22426: Increase amount of yeast extract to 1.00 g/l.

Trace element solution SL-4 (from medium 14)

Na_2-EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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509: SPIROCHAETA THERMOPHILA MEDIUM

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 -45 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C, E and F are autoclaved under 100% N_2 gas atmosphere. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.2 - 7.4.

Solution A

NaCl	15.00	g
$MgCl_2$ x 6 H_2O	2.30	g
KCl	0.50	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
$CaCl_2$ x 2 H_2O	0.03	g
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Trace element solution SL-10	1.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

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Solution E

Starch	1.00	g
Distilled water	10.00	ml

Solution F

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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511: METHANOPYRUS MEDIUM

NH_4Cl	0.25	g
K_2HPO_4 x 3 H_2O	0.07	g
KH_2PO_4	0.09	g
NaCl	11.80	g
$MgSO_4$ x 7 H_2O	1.75	g
$MgCl_2$ x 6 H_2O	4.50	g
Na_2SO_4	0.81	g
$CaCl_2$ x 2 H_2O	0.78	g
KCl	0.30	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
$(NH_4)_2Ni(SO_4)_2$ (0.1% w/v)	2.00	ml
Na_2WO_4 x 2 H_2O (0.1% w/v)	2.00	ml
Marine trace elements solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	980.00	ml

1. Dissolve ingregients except bicarbonate, vitamins and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into serum vials to 30% of their volume and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.5, if necessary.

2. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Marine trace elements solution

NaBr	4.00	g
SrCl2 x 6 H_2O	1.80	g
H_3BO_3	1.30	g
KI	1.25	mg
Na-silicate	100.00	mg
NaF	60.00	mg
KNO_3	40.00	mg
Na_2HPO_4 x 3 H_2O	0.25	mg
Distilled water	1000.00	ml

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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513. PP-MEDIUM

Polypeptone	10.0	g
Yeast extract	2.0	g
$MgSO_4$ x 7 H_2O	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH of medium should be 7.0.

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514. BACTO MARINE BROTH (DIFCO 2216)

Bacto peptone	5.00	g
Bacto yeast extract	1.00	g
Fe(III) citrate	0.10	g
NaCl	19.45	g
$MgCl_2$ (anhydrous)	5.90	g
Na_2SO_4	3.24	g
$CaCl_2$	1.80	g
KCl	0.55	g
$NaHCO_3$	0.16	g
KBr	0.08	g
$SrCl_2$	34.00	mg
H_3BO_3	22.00	mg
Na-silicate	4.00	mg
NaF	2.40	mg
$(NH_4)NO_3$	1.60	mg
Na_2HPO_4	8.00	mg
Distilled water	1000.00	ml

Final pH should be 7.6 ± 0.2 at 25°C. If using the complete medium from Difco add 37.40 g to 1 litre water.

For DSM 11879 and DSM 16960 add to the medium after autoclaving 1.00 g/l $Na_2S_2O_3$ x 5 H_2O from a stock solution sterilized by filtration. Check medium pH after autoclaving and adjust if necessary.

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516: ANAEROCELLUM MEDIUM

NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Trace element solution SL-10	1.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Cellobiose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, carbonate, cellobiose and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add vitamins, cellobiose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of vitamins and cellobiose should be sterilized by filtration. The pH of the complete medium should be at 7.1 - 7.3.

For DSM 6724: Replace cellobiose with 5.00 g/l soluble starch as substrate added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 9003: Supplement medium with 1.00 g/l Trypticase peptone and increase amount of yeast extract to 2.00 g/l.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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517: DESULFOSUDIS MEDIUM

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	11.00	ml
Solution D	1.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 45 min), then distributed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and F are autoclaved under 100% N_2 gas. Solutions D and E are prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.1 - 7.4, if necessary.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% inoculum.

Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
Na_2SO_4	4.00	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.40	g
$CaCl_2$ x 2 H_2O	0.25	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Stearic acid	0.36	g
NaOH (2 N)	0.63	ml

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Distilled water	10.00	ml

Heat the suspension in a closed bottle (with 100% N_2 gas atmosphere in head space) in a boiling water bath. Shake until stearate has dissolved, then autoclave. Stored stearate solution has to be remelted before use.

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Vitamin B_{12} (50 µg/ml)	1.00	ml

Solution F

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg

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Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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518: GUYPARKERIA MEDIUM

Na_2HPO_4 x 2 H_2O	7.90	g
KH_2PO_4	1.50	g
NaCl	50.00	g
$MgSO_4$ x 7 H_2O	0.10	g
NH_4Cl	0.40	g
Phenol red (saturated aq. solution)	12.50	ml
Trace element solution	10.00	ml
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Agar, for solid medium (optional)	15.00	g
Distilled water	1000.00	ml

Dissolve ingredients except thiosulfate and adjust pH of medium to 7.3 - 7.5. Add thiosulfate to the medium after autoclaving from a sterile stock solution sterilized by filtration. For liquid medium also sterilize the phosphates separately in 1/10 of medium volume.

Trace element solution (from medium 333)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
NaOH	11.00	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds. Adjust pH of final solution to 6.0 with NaOH.

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519: ARCHAEOGLOBUS PROFUNDUS MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
Na_2SO_4	2.70	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
Yeast extract (OXOID)	0.50	g
Na-acetate	1.00	g
$Fe(NH_4)_2(SO_4)_2$ x 7 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 6.5 and dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic serum vials (e.g., 20 ml medium per 100 ml bottle), pressurize bottles up to 2 bar overpressure, then autoclave. Before use, release excess pressure and reduce the medium with sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas.

2. After inoculation, add 2 bar overpressure of sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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520: RUMINOCLOSTRIDIUM CELLULOLYTICUM (CM3) MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	1.50	g
K_2HPO_4 x 3 H_2O	2.90	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.25	ml
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	75.00	mg
Cellobiose	6.00	g
Cellulose, MN 301 (optional)	10.00	g
Na_2CO_3	1.50	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except magnesium chloride, calcium chloride, cellobiose, cysteine and carbonate, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add magnesium chloride, calcium chloride and cellobiose from anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2. Cellobiose has to be sterilized by filtration. Prior to inoculation add cysteine from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH to 7.2.

2. Note: Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose powder MN 301 (MACHEREY-NAGEL).

For DSM 5974, DSM 9801, DSM 17427: Use 5.00 g/l D-glucose as the substrate. Supplement medium after autoclaving with 0.50 g/l Na_2S x 9 H_2O added from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH of complete medium to 6.8 - 7.0, if necessary.

For DSM 27016: Adjust pH of final medium to 8.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg

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$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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521: DESULFOMONILE MEDIUM

NH_4Cl	1.00	g
Na_2SO_4	2.00	g
$Na_2S_2O_3$ x 5 H_2O	1.00	g
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KH_2PO_4	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Vitamin solution	10.00	ml
Na-pyruvate	2.50	g
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, vitamins, pyruvate and sulfide), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete the medium by adding vitamins, pyruvate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of vitamins and pyruvate are sterilized by filtration. Adjust pH of the complete medium to 6.8 - 7.0.

2. Note: Prior to inoculation 10-20 mg/l sodium dithionite (added from a 5% w/v solution freshly prepared under N_2 and filter-sterilized) can be added to the medium to stimulate growth at the beginning.

Vitamin solution

Pyridoxine hydrochloride	5.00	mg
Riboflavin	5.00	mg
Calcium pantothenate	5.00	mg
p-Aminobenzoate	5.00	mg
Thioctic acid	5.00	mg
Nicotinamide	50.00	mg
Biotin	5.00	mg
Folic acid	5.00	mg
Vitamin B_{12}	5.00	mg
Haemin (dissolved in 0.1 N NaOH)	5.00	mg
1,4-Naphthoquinone (dissolved in 0.1 N NaOH)	20.00	mg
Distilled water	1000.00	ml

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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523: CLOSTRIDIUM CHARTATABIDUM MEDIUM

Mineral solution	38.00	ml
Clarified rumen fluid	200.00	ml
K_2HPO_4	0.30	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	0.50	g
Haemin solution (0.05% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
Cellobiose	5.00	g
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	760.00	ml

Dissolve ingredients (except carbonate, cellobiose, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 100% CO_2 gas atmosphere. Add the carbonate and equilibrate the medium with the CO_2 gas to pH 6.8. Distribute under CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

Mineral solution (from medium 330)

KH_2PO_4	6.00	g
NaCl	12.00	g
$(NH_4)_2SO_4$	6.00	g
$CaCl_2$ x 2 H_2O	1.60	g
$MgSO_4$ x 7 H_2O	2.50	g
Distilled water	1000.00	ml

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml

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Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

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524: FLEXISTIPES MEDIUM

NaCl	58.35	g
$MgCl_2$ x 6 H_2O	37.80	g
Na_2SO_4	9.72	g
$CaCl_2$ x 2 H_2O	7.14	g
KCl	1.65	g
KBr	0.24	g
SrCl2 x 6 H_2O	0.17	g
H_3BO_3	0.66	g
Na_2HPO_4 x 2 H_2O	0.03	g
Na-meta silicate	12.00	mg
NaF (0.1% w/v)	7.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	0.50	g
Yeast extract	1.00	g
KNO_3	0.25	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, yeast extract, nitrate and sulfide, adjust pH to 6.0 - 6.2 using sulfuric acid and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete medium by adding yeast extract, nitrate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2. Adjust pH of complete medium to 6.5.

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525: METHANOHALOPHILUS (MG) MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	2.75	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
K_2HPO_4 x 3 H_2O	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
NaCl	100.00	g
Modified Wolin's mineral solution	10.00	ml
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Na_2CO_3	0.50	g
Trimethylamine-HCl	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonates, trimethylamine, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete medium by adding sterile anoxic stock solutions of trimethylamine, vitamins (sterilized by filtration), cysteine and sulfide prepared under 100% N_2 gas atmosphere and bicarbonate and carbonate from sterile anoxic stock solutions prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 6.9 - 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g

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Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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526: CLOSTRIDIUM VIRIDE MEDIUM

5-Aminovaleric acid	15.00	g
K_2HPO_4	8.70	g
Yeast extract	7.50	g
Trace elements solution VR	4.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
L-Cysteine HCl x H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate and cysteine), adjust pH to 7.0 with NaOH and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min. Add and dissolve solid bicarbonate and cysteine, dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving adjust pH of medium to 7.6 - 7.8 using a sterile anoxic stock solution of Na_2CO_3 (5% w/v) prepared under 80% N_2 and 20% CO_2 gas atmosphere.

Trace elements solution VR

Nitrilotriacetic acid (NTA)	5.00	g
$MgSO_4$ x 7 H_2O	24.00	g
$CaCl_2$ x 2 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	0.50	g
$NiCl_2$ x 6 H_2O	0.25	g
$ZnSO_4$ x 7 H_2O	0.44	g
$MnSO_4$ x H_2O	0.25	g
$CoCl_2$ x 6 H_2O	0.25	g
$VOSO_4$ x 5 H_2O	0.25	g
Na_2MoO_4 x 2 H_2O	0.25	g
$CuSO_4$ x 5 H_2O	0.12	g
Distilled water	1000.00	ml

Dissolve mineral salts in 700 ml water acidified with 2 ml of conc. HCl. Separately dissolve 5.00 g of NTA in 300 ml water add 10 N NaOH until all the NTA has dissolved and the pH is 7.0. While stirring, slowly add the NTA solution to the salt solution. Stir until the solution is almost clear. Store cool and dark.

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528. NAPHTHALENE SULFONIC ACID MEDIUM

Na_2HPO_4 x 2 H_2O	3.50	g
KH_2PO_4	1.00	g
NH_4Cl	0.31	g
$MgCl_2$ x 6 H_2O	0.10	g
$Ca(NO_3)_2$ x 4 H_2O	0.05	g
Distilled water	1000.00	ml

pH 7.0.

After autoclaving add 1 ml sterile trace element solution SL-4 (medium 14), 100 ml solution A and 3 ml solution B per liter.

Solution A:

Glucose	3.0	g
Na-succinate	3.0	g
Glycerol	3.0	g
Distilled water	1000.0	ml

Sterilize by filtration.

Solution B:

Naphthalene sulfonic acid	2.3	g
Distilled water	1000.0	ml

Sterilize by filtration.

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529. AMPHIBACILLUS MEDIUM

Glucose	10.000	g
K_2HPO_4	1.000	g
NH_4NO_3	2.000	g
$MgSO_4$ x 7 H_2O	0.200	g
$MnSO_4$ x 7 H_2O	0.005	g
$FeSO_4$ x 7 H_2O	0.005	g
$CaCl_2$ x 2 H_2O	0.100	g
Yeast extract	3.000	g
Polypeptone (BBL)	0.300	g
Agar	15.000	g
Distilled water	1000.000	ml

After sterilization add sterile 1 M Na-sesquicarbonate solution (1 ml in 10 ml) to achieve a pH of 9.7 (see medium 31).

Na-sesquicarbonate solution:

$NaHCO_3$	4.200	g
Na_2CO_2 (anhydrous)	5.300	g
Distilled water	100.000	ml

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530. THIOGLYCOLATE MEDIUM

Peptone from casein	5.0	g
Meat extract	3.0	g
Na-Thioglycolate	0.5	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 5.5.

Sterilize Na-Thioglycolate separately by filtration.

Optimal pO_2 for growth on solid media is 0.03 - 0.08 atm.

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531. SPORULATION MEDIUM

Nutrient Broth (Difco)	6.0	g
Yeast extract	1.0	g
Trypticase (BBL)	2.0	g
$MnSO_4$ x H_2O	0.5	mg
Marine Broth (Difco)	0.5	g
Agar	10.0	g
Inosin (5 mM)	1.0	ml
Distilled water	1000.0	ml

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533. THERMOPHILIC HYDROGEN BACTERIA MEDIUM

NH_4NO_3	1.0	g
Na_2HPO_4 x 12 H_2O	4.5	g
KH_2PO_4	1.5	g
$MgSO_4$ x 7 H_2O	0.2	g
$FeSO_4$ x 7 H_2O	10.0	mg
$CaCl_2$ x 2 H_2O	10.0	mg
NaCl	1.0	g
Trace element solution	0.5	ml
H_2O (deionized)	1000.0	ml

pH 7.0

Trace element solution:

H_2O (deionized)	1000.0	ml
MoO_3	4.0	mg
$ZnSO_4$ x 7 H_2O	28.0	mg
$CuSO_4$ x 5 H_2O	2.0	mg
H_3BO_3	4.0	mg
$MnSO_4$ x 5 H_2O	4.0	mg
$CoCl_2$ x 6 H_2O	4.0	mg

Incubate under 5% (v/v) O_2, 80% H_2, 10% CO_2.

Note: DSM 6534 (at 70°C) and DSM 6765 (at 50°C) will also show good growth in medium 81 under 5% (v/v) O_2, 10% $_CO_2$, 45% H_2.

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534. MODIFIED SME MEDIUM

NaCl	30.00	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_20	5.50	g
KCl	0.65	g
NaBr	0.10	g
NH_4Cl	0.15	g
K_2HPO_4	0.15	g
$CaCl_2$ x 2 H_2O	0.50	g
Trace element solution(see medium 141)	10.00	ml
Distilled water	1000.00	ml

Gas with nitrogen gas for about 20 min and then adjust the pH to 6.5 - 6.8 with H_2SO_4. Fill 10 ml of medium in serum bottles (100 ml) under a protective CO_2 atmosphere and tightly stopper and autoclave at 121°C for 15 min. Add 0,3 ml filter-sterilised solution A (gassed with N_2) and sterile solution B (gassed with CO_2) in 10 ml medium . Exchange the gas phase by a mixture of H_2 : CO_2 : O_2 (300 KPa; each mixture 79.75 : 19.75 : 0.5 by volume). For more details see the original description (Huber et al. System. Appl. Microbiol. 15:340-351 (1992)).

Solution A:

$NaHCO_3$	2,0	g
Distilled water	30	ml

prepared under CO_2 in an ice-bath, filter-sterilise

Solution B:

$Na_2S_2O_3X5H_2O$	2,0	g
Distilled water	30	ml

Prepared under N_2,autoclave 121°C, 15 min

Trace element solution:
To 1000.0 ml trace element solution in medium 141 add:

$(NH_4)_2Ni(SO_4)_2$	2.00	g
Na_2WO_4	10.00	mg
Na_2SeO_4	10.00	mg

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535. TRYPTICASE SOY BROTH AGAR

Trypticase Soy Broth		
(BBL 11768, Oxoid CM129 or Merck 5459)	30.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.3

Autoclave at 121°C for 15 min.

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538. HERBASPIRILLUM MEDIUM

KH_2PO_4	0.400	g
K_2HPO_4	0.100	g
$MgSO_4$ x 7 H_2O	0.200	g
NaCl	0.100	g
$CaCl_2$	0.020	g
$FeCl_2$ x 6 H_2O	0.010	g
$NaMoO_4$ x 2 H_2O	0.002	g
Yeast extract	0.050	g
Distilled water	950.000	ml
Agar	15.000	g

pH 7.0

Autoclave at 121°C for 15 min and after sterilization add filter sterilized solution A.

Solution A:

Na-Malate	5.0	g
H_2O	50.0	ml

pH 7.0.

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539: RUMINICLOSTRIDIUM CELLOBIOPARUM MEDIUM

KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	1.00	g
$MgCl_2 \times 6\ H_2O$	0.40	g
KCl	0.50	g
$CaCl_2 \times 2\ H_2O$	0.15	g
Trace element solution SL-10	1.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Cellobiose	5.00	g
Cellulose, MN 301 (optional)	5.00	g
$Na_2S \times 9\ H_2O$	0.40	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, cellobiose and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cellobiose (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.0, if necessary.

2. Note: Some strains can be adapted to cellulose as substrate using 5.00 g/l cellulose powder MN 301 (MACHEREY-NAGEL).

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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540: ACETITOMACULUM MEDIUM

KH_2PO_4	0.20	g
Na_2SO_4	0.10	g
NH_4Cl	0.30	g
KCl	0.50	g
NaCl	7.00	g
$MgCl_2$ x 6 H_2O	1.20	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	1.50	g
Modified Wolin's mineral solution	10.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	1.00	ml
Na_2WO_4 x 2 H_2O (0.1% w/v)	0.10	ml
Ascorbic acid (0.01% w/v)	0.50	ml
Myo-inositol (0.01% w/v)	0.50	ml
Niacinamide (0.01% w/v)	0.50	ml
Choline chloride (0.01% w/v)	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose, vitamins and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate and equilibrate pH to 6.8 with 80% N_2 and 20% CO_2 gas mixture, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g

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$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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541: SULFUROSPIRILLUM MEDIUM

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	2.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C should be prepared under 100% N_2 gas and sterilized by filtration. Solution D is autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to D are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.2, if necessary.

For DSM 18149: Supplement medium after autoclaving with 1.00 ml/l Wolin's vitamin solution 10x (see medium 120) sterilized by filtration. Prior to inoculation reduce the complete medium with 10 - 20 mg/l sodium dithionite (added from a 5% w/v stock solution freshly prepared under 100% N_2 gas and filter-sterilized) instead of using cysteine.

For DSM 22742: Supplement medium with 1.00 ml/l Wolin's vitamin solution 10x (see medium 120) and 0.50 g/l Na-thiosulfate added after autoclaving from anoxic stock solutions sterilized by filtration.

Solution A

KH_2PO_4	1.36	g
$MgSO_4$ x 7 H_2O	0.37	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.27	g
Trace element solution SL-10	2.00	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Na_2-fumarate	4.00	g
Distilled water	20.00	ml

Solution D

L-Cysteine HCl x H_2O	0.07	g

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Distilled water	2.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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542. PENNASSAY G-THY MEDIUM

Pennassay Broth	17.50	g
Glucose	20.00	g
Thymine	0.05	g
Distilled water	1000.00	ml

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544. NZCYM-MEDIUM

Casein hydrolysate	10.0	g
NaCl	5.0	g
Casamino acids (Difco)	1.0	g
Yeast extract	5.0	g
$MgSO_4$ x 7 H_2O	2.0	g
Maltose	2.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5.

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545: TRYPTONE SOYA BROTH (TSB)

Final pH: 7.3
Final volume: 1000 ml

Casein peptone	17.00	g
Soy peptone	3.00	g
D(+)-Glucose	2.50	g
NaCl	5.00	g
K_2HPO_4	2.50	g
Distilled water	1000.00	ml

1. Adjust pH to 7.3.

2. The same as Caso Bouillon (Merck or Oxoid, Germany).

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546. LC BROTH MEDIUM

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	10.0	g
Glucose	1.0	g
Distilled water	990.0	ml

Adjust pH to 7.4.
Sterilize at 121°C for 15 min. Cool to 50°C and add 5 ml 1 M $CaCl_2$ x 2 H_2O and 5 ml 1 M $MgSO_4$ x 7 H_2O as sterile solutions.

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547. ISP MEDIUM 4

Solution I:
Difco soluble starch, 10.0 g. Make a paste of the starch with a small amount of cold distilled water and bring to a volume of 500 ml.

Solution II:

$CaCO_3$	2.0	g
K_2HPO_4 (anhydrous)	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
NaCl	1.0	g
$(NH_4)_2SO_4$	2.0	g
Distilled water	500.0	ml
Trace salt solution	1.0	ml

The pH should be between 7.0 and 7.4. Do not adjust if it is within this range. Mix solutions I and II together. Add 20.0 g agar. Liquify agar by steaming at 100°C for 10 to 20 min.

Trace salt solution:

$FeSO_4$ x 7 H_2O	0.1	g
$MnCl_2$ x 4 H_2O	0.1	g
$ZnSO_4$ x 7 H_2O	0.1	g
Distilled water	100.0	ml

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548. BENNETT'S AGAR

Beef extract	1.0	g
Glucose	10.0	g
N-Z amine A (enzymatic digest of casein)	2.0	g
Yeast extract	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.3 with NaOH.
Modified Bennett's agar can also be used. In it, glucose is replaced by glycerol (10.0 g) and N-Z amine by Bacto casein (2.0 g, Difco).

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549. CM + YE MEDIUM

Casamino acids Difco (vitamin free)	7.5	g
Yeast extract	10.0	g
$MgSO_4$ x 7 H_2O	20.0	g
Na_3Citrat	3.0	g
KCl	2.0	g
NaCl	200.0	g
Fe*	10.0	ppm
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.4 (NaOH)

* Prepare 4.98% $FeSO_4$ x 7 H_2O (= 10 g Fe in 1l 0.001 M HCl); add 1 ml per liter medium.

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550. CYC-MEDIUM (modified following Cross and Attwell,1973)

Czapek Dox agar (Merck)	48.00	g
Yeast extract (Oxoid)	2.00	g
Casamino acids (Difco)	6.10	g
Tryptophan	0.02	g
Distilled water	1000.00	ml

pH 7.2

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553. GPHF-MEDIUM

Glucose	10.00	g
Peptone from Casein	5.00	g
Yeast extract (Oxoid)	5.00	g
Beef extract (Oxoid)	5.00	g
$CaCl_2$ x 2 H_2O	0.74	g
Agar (Oxoid)	15.00	g
Distilled water	1000.00	ml

pH 7.2

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554. N-Z-AMINE-MEDIUM

Glucose	10.0	g
Starch, soluble	20.0	g
Yeast extract	5.0	g
N-Z-Amine	5.0	g
$CaCO_3$	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.2

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555. SPORULATION-AGAR (SA)

Yeast extract	1.0	g
Beef extract	1.0	g
Trypt. Peptone	2.0	g
$FeSO_4$	trace	-
Glucose	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.2

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557: CLOSTRIDIUM SP. MEDIUM (CREATININE)

$CaSO_4$ x 2 H_2O	0.05	g
$MgSO_4$ x 7 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	0.01	g
$MnCl_2$ x 4 H_2O	0.06	g
NaCl	1.00	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
KH_2PO_4	2.64	g
K_2HPO_4	5.33	g
$NaHCO_3$	1.00	g
Creatinine	5.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except hydrogenphosphates, bicarbonate, creatinine, vitamins, cysteine and sulfide, adjust pH to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete medium by adding hydrogenphosphates, creatinine, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of creatinine (5% w/v) and vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

For DSM 6877: Replace creatinine with 6.80 g/l N-methylhydantoin (SIGMA) as substrate.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

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Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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558: CLOSTRIDIUM (RB-1) MEDIUM

KH_2PO_4	1.50	g
Na_2HPO_4 x 12 H_2O	4.20	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	0.36	g
Na_2SO_4	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	10.00	g
Yeast extract	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, yeast extract, vitamin solution and reducing agents), adjust pH to 3.5 using HCl and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials. After autoclaving add glucose, yeast extract, vitamin solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 3.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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559. TMBS4 MEDIUM

Solution A:		
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.15	g
KCl	0.50	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite/tungstate solution (see medium 385)	1.00	ml
Resazurin	0.50	mg
Distilled water	930.00	ml
Solution B:		
$NaHCO_3$, 5% w/v solution	30.00	ml
Solution C:		
Vitamin solution (see next page)	10.00	ml
Solution D:		
Na-thiosulfate, 5% w/v solution	2.00	ml
Solution E:		
Yeast extract, 10% w/v solution	10.00	ml
Solution F:		
Syringate solution (see next page)	10.00	ml
Solution G:		
Dithiothreitol, 100 mM solution	10.00	ml

Boil solution A for 3 min., then cool to room temperature under 100% N_2 gas atmosphere. Dispense the medium under same gas atmosphere in culture vessels and autoclave. Autoclave separately solutions E and F under 100% N_2 and solution B under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C, D, and G are prepared under N_2 gas atmosphere and sterilized by filtration.

Solutions B to G are added to the sterile, cooled solution A in the sequence as indicated. The final pH of the medium should be 7.2-7.4. After inoculation add 25 mg sodium dithionite per liter medium from a 2.5% (w/v) solution, freshly prepared under N_2 and filter-sterilized.

Vitamin solution and syringate solution: *see next page*

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Vitamin solution:
Use vitamin solution of medium 141, but increase amount of vitamin B_{12} to 5.0 mg/l and adjust final pH of solution to 7.2 using 50 mM sodium phosphate buffer.

Syringate solution:
Dissolve syringic acid in distilled water and neutralize solution with 2 N NaOH until a pH of 7.5 is reached. The final concentration of syringate in the solution should be 6% w/v.

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561: SULFURIFERULA MEDIUM

KCl	0.30	g
$MgCl_2$ x 6 H_2O	2.75	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	1.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	0.50	g
Modified Wolin's mineral solution	10.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	5.00	ml
Na_2WO_4 x 2 H_2O (0.1% w/v)	0.50	ml
Distilled water	1000.00	ml

1. Dissolve ingredients, adjust pH to 6.0 with H_2SO_4 and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium in 100 ml bottles) and autoclave. Adjust medium pH to 6.0, if necessary.

2. After inoculation, add by injection 3% (v/v) sterile air (usually 9 ml per bottle) and pressurize bottles with sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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563. Z MEDIUM

Glucose	1.000	g
$CaCl_2$ x 2 H_2O	0.367	g
NaCl	10.000	g
Casein hydrolysate	10.000	g
Yeast extract	5.000	g
Distilled water	1000.000	ml

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569. MEDIUM 125 WITH METHANOL

To 1 l of medium 125 pH adjusted to 4.0-5.4 add 10 ml CH_3OH.

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570. MEDIUM FOR THERMOPHILIC ACTINOMYCETES (AGRE 1964)

Soluble starch	10.0	g
NaCl	5.0	g
$CaCl_2$	0.5	g
Peptone	5.0	g
Maize extract	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Final pH should be 6.5.

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572. GYE - MEDIUM

Glucose	15.0	g
Yeast extract	5.0	g
$CaCl_2$ x 2 H_2O	0.2	g
Tap water	1000.0	ml

pH 7.0

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573. THIOTHRIX MEDIUM

NH_4Cl	0.20	g
K_2HPO_4	0.01	g
$MgSO_4$ x 7 H_2O	0.01	g
$CaSO_4$ (saturated solution)	20.00	ml
Trace element solution (see medium 155)	5.00	ml
Na-acetate	0.10	g
Agar (if necessary)	12.00	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Adjust pH to 7.5 before autoclaving. Sterilize separately a 10% (w/v) solution of Na_2S x 9 H_2O; add 3 ml/l of this solution to the medium after autoclaving.

For DSM 113264 add 0.3 g/L $NaNO_3$.

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574: GUYPARKERIA HYDROTHERMALIS MEDIUM

NaCl	25.00	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_2O	1.50	g
KH_2PO_4	0.40	g
$NaHCO_3$	0.20	g
$CaCl_2$ x 2 H_2O	0.30	g
Phenol red (0.5% w/v)	2.00	ml
Trace element solution	1.00	ml
Tris-HCl buffer, 0.1 M, pH 7.5	200.00	ml
Distilled water	800.00	ml

Dissove ingredients and adjust pH to 7.5. Sterilize the medium by filtration.

Trace element solution

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	2.20	g
$CaCl_2$ x 2 H_2O	5.50	g
$MnCl_2$ x 4 H_2O	5.10	g
$FeSO_4$ x 7 H_2O	5.00	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1.10	g
$CuSO_4$ x 5 H_2O	1.60	g
$CoCl_2$ x 6 H_2O	1.60	g
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7 using 2 N KOH. Adjust pH of final solution to 6.0 with KOH.

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578. TRYPTONE-YEAST EXTRACT MEDIUM

Tryptone	8.0	g
Yeast extract	5.0	g
NaCl	5.0	g
Distilled water	1000.0	ml

pH 7.0

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579: GEOBACTER MEDIUM

Final pH: 6.7 - 7.0
Final volume: 1011 ml

Ferric citrate monohydrate	13.70	g
NH_4Cl	1.50	g
NaH_2PO_4	0.60	g
KCl	0.10	g
Na-acetate	2.50	g
Modified Wolin's mineral solution	10.00	ml
$NaHCO_3$	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. First dissolve ferric citrate in water by heating and adjust to pH 6.0, then add other medium ingredients except bicarbonate and vitamins. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then add and dissolve bicarbonate and adjust pH to 6.8. Thereafter, dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins to the sterile medium from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 6.7 - 7.0, if necessary.

2. Note: Use 5 - 10% (v/v) inoculum.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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581. GLYCEROL CORNSTEEP AGAR

Glycerol	5.0	g
Cornsteep powder	5.0	g
Yeast extract	3.0	g
Beef extract	1.0	g
Peptone from casein	3.0	g
NaCl	3.0	g
Agar	12.0	g
Distilled water	1000.0	ml

pH 7.2

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582. 15% MH MEDIUM

NaCl	121.500	g
$MgCl_2$	10.500	g
$MgSO_4$	14.400	g
$CaCl_2$	0.540	g
KCl	3.000	g
$NaHCO_3$	0.090	g
NaBr	0.039	g
Proteose peptone no. 3 (Difco)	5.000	g
Yeast extract (Difco)	10.000	g
Glucose	1.000	g
Distilled water	1000.000	ml

Final pH 7.5

$NaHCO_3$ is added from a sterilized stock solution when the medium has cooled. Agar is added at a concentration of 20 g/l for solid media.

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583. CORYNEBACTERIUM MEDIUM CII

Yeast extract	4.0	g
Sucrose	10.0	g
$CaCO_3$	20.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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585. BCYE AGAR

Dehydrated media preparations are available from media distributors.

We use OXOID Legionella CYE-Agar base with Legionella BCYE supplement.

For solubilization of the lyophilized pellet use standard liquid media. Although Legionellae do not grow in those media they are suitable for resuspension and transfer onto agar plates.

Alternatively:

CYE-ACES AGAR

Solution A:

Yeast extract (Difco 0127)	10.00	g
ACES (N-2-acetamido-2-aminoethane-sulfonic acid)	10.00	g
Activated charcoal (Sigma C 5510)	2.00	g
Distilled water	490.00	ml

Dissolve ACES and bring to pH 6.9 by slowly adding 1 N KOH. Add the other components and dissolve by boiling. Autoclave at 121°C for 15 minutes. Cool to 50 - 55°C.

Solution B:

Agar	15.00	g
Distilled water	490.00	ml

Autoclave at 121°C and cool to 50 - 55°C.

Solution C:

Cysteine-HCl x H_2O	0.40	g
Distilled water	10.00	ml

Prepare a fresh solution and filter-sterilize it.

Solution D:

$Fe_4(PO_4)_2$	0.25	g
Distilled water	10.00	ml

Prepare a fresh solution by heating to 50 - 55°C in a water-bath. Filter-sterilize solution separately.
Please note: Soluble ferric pyrophosphate must be kept dry and in the dark. Do not use if chemical looses its green colour and becomes brown or yellow.

Add Cysteine-HCl, then ferric pyrophosphate solution to solution A. Adjust the complete medium to pH 6.9 ± 0.5 at 50°C with sterile 1.0 N KOH or HCl. The pH of the medium is critical. Finally, add solution B. Swirl medium in flask during dispensing to petri dishes or tubes in order to keep charcoal suspended.

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586. THAUERA AROMATICA MEDIUM

Solution A:

KH_2PO_4	0.816	g
K_2HPO_4	5.920	g
Distilled water	500.000	ml

Solution B:

NH_4Cl	0.530	g
$MgSO_4$ x 7 H_2O	0.200	g
KNO_3	2.000	g
$CaCl_2$ x 2 H_2O	0.025	g
Na-benzoate	0.720	g
Distilled water	500.000	ml

Adjust solutions A and B to pH 7.8 for DSM 6898, or to pH 7.2 for other DSMZ strains, autoclave separately and combine after cooling. Add 10 ml of sterile trace elements solution SL-10 (see below) and 5 ml vitamin solution (see below).

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Vitamin solution:

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

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587. XYLOPHILUS MEDIUM

Yeast extract	10.0	g
D-Galactose	10.0	g
$CaCO_3$	20.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Add 0.25 g/l ferric ammonium citrate from a separately autoclaved stock solution.

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588. HALOBACTEROIDES HALOBIUS MEDIUM

NaCl	87.8000	g
$MgCl_2$ x 6 H_2O	20.3000	g
$CaCl_2$ x 2 H_2O	7.3500	g
KCl	3.7000	g
Yeast extract	5.0000	g
Cysteine-HCl x H_2O	0.5000	g
Resazurin	0.0001	g
Glucose	5.0000	g
PIPES	12.0960	g
Distilled water	1000.0000	ml

pH 6.5

Prepare salts (in 850 ml distilled water, do not adjust the pH), glucose (50 ml of a 10% solution), and PIPES (100 ml of 400 mM solution, 12.096 g/100 ml), separately under nitrogen using normal anaerobic techniques. Add glucose and PIPES using anaerobic techniques.

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589. HALOBACTERIUM LACUSPROFUNDII MEDIUM

NaCl	181.0	g
$MgCl_2$ x 6 H_2O	75.0	g
$MgSO_4$ x 7 H_2O	7.4	g
KCl	7.4	g
$CaCl_2$ x 2 H_2O	1.0	g
Yeast extract	1.0	g
Na-succinate	10.0	g
Vitamin solution	1.0	ml
Distilled water	1000.0	ml

pH 7.4

Vitamin solution:

Biotin	10.0	mg
Vitamin B_{12}	10.0	mg
Thiamine-HCl x 2 H_2O	10.0	mg
Distilled water	100.0	ml

Filter-sterilize and add to the medium when it has cooled to about 55 - 60°C. Add agar at a concentration of 15 g/l for solid media.

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590. DICHOTOMICROBIUM THERMOHALOPHILUM MEDIUM

Bacto Yeast extract (Difco)	1.000	g
Na_2-DL-malate	1.000	g
Hutner's basal salts	20.000	ml
Artifical sea water, 3 times conc.	980.000	ml

pH 7.0-7.2

Agar at a concentration of 18 g/l is added for solid media.

Prepare an 8% solution of $NaHCO_3$ in half full 100ml serum bottles closed with a rubber septum and autoclave. Add 28.4 ml of the sterile 8% solution of $NaHCO_3$ (2.822g/l) per litre of the sterile, cooled medium.

Artificial sea water, 3 times concentrated:

NaCl	70.430	g
Na_2SO_4	11.750	g
KCl	1.990	g
KBr	0.290	g
$MgCl_2$ x 6 H_2O	31.860	g
$CaCl_2$ x 2 H_2O	4.350	g
H_3BO_3	0.080	g
Distilled water	1000.000	ml

Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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591. HALOBACTEROIDES/HALOINCOLA MEDIUM

NH_4Cl	0.33	g
$CaCl_2$	0.33	g
$MgCl_2$	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
NaCl	100 - 150.00	g
$NaHCO_3$	1.50	g
Na_2S x 9 H_2O	0.50	g
Glucose	5.00	g
Peptone (Bacto Proteose Peptone No.3)	5.00	g
Trace elements (SL 10, see medium 320)	1.00	ml
Vitamin solution (see medium 141)	10.00	ml
Resazurin	2.00	mg
Distilled water	990.00	ml

Prepare the medium without $NaHCO_3$, Na_2S, glucose and vitamins, boil and cool under a nitrogen:carbon dioxide (80:20 v/v) atmosphere. Use 100.0 g/l NaCl for DSM 6641, DSM 6642, DSM 6638, DSM 6639, DSM 6643, and DSM 7212 and 150.0 g/l for DSM 7379. Glucose (filter-sterilized and stored under nitrogen), vitamins (filter sterilized and stored under nitrogen), and sodium sulphide (autoclaved under nitrogen) are added from sterile stock solutions. The pH should be pH 7.5.

For DSM 12596 use 100 g/l NaCl, replace the peptone with 0.5 g/l yeast extract (Oxoid)and adjust the pH to 6.6.

For DSM 6640 use 150 g/l NaCl, replace the peptone with 0.5 g/l yeast extract (Difco) and adjust the pH to 7.5.

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592. THERMOMICROBIUM ROSEUM MEDIUM

Yeast extract	1.000	g
Tryptone (BD 211705)	1.000	g
$(NH_4)_2SO_4$ (sublimed)	1.300	g
$MgSO_4$ x 7 H_2O	0.247	g
KH_2PO_4	0.280	g
$CaCl_2$ x 2 H_2O	0.074	g
$FeCl_3$ x 6 H_2O	0.019	g
Trace elements (see below)	1.000	ml
Distilled water	999.000	ml

Adjust pH to 8.5.
Add 20.0 g/l agar for solid media and pour short slants with a long butt in screw-capped tubes.

Trace elements:

$MnCl_2$ x 4 H_2O	1.800	g
$Na_2B_4O_7$ x 10 H_2O	4.400	g
$ZnSO_4$ x 7 H_2O	0.220	g
$CuCl_2$ x H_2O	0.050	g
Na_2MoO_4 x 2 H_2O	0.030	g
$VOSO_4$ x 2 H_2O	0.030	g
Distilled water	100.000	ml

pH adjusted to 2.0 with H_2SO_4.

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593. 10% MH MEDIUM

NaCl	81.000	g
$MgCl_2$	7.000	g
$MgSO_4$	9.600	g
$CaCl_2$	0.360	g
KCl	2.000	g
$NaHCO_3$	0.060	g
NaBr	0.026	g
Proteose peptone no. 3 (Difco)	5.000	g
Yeast extract (Difco)	10.000	g
Glucose	1.000	g
Distilled water	1000.000	ml

Final pH 7.5
$NaHCO_3$ is added from a sterilized stock solution when the medium has cooled. Agar is added at a concentration of 15.0 g/l for solid media.

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595. CAULOBACTER MEDIUM

Bacto peptone	2.0	g
Yeast extract	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Tap water	1000.0	ml

Add 15.0 g/l agar for solid medium.

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596. BLASTOCOCCUS AGGREGATUS MEDIUM

KNO_3	0.50	g
Na-glycerophosphate	0.10	g
Tris HCl	1.00	g
Tryptone (Difco 0123)	2.00	g
Yeast extract	2.00	g
Trace elements (see below)	1.00	ml
Artificial sea water	999.00	ml

pH 7.0

Artificial sea water is prepared from a marine aquarium salts mixture.

Trace elements:

H_3BO_3	2.85	g
$MnCl_2$ x 4 H_2O	1.80	g
$FeSO_4$	1.36	g
Na-tartrate	1.77	g
$CuCl_2$ x 2 H_2O	26.90	mg
$ZnCl_2$	20.80	mg
$CoCl_2$ x 6 H_2O	40.40	mg
Na_2MoO_4 x 2 H_2O	25.20	mg
Distilled water	1000.00	ml

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597: SALINIVIBRIO COSTICOLA SUBSP. VALLISMORTIS MEDIUM

$(NH_4)Cl$	1.00	g
NaCl	25.00	g
$MgCl_2$ x 6 H_2O	7.00	g
$MgSO_4$ x 7 H_2O	9.60	g
$CaCl_2$ x 2 H_2O	0.50	g
KCl	3.80	g
K_2HPO_4 x 3 H_2O	0.40	g
Yeast extract	1.00	g
Glucose	5.00	g
Modified Wolin's mineral solution II	10.00	ml
Distilled water	1000.00	ml

1. Final pH 7.0.

2. 3 g/l $NaHCO_3$ are added from a filter-sterilized stock solution when the medium has cooled.

Modified Wolin's mineral solution II (from medium 700)

Nitrilotriacetic acid	1.500	g
$MgSO_4$ x 7 H_2O	3.000	g
$MnSO_4$ x H_2O	0.500	g
NaCl	1.000	g
$FeSO_4$ x 7 H_2O	0.100	g
$CoSO_4$ x 7 H_2O	0.180	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnSO_4$ x 7 H_2O	0.180	g
$CuSO_4$ x 5 H_2O	0.010	g
$AlK(SO_4)_2$ x 12 H_2O	0.020	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.010	g
$NiCl_2$ x 6 H_2O	0.025	g
Na_2SeO_3 x 5 H_2O	0.300	mg
Distilled water	1000.000	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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598. HALOVIBRIO VARIABILIS MEDIUM

NaCl	95.00	g
$MgSO_4$ x 7 H_2O	81.00	g
KCl	1.00	g
Yeast extract	7.50	g
Proteose peptone	2.50	g
Trace element sol. SL-4 (see medium 14)	10.00	ml
Vitamin solution (see below)	10.00	ml
Distilled water	980.00	ml
pH 7.5		

Vitamin solution:

Biotin	0.20	mg
Folic acid	0.20	mg
Pyridoxine-HCl	1.00	mg
Thiamine-HCl x 2 H_2O	0.50	mg
Riboflavin	0.50	mg
Nicotinic acid	0.50	mg
Pantothenic acid	0.50	mg
Vitamin B_{12}	0.01	mg
p-Aminobenzoic acid	0.50	mg
Lipoic acid	0.50	mg
Distilled water	1000.00	ml

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600. M14 (PIRELLULA MARINA) MEDIUM

Yeast extract	1.0	g
Glucose	1.0	g
Vitamin solution	10.0	ml
Hutner's basal salts (see medium 590)	20.0	ml
0.1M Tris/HCl buffer pH 7.5	50.0	ml
Artifical sea water	250.0	ml
Distilled water	680.0	ml

Vitamin solution:

Vitamin B_{12}	0.1	mg
Biotin	2.0	mg
Thiamine-HCl x 2 H_2O	5.0	mg
Ca-pantothenate	5.0	mg
Folic acid	2.0	mg
Riboflavin	5.0	mg
Nicotinamide	9.0	mg
Distilled water	1000.0	ml

Filter-sterilized.

Artifical sea water:

NaCl	23.47	g
Na_2SO_4	3.92	g
$MgCl_2$ x 6 H_2O	10.64	g
$CaCl_2$	1.10	g
$NaHCO_3$	192.0	mg
KCl	664.0	mg
KBr	96.0	mg
H_3BO_3	26.0	mg
$SrCl_2$	24.0	mg
NaF	3.0	mg
Distilled water	1000.0	ml

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601. MARINE CAULOBACTER MEDIUM

Yeast extract	3.0	g
Proteose peptone (Difco 0120)	10.0	g
Artificial sea water	1000.0	ml

pH 7.2-7.4

Artificial sea water is prepared from marine aquarium salt mixtures.

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602. HALOMONAS SUBGLACIESCOLA (ARTIFICAL ORGANIC LAKE MEDIUM)

NaCl	80.0	g
$MgSO_4$ x 7 H_2O	9.5	g
KCl	0.5	g
$CaCl_2$ x 2 H_2O	0.2	g
$(NH_4)_2SO_4$	0.1	g
KNO_3	0.1	g
Yeast extract	1.0	g
Distilled water	960.0	ml
pH 8.0		

Autoclave and add, on cooling 20.0 ml sterile Hutner's salts (see medium 590), 1.0 ml vitamin solution, and 20.0 ml sterile phosphate supplement.

Vitamin solution:

Vitamin B_{12}	10.0	mg
Biotin	2.0	mg
Thiamine-HCl x 2 H_2O	10.0	mg
Ca-pantothenate	5.0	mg
Folic acid	2.0	mg
Nicotinamide	5.0	mg
Pyridoxine-HCl	10.0	mg
Distilled water	1000.0	ml

Filter-sterilized.

Phosphate supplement:

K_2HPO_4	50.0	mg
KH_2PO_4	50.0	mg
Distilled water	20.0	ml

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603. ANCALOMICROBIUM MEDIUM

$(NH_4)_2SO_4$	0.250	g
Glucose	0.250	g
Vitamin solution (see below)	10.000	ml
Hutner's basal salts (see below)	20.000	ml
Na_2HPO_4	0.071	g
Distilled water	980.000	ml
pH 7		

Make up the medium without the vitamin solution and autoclave it. The filter-sterilized vitamin solution is added to the cooled medium. The medium may be solidified by adding 15 g/l agar. For DSM 3698, DSM 3699, DSM 5900 and DSM 5901 add 0.1 g/l yeast extract.

Vitamin solution:

Vitamin B_{12}	0.100	mg
Biotin	2.000	mg
Thiamine-HCl x 2 H_2O	5.000	mg
Ca-pantothenate	5.000	mg
Folic acid	2.000	mg
Riboflavin	5.000	mg
Nicotinamide	5.000	mg
Distilled water	1000.000	ml

Filter-sterilized

Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44" (see below)	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

(continued on next page)

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"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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605. NUTRIENT AGAR (OXOID CM3)

Lab-Lemco beef extract	1.0	g
Yeast extract	2.0	g
Peptone	5.0	g
NaCl	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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606. COLBY AND ZATHMAN MEDIUM

K_2HPO_4	1.20	g
KH_2PO_4	0.62	g
$CaCl_2$ x 6 H_2O	0.05	g
$MgSO_4$ x 7 H_2O	0.20	g
NaCl	0.10	g
$(NH_4)_2SO_4$	0.50	g
Trace element solution (see below)	1.00	ml
Purified agar	15.00	g
Methanol	2.00	ml
Distilled water	1000.00	ml

Adjust pH to 7.0. Autoclave at 121°C for 15 min. Cool to 50°C. Add a filter-sterilized solution of methanol to give a final concentration of 0.2%.

For DSM 15625 replace the methanol with a filter-sterilized solution of trimethylammonium chloride to give a final concentration of 0.1%.

Trace elements solution

$CuSO_4$ x 5 H_2O	5.0	mg
$MnSO_4$ x 5 H_2O	10.0	mg
Na_2MoO_4 x 2 H_2O	10.0	mg
H_3BO_3	10.0	mg
$ZnSO_4$ x 7 H_2O	70.0	mg
$CoCl_2$ x 6 H_2O	5.0	mg
$FeCl_3$ x 6 H_2O	1.0	g
Distilled water	1000.0	ml

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607. M13 VERRUCOMICROBIUM MEDIUM

Peptone	0.250	g
Yeast extract	0.250	g
Glucose	0.250	g
Vitamin solution (see medium 603)	10.000	ml
Hutner's basal salts (see medium 590)	20.000	ml
0.1M Tris/HCl at pH 7.5	50.000	ml
Artificial sea water (see below)	250.000	ml
Distilled water	670.000	ml

Autoclave at 121°C for 15 min.

Artifical sea water:

NaCl	23.477	g
Na_2SO_4	3.917	g
$MgCl_2$ x 6 H_2O	4.981	g
$CaCl_2$	1.102	g
$NaHCO_3$	192.000	mg
KCl	664.000	mg
KBr	6.000	mg
H_3BO_3	26.000	mg
$SrCl_2$	24.000	mg
NaF	3.000	mg
Distilled water	1000.000	ml

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610. BACILLUS THERMOALCALOPHILUS MEDIUM

Na-acetate	3.00	g
KCl	1.80	g
Na_2SO_4	0.40	g
$MgSO_4$	0.20	g
KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
$FeSO_4$	0.01	g
Yeast extract	10.00	g
Distilled water	1000.00	ml
pH 8.2		

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611: PYROBACULUM (BS) MEDIUM

Synthetic seawater mix	125.00	ml
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.01% w/v)	2.00	ml
NH_4Cl	0.25	g
KH_2PO_4	0.07	g
Sodium resazurin (0.1% w/v)	0.50	ml
KNO_3	1.00	g
Yeast extract	0.50	g
$NaHCO_3$	2.00	g
Distilled water	875.00	ml

Dissolve ingredients (except nitrate, yeast extract and bicarbonate) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Adjust pH to 7.0 and dispense under 100% N_2 gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle). Replace gas phase with 80% H_2 and 20% CO_2 gas mixture and autoclave. Prior to inoculation add nitrate and yeast extract from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.0 - 7.2.

Synthetic seawater mix

NaCl	47.15	g
$MgCl_2$ x 6 H_2O	18.10	g
$MgSO_4$ x 7 H_2O	7.00	g
$CaCl_2$ x 2 H_2O	3.13	g
Na_2SO_4	3.24	g
KCl	1.20	g
Na_2CO_3	0.10	g
NaBr	0.10	g
KBr	80.00	mg
SrCl2 x 6 H_2O	72.00	mg
H_3BO_3	52.00	mg
Na_2HPO_4	8.10	mg
NaF	2.40	mg
Na-silicate	0.40	mg
KI	50.00	µg
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g

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611: PYROBACULUM (BS) MEDIUM

$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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612: DESULFOSARCINA CETONICA MEDIUM

Solution A	962.00	ml
Solution B	20.00	ml
Solution C	10.00	ml
Solution D	10.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to D are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.2 – 7.4.

Solution A

KH_2PO_4	0.70	g
NH_4Cl	0.30	g
Na_2SO_4	2.80	g
$MgCl_2$ x 6 H_2O	1.70	g
$CaCl_2$ x 2 H_2O	0.05	g
NaCl	10.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	960.00	ml

Solution B

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution C

Na-butyrate	1.20	g
Distilled water	10.00	ml

Solution D

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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613: THERMOTOGA TF(C) MEDIUM

NH_4Cl	0.50	g
K_2HPO_4	1.60	g
$Na_2HPO_4 \times H_2O$	1.00	g
NaCl	7.00	g
$MgCl_2 \times 6\ H_2O$	1.40	g
$MgSO_4 \times 7\ H_2O$	1.96	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	3.00	g
$CaCl_2 \times 2\ H_2O$	0.06	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl $\times H_2O$	0.30	g
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, calcium chloride, vitamins and reducing agents), adjust pH to 6.5 - 6.8 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add glucose, calcium chloride, vitamins (sterilized by filtration), sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere.

For DSM 4359: Replace glucose with 0.50 g/l soluble starch added to the autoclaved medium from a sterile anoxic stock solution prepared under 100% N_2 gas.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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614: ACETOBACTERIUM FIMETARIUM MEDIUM

KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.52	g
$CaCl_2$ x 2 H_2O	0.22	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
Yeast extract	0.50	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
D-Fructose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.70	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, fructose and sulfide) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add vitamins, fructose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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616: ALKALITHERMOBACTER MEDIUM

Final pH: 10.0
Final volume: 1000 ml

Na_2HPO_4 x 2 H_2O	0.36	g
KCl	0.08	g
Yeast extract (OXOID)	5.00	g
Tryptone (BD Bacto)	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	5.30	g
D-Glucose	3.00	g
L-Cysteine HCl x H_2O	0.20	g
Na_2S x 9 H_2O	0.20	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, glucose, cysteine and sulfide) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonate, dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and adjust pH to 10 by adding sterile anoxic 3 N NaOH.

For DSM 8682: Reduce amount of carbonate to 4.40 g/l and adjust pH of complete medium to 9.0.

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617. MARINOMONAS VAGA MEDIUM

Beef extract (Lab Lemco)	10.0	g
Peptone	10.0	g
NaCl	30.0	g
Agar	15.0	g
Tap water	1000.0	ml

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618. TWEEN 80A MEDIUM

Casitone (Difco)	5.0	g
Yeast extract (Difco)	3.0	g
$MgSO_4$ x 7 H_2O	2.0	g
Distilled water	1000.0	ml

For solid media add 15.0 g agar. Dissolve ingredients and adjust the pH to 7.2. Sterilize by autoclaving. After sterilization add sterile Tween 80 to a final concentration of 1.0%.

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619. HYPHOMICROBIUM METHYLOVORUM MEDIUM

Methanol	10.0	ml
$(NH_4)_2HPO_4$	3.0	g
NaCl	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
$FeSO_4$ x 7 H_2O	10.0	mg
$MnSO_4$ x 1 H_2O	5.0	mg
Vitamin mixture	5.0	ml
Agar	15.0	g
Tap water	1000.0	ml

Sterilize methanol by filtration, and add to other components sterilized by autoclaving.

Vitamin mixture:

Biotin	0.2	mg
Ca-pantothenate	40.0	mg
Folic acid	0.2	mg
Inositol	200.0	mg
Niacin	40.0	mg
p-Aminobenzoic acid	20.0	mg
Pyridoxine-HCl	40.0	mg
Riboflavin	40.0	mg
Thiamine-HCl x 2 H_2O	20.0	mg
Choline	100.0	mg
Vitamin B_{12}	2.0	µg
Distilled water	1000.0	ml

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620. 2% MH MEDIUM

Glucose (filter-sterilized)	1.500	g
KH_2PO_4	2.000	g
$(NH_4)_2SO_4$	2.000	g
$MgSO_4$ x 7 H_2O	0.025	g
NaCl	0.500	g
$FeSO_4$ x 7 H_2O	2.000	mg
Distilled water	1000.000	ml
Methanol (filter-sterilized)	2.000	%
pH 7-7.5		

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621. PYGV AGAR

Mineral salt sol. ("Hutner/Cohen-Bazire")	20.00	ml
Peptone (Bacto)	0.25	g
Yeast extract (Bacto)	0.25	g
Agar (Bacto)	15.00	g
Distilled water	965.00	ml

Sterilize 20 min./121°C. After cooling to 60°C add to the medium:

Glucose sol. (2.5%, sterile-filtered)	10.00	ml
Vitamin sol. (double conc.)	5.00	ml

Adjust pH to 7.5 (the medium is only weakly buffered; one needs approx. 10 drops/l medium of 6 N KOH).

Mineral salt solution:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_2O	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
Na_2MoO_4 x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
Metall salt sol. "44" (see medium 590)	50.00	ml
Distilled water	900.00	ml

Dissolve NTA first by neutralizing with KOH, then add other salts. Adjust pH to 7.2 with KOH or H_2SO_4. Adjust volume to 1000.0 ml with distilled water.

Vitamin solution (double conc.):

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Distilled water	1000.00	ml

Store in the dark and cold (5°C).

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623. THERMOCOCCUS LITORALIS MEDIUM

Bactopeptone	5.0000	g
Yeast extract	1.0000	g
NaCl	19.4500	g
$MgCl_2$ x 6 H_2O	12.6000	g
Na_2SO_4	3.4200	g
$CaCl_2$ x 2 H_2O	2.3800	g
KCl	0.5500	g
Na_2CO_3	0.6100	g
KBr	0.0800	g
$SrCl_2$ x 6 H_2O	0.0570	g
Boric acid	0.0220	g
Na-meta-silicate	0.0040	g
NaF	0.0024	g
KNO_3	0.0016	g
Na_2HPO_4	0.0100	g
S	10.0000	g
Resazurin	0.1000	g
Distilled water	1000.0000	ml

Prepare the medium under 100% N_2 and adjust final pH to 6.5. The medium, containing sulphur, is sterilized by steaming on three consecutive days for 3 h. Reduce the medium with a sterile Na_2S stock solution to give a final concentration of 0.5 g/l.

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624. MH MEDIUM

NaCl	60.700	g
$MgCl_2$ x 6 H_2O	15.000	g
$MgSO_4$ x 7 H_2O	7.400	g
$CaCl_2$	0.270	g
KCl	1.500	g
$NaHCO_3$	0.045	g
NaBr	0.019	g
Proteose Peptone No. 3	5.000	g
Yeast extract	10.000	g
Glucose	1.000	g
Distilled water	1000.000	ml

Adjust pH to 7.2.

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625. HP 101 HALOPHILE MEDIUM

Peptone	10.0	g
Yeast extract	1.0	g
$MgSO_4$ x 7 H_2O	4.3	g
$NaNO_3$	2.0	g
NaCl	100.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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626. 5% SORBITOL MEDIUM

D-Sorbitol	50.0	g
Yeast extract	10.0	g
Peptone	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust medium for final pH 6.0 with HCl.

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627. SEAWATER LEMCO

Beef extract	10.0	g
Peptone	10.0	g
Filtered aged sea water	750.0	ml
Distilled water	250.0	ml

Dissolve ingredients, by heating if necessary. Adjust pH to 7.8, boil 3-5 min, filter. Readjust pH to 7.3. Sterilize by autoclaving for 15 min at 121°C.
For solid medium add 15.0 g agar after readjusting pH and steam to dissolve the agar prior to autoclaving.

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628. MMB MEDIUM

Peptone	0.15	g
Yeast extract	0.15	g
Glucose	1.00	g
$(NH_4)_2SO_4$	0.25	g
Hutner's salts (see medium 590)	20.00	ml
Vitamin solution	10.00	ml
Distilled water	970.00	ml
pH 7.2		

Glucose and vitamin solution should be filter-sterilized separately.

Vitamin solution:

Vitamin B_{12}	0.01	mg
Biotin	0.20	mg
Ca-pantothenate	0.50	mg
Folic acid	0.20	mg
Nicotinamide	0.50	mg
Pyridoxine-HCl	0.50	mg
Riboflavin	0.50	mg
Thiamine-HCl x 2 H_2O	0.50	mg
Distilled water	100.00	ml

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629. STALEY'S MAINTENANCE MEDIUM

Peptone	5.0	g
Yeast extract	0.5	g
Hutner's salts (see medium 590)	20.0	ml
Staley's vitamins (filter-sterilized) (see medium 600)	10.0	ml
Distilled water	970.0	ml

Dissolve peptone and yeast extract in the distilled water, adjust the pH to 7.2 and sterilize. After the medium has cooled add the Hutner's salts and Staley's vitamins. For solid medium use 15.0 g agar.

Hutner's salts:

Nitrilotriacetic acid	10.0	g
$MgSO_4$ x 7 H_2O	29.7	g
$CaCl_2$ x 2 H_2O	3.3	g
$NaMoO_4$ x 2 H_2O	12.7	mg
$FeSO_4$ x 2 H_2O	99.0	mg
"Metals 44" (see medium 600)	50.0	ml
Distilled water	950.0	ml

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630. MODIFIED THERMUS 162 MEDIUM

Yeast extract	2.50	g
Tryptone	2.50	g
Agar	28.00	g
Nitrilotriacetic acid	100.00	mg
$CaSO_4$ x 2 H_2O	40.00	mg
$MgCl_2$ x 6 H_2O	200.00	mg
0.01 M Fe citrate	0.50	ml
Trace element solution (see below)	0.50	ml
Phosphate buffer (see below)	100.00	ml
Distilled water	900.00	ml

Adjust pH to 7.2 with NaOH. Autoclave at 121°C for 15 min. Autoclave the phosphate buffer separately and then add to the medium.

Phosphate buffer:

KH_2PO_4	5.44	g
Na_2HPO_4 x 12 H_2O	43.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2

Trace element solution:

Nitrilotriacetic acid	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.50	g
$CoCl_2$ x 4 H_2O	0.30	g
$CuCl_2$ x 2 H_2O	50.00	mg
Na_2MoO_4 x 2 H_2O	50.00	mg
H_3BO_3	20.00	mg
$NiCl_2$ x 6 H_2O	20.00	mg
Distilled water	1000.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 8 - 9 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

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631. ACETOBACTER EUROPAEUS MEDIUM

Yeast extract	2.0	g
Peptone	3.0	g
Glucose	5.0	g
Acetic acid	40.0	ml
Ethanol	30.0	ml
Distilled water	930.0	ml

The ethanol and acetic acid are sterilized separately by filtration (use filter resistant to these solvents), and added aseptically to the sterile medium. *Acetobacter europaeus* will not grow on plates containing 1-2% agar, and a double layer system has to be used. A layer of growth medium containing 0.5% agar (about 35 ml) is poured into a plate and allowed to solidify. A thin layer (5-10 ml) of the growth medium containing 1% agar is then poured on top. Carefully streak the plates, which are best incubated in closed containers (e.g. plastic boxes) at 30°C.

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632. NMS MEDIUM

Methanol	1.000	ml
$MgSO_4$ x 7 H_2O	1.000	g
$CaCl_2$ x 6 H_2O	0.200	g
Fe(III)NH_4-EDTA	4.000	mg
KNO_3	1.000	g
Trace element solution (see below)	0.500	ml
KH_2PO_4	0.272	g
Na_2HPO_4 x 12 H_2O	0.717	g
Purified agar (e.g. Oxoid L28)	12.500	g
Distilled water	1000.000	ml

Adjust pH to 6.8. Autoclave at 121°C for 15 min. Methanol is added from a sterile stock solution.

Trace element solution:

Na_2-EDTA	500.000	mg
$FeSO_4$ x 7 H_2O	200.000	mg
$ZnSO_4$ x 7 H_2O	10.000	mg
$MnCl_2$ x 4 H_2O	3.000	mg
H_3BO_3	30.000	mg
$CoCl_2$ x 6 H_2O	20.000	mg
$CaCl_2$ x 2 H_2O	1.000	mg
$NiCl_2$ x 6 H_2O	2.000	mg
Na_2MoO_4 x 2 H_2O	3.000	mg
Distilled water	1000.000	ml

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634. RCM MEDIUM

Dissolve dehydrated RCM medium (available from BD-BBL, OXOID or MERCK) in distilled water and add 0.5 ml/l Na-resazurin solution (0.1% w/v), bring to the boil and cool to room temperature while sparging with 100% N_2 gas. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

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635: GRACILINEMA MEDIUM

D-Glucose	2.00	g
Tryptone (BD BACTO)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.25	g
Trace element solution SL-10	10.00	ml
Modified Wolin's mineral solution	25.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	970.00	ml

Dissolve ingredients (except cysteine, trace elements and vitamins), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Then add cysteine, adjust pH to 7.0, dispense medium under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add trace elements and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Check pH of complete medium and adjust to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g

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$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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636. ISP MEDIUM 2 WITH 5% NaCl

Yeast extract (Difco)	4.0	g
Malt extract (Difco)	10.0	g
Dextrose (Difco)	4.0	g
Agar	20.0	g
NaCl 5%	50.0	g
Distilled water	1000.0	ml

pH 7.3 - 7.5

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638. LACTOBACILLUS MEDIUM III

Tryptone	10.00	g
Meat extract	5.00	g
Yeast extract	5.00	g
Glucose	7.00	g
Fructose	7.00	g
Maltose	7.00	g
Na-gluconate	2.00	g
Na-acetate x 3 H_2O	5.00	g
$(NH_4)_2$ citrate	2.00	g
K_2HPO_4 x 3 H_2O	2.60	g
$MgSO_4$ x 7 H_2O	0.10	g
$MnSO_4$ x 4 H_2O	0.05	g
Cysteine-HCl x H_2O	0.50	g
Tween 80	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 6.3

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640: CALDICELLULOSIRUPTOR MEDIUM

NH_4Cl	0.90	g
NaCl	0.90	g
$MgCl_2$ x 6 H_2O	0.40	g
KH_2PO_4	0.75	g
K_2HPO_4	1.50	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	1.00	g
Trace element solution SL-10	1.00	ml
$FeCl_3$ x 6 H_2O (0.1% w/v in 0.2 N HCl)	2.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.75	g
Cellobiose	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine and cellobiose. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then add cysteine and adjust pH to 7.2. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose after autoclaving from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of the complete medium to 7.2, if necessary.

For DSM 8685, DSM 8691, DSM 8692, DSM 10170: Replace cellobiose with 5.00 g/l D-xylose added to the autoclaved medium from an anoxic stock solution sterilized by filtration. Adjust pH of the complete medium to 5.2 - 5.4!

For DSM 10319, DSM 11055, DSM 11056, DSM 11057, DSM 11426, DSM 22141, DSM 101588: Replace cellobiose with 5.00 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 13528: Replace cellobiose with 5.00 g/l D-fructose added to the autoclaved medium from a sterile anoxic stock solution. Adjust pH of the complete medium to 6.0!

For DSM 16488, DSM 24455: Replace cellobiose with 2.00 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 22658: Replace cellobiose with 5.00 g/l D-fructose added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 24750: Replace cellobiose with 2.00 g/l Na-pyruvate added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg

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$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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641: DESULFOVIBRIO (MV) MEDIUM

NH_4Cl	1.00	g
Na_2SO_4	2.00	g
$Na_2S_2O_3$ x 5 H_2O	1.00	g
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KH_2PO_4	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na-DL-lactate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, lactate and sulfide), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding vitamins (sterilized by filtration), lactate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

2. Note: Prior to inoculation 10-20 mg/l sodium dithionite (added from a 5% w/v solution freshly prepared under N_2 and filter-sterilized) can be added to the medium to stimulate growth at the beginning.

For DSM 7073, DSM 101608, DSM 101609: Replace lactate with 0.9 g/l glycerol and adjust pH of complete medium to 7.5.

For DSM 10017: Replace lactate with 2.5 g/l Na-pyruvate added to the autoclaved medium from an anoxic stock solution sterilized by filtration and replace sodium sulfide with 0.15 g/l DL-dithiothreitol (DTT) added from an anoxic stock solution sterilized by filtration.

For DSM 10349: Replace lactate with 2.5 g/l Na-pyruvate added to the autoclaved medium from an anoxic stock solution sterilized by filtration and supplement medium with an additional amount of 1.0 g/l $Na_2S_2O_3$ x 5 H_2O added from an anoxic stock solution sterilized by filtration.

For DSM 13257, DSM 16036, DSM 16058, DSM 24088, DSM 24093, DSM 24523, DSM 26885: Replace lactate with 2.5 g/l Na-pyruvate added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

For DSM 18151: Replace lactate with 0.2 g/l Na-acetate. After inoculation pressurize

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culture vessels to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 21394: Replace lactate with 5.0 ml/l methanol added to the autoclaved medium from an anoxic stock solution prepared under 100% N_2 gas.

For DSM 24089, DSM 102940: Replace lactate with 3.5 g/l D-fructose added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

For DSM 25471: Replace lactate with 2.0 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 28127: Replace lactate with 0.4 g/l Na-benzoate added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 111569: Supplement medium with 2.20 g/l pyruvate, 1.20 g/l taurine and 0.20 mg/l 1,4-naphthochinone added to the autoclaved medium from anoxic stock solutions sterilized by filtration,

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg

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641: DESULFOVIBRIO (MV) MEDIUM

Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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643: SEDIMENTIBACTER MEDIUM

Sodium Potassium phosphate buffer (0.2 M, pH 7.0)	100.00	ml
NaCl	10.00	g
NH_4Cl	0.50	g
$MgCl_2 \times 7\ H_2O$	0.10	g
$CaCl_2 \times 2\ H_2O$	0.03	g
Modified Wolin's mineral solution	5.00	ml
Yeast extract	10.00	g
Glycine	0.75	g
L-Arginine x HCl	2.10	g
L-Lysine HCl	1.80	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.40	g
$Na_2S \times 9\ H_2O$	0.40	g
Distilled water	900.00	ml

Dissolve ingredients except vitamins, cysteine and sulfide, sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Check pH of complete medium and adjust to pH 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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644: CATENOCOCCUS MEDIUM

NaCl	15.00	g
K_2HPO_4	1.00	g
KH_2PO_4	0.15	g
NH_4Cl	0.50	g
Na-acetate	0.82	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
Yeast extract	0.20	g
D-Glucose	1.00	g
Agar, for solid medium	15.00	g
Distilled water	1000.00	ml

Dissolve sodium chloride, hydrogenphosphates, ammonium chloride and acetate, adjust pH of solution to 7.0 - 7.2 and autoclave. After autoclaving add trace elements, magnesium sulfate, calcium chloride, yeast extract and glucose from sterile stock solutions. Adjust pH of complete medium to 7.0, if necessary.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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645. MIDDLEBROOK MEDIUM

Bacto Middlebrook 7H10 agar (Difco 262710)	19.000	g
Glycerol	5.000	ml
Distilled water	900.000	ml

Sterilize for 15 min by 121°C cool to 50 - 55°C. Add 100 ml Middlebrook OADC enrichment (Becton Dickinson 212240) under aseptic conditions and mix thoroughly.

If you can´t order the commercial media then prepare the media in this way:

Bacto Middlebrook 7H10 agar:

$(NH_4)_2SO_4$	0.500	g
KH_2PO_4	1.500	g
Na_2HPO_4	1.500	g
Na-citrate	0.400	g
$MgSO_4$	0.025	g
$CaCl_2$	0.500	mg
$ZnSO_4$	1.000	mg
$CuSO_4$	1.000	mg
L-Glutamic acid	0.500	g
Ferric ammonium citrate	0.040	g
Pyridoxine	1.000	mg
Biotin	0.500	mg
Malachite green	0.250	mg
Bacto agar	15.000	g
Distilled water	1000.000	ml

Final pH at 25°C 6.6 ± 0.2.

Bacto Middlebrook OADC enrichment:

Oleic acid	0.050	g
Albumin fraction V, bovine	5.000	g
Dextrose	2.000	g
Catalase (beef)	4.000	mg
NaCl	0.850	g
Distilled water	100.000	ml

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647: DESULFUROMONAS THIOPHILA MEDIUM

KH_2PO_4	1.00	g
NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	5.00	g
Na_2CO_3	1.50	g
Na-pyruvate	0.60	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, carbonate, pyruvate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Sulfur is sterilized by steaming for 3 hours on each of 3 successive days (see medium 35) and added aseptically to the sterile medium while retaining anoxic conditions. Complete the medium by adding pyruvate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of pyruvate and vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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647: DESULFUROMONAS THIOPHILA MEDIUM

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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648: CLOSTRIDIUM GRANTII MEDIUM

Marine Broth (BD Difco)	18.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
D-Glucose	5.00	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve DIFCO Marine Broth powder and resazurin, bring the medium to a boil, then cool to room temperature under 80% N_2 and 20% CO_2 gas atmosphere. Dispense the medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete the medium by adding glucose, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be around 6.6.

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649: DESULFUROMONAS SUCCINOXIDANS MEDIUM

KH_2PO_4	1.00	g
NH_4Cl	0.30	g
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	2.00	g
NaCl	20.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-acetate	0.80	g
Na_2-fumarate	1.60	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, acetate, fumarate, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, fumarate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of fumarate and vitamins should be sterilized by filtration. The pH of the complete medium should be 7.0 - 7.2.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg

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649: DESULFUROMONAS SUCCINOXIDANS MEDIUM

p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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650. CENTENUM MEDIUM

K_2HPO_4	1.0	g
$MgSO_4$	0.5	g
Yeast extract	10.0	g
Vitamin B_{12}	20.0	µg
Tap water	1000.0	ml

Adjust pH to 7.0.

Boil under nitrogen gas and autoclave at 121°C for 15 min. Add 20 mM pyruvate after autoclaving.

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651. R 8 A H MEDIUM

Malic acid	2.500	g
Yeast extract (Difco 0127)	1.000	g
$(NH_4)_2SO_4$	1.250	g
$MgSO_4$ x 7 H_2O	0.200	g
$CaCl_2$ x 2 H_2O	0.070	g
Fe(III) citrate	0.010	g
Na_2-EDTA	0.020	g
KH_2PO_4	0.600	g
K_2HPO_4	0.900	g
Trace elements (see below)	1.000	ml
Vitamin solution	7.500	ml
Distilled water	1000.000	ml

Neutralize malic acid with NaOH and adjust the pH of the completed medium for a final pH to 6.9.

Trace elements:

Fe(III) citrate	0.300	g
$MnSO_4$x H_2O	0.002	g
H_3BO_3	0.001	g
$CuSO_4$ x 5 H_2O	0.001	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.002	g
$ZnSO_4$	0.001	g
EDTA	0.050	g
$CaCl_2$ x 2 H_2O	0.020	g
Distilled water	100.000	ml

Vitamin solution:

Nicotinic acid	0.200	g
Nicotinamide	0.200	g
Thiamine-HCl x 2 H_2O	0.400	g
Biotin	0.008	g
Distilled water	1000.000	ml

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652. HALOPHILE MEDIUM

NaCl	100.0	g
KCl	5.0	g
$MgCl_2$ x 6 H_2O	5.0	g
NH_4Cl	5.0	g
$MgSO_4$ x 7 H_2O	5.0	g
Yeast extract	5.0	g
Peptone (Difco)	5.0	g
Trace element solution	5.0	ml
1% Fe(III) citrate solution	5.0	ml
Distilled water	990.0	ml

Adjust pH to 7.5-8.0. Boil the medium for few minutes. Fill in tubes with rubber stoppers under nitrogen gas. Sterilize at 121°C for 15 min.

Trace element solution:

$CuSO_4$ x 5 H_2O	1.0	mg
$ZnSO_4$ x 7 H_2O	220.0	mg
$CoCl_2$ x 6 H_2O	10.0	mg
$MnCl_2$ x 4 H_2O	180.0	mg
Na_2MoO_4 x H_2O	6.3	mg
Distilled water	1000.0	ml

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653. PEPTONE-SUCCINATE AGAR

NH_4SO_4	1.00	g
$MgSO_4$ x 7 H_2O	1.00	g
$MnSO_4$ x H_2O	2.00	mg
$FeCl_3$ x 6 H_2O	2.00	mg
Succinic acid	1.68	g
Peptone	5.00	g
Agar	1.50	g
Distilled water	1000.00	ml

Adjust pH to 7.0.

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654. SPIRILLUM GRACILE MEDIUM

Peptone	5.00	g
Yeast extract	0.50	g
Tween 80	0.02	g
K_2HPO_4	0.10	g
Agar (if needed)	15.00	g
Tap water	1000.00	ml

Adjust pH to 7.2.

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655. PYE MEDIUM

K_2HPO_4	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.02	g
$Na_2S_2O_3$ x 5 H_2O	0.20	g
Na-pyruvate	2.20	g
Yeast extract (Difco)	4.00	g
$(NH_4)_2SO_4$	1.00	g
Trace element solution SL6 (see Medium 27)	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 6.8.
Boil the medium for few minutes. Fill in tubes with rubber stoppers under nitrogen gas.
Sterilize at 121°C for 15 min.

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656. YEAST-STARCH-AGAR(B)

Soluble starch	15.0	g
Yeast extract (Bacto)	4.0	g
K_2HPO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.5	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.4.

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657. COW MANURE AGAR

Suspend 50 g of dry cow-manure in one liter of tap water. Boil for one hour. Filter, first through cheesecloth and then paper. Make up volume to one liter. Add 20 g agar.

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658. HICKEY-TRESNER-AGAR

Dextrin	10.0	g
Enzymatic digest of casein	2.0	g
Meat extract	1.0	g
Yeast extract	1.0	g
$CoCl_2$ x 6 H_2O	2.0	mg
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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659. M17 MEDIUM (modified)

Casein peptone	5.00	g
Soya peptone	5.00	g
Meat peptone	5.00	g
Yeast extract	2.50	g
Ascorbic acid	0.50	g
$MgSO_4$ x 7 H_2O	0.25	g
Na_2-ß-glycerolphosphate	9.50	g
Distilled water	1000.00	ml

After autoclaving add a lactose solution (sterilized by filtration) up to a concentration of 8.0 g/l, and 1.2 ml 1M $CaCl_2$ x 2 H_2O. Adjust pH to 7.15 ± 0.05.

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660. ALCALIGENES MEDIUM

Tris	6.060	g
NaCl	4.680	g
KCl	1.490	g
NH_4Cl	1.070	g
Na_2SO_4	0.430	g
$MgCl_2$ x 6 H_2O	0.200	g
$CaCl_2$ x 2 H_2O	0.030	g
Na_2HPO_4 x 12 H_2O	0.230	g
Fe(III)(NH_4)citrate	0.005	g
Trace element solution SL 7	1.000	ml
Distilled water	988.500	ml

Trace element solution SL-7:

HCl (25%)	1.000	ml
$ZnCl_2$	70.000	mg
$MnCl_2$ x 4 H_2O	100.000	mg
H_3BO_3	60.000	mg
$CoCl_2$ x 6 H_2O	200.000	mg
$CuCl_2$ x 2 H_2O	20.000	mg
$NiCl_2$ x 6 H_2O	20.000	mg
Na_2MoO_4 x 2 H_2O	40.000	mg
Distilled water	1000.000	ml

After autoclaving add 2.5 ml 1.0 M $CuSO_4$ and 10 ml of a 40% Na-succinate solution, both sterilized by filtration.

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661. DP MEDIUM

Tryptone	10.0	g
Yeast extract	10.0	g
Glucose	5.0	g
KH_2PO_4	3.0	g
K_2HPO_4	7.0	g
Na_3-citrate x 3 H_2O	0.5	g
$MgSO_4$ x 7 H_2O	0.1	g
Agar	2.0	%(w/v)
Distilled water	1000.0	ml

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662. YEPG MEDIUM

Glucose	1.0	g
Polypeptone	2.0	g
Yeast extract	0.2	g
NH_4NO_3	0.2	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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663: DESULFITOBACTERIUM MEDIUM

KH_2PO_4	5.44	g
$MgCl_2$ x 2 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.03	g
NH_4Cl	0.50	g
Trace element solution	5.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-pyruvate	2.50	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
Wolin's vitamin solution (10x)	2.00	ml
L-Cysteine HCl x H_2O	0.40	g
Na_2S x 9 H_2O	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except pyruvate, thiosulfate, vitamins, cysteine and sulfide), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, thiosulfate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of pyruvate and vitamins are sterilized by filtration. Adjust medium pH to 7.5 by adding sterile anoxic 1 N NaOH.

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	0.20	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.17	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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664: PSEUDOTHERMOTOGA ELFII MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	10.00	g
Modified Wolin's mineral solution	10.00	ml
Na-acetate	0.50	g
Yeast extract (OXOID)	5.00	g
Trypticase peptone (BD BBL)	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
D-Glucose	4.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, thiosulfate, glucose and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate, glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The pH of the complete medium should be adjusted to 7.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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665. SULFOBACILLUS MEDIUM

Solution A:

$(NH_4)_2SO_4$	3.00	g
KCl	0.10	g
K_2HPO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$Ca(NO_3)_2$	0.01	g
Distilled water	700.00	ml

Adjust pH to 2.0 - 2.2 with sulfuric acid.

Solution B:

$FeSO_4$ x 7 H_2O	44.20	g
Distilled water	300.00	ml
H_2SO_4, 10 N	1.00	ml

(sterilize by filtration or handle under N2 before autoclaving to prevent oxidation of the iron)

Solution C:

Yeast extract (1% w/v in water)	20.00	ml

After autoclaving, combine the three solutions. Medium pH 1.9 - 2.4.

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666: SUCCINICLASTICUM MEDIUM

Clarified rumen fluid	400.00	ml
K_2HPO_4	0.23	g
KH_2PO_4	0.23	g
NaCl	0.45	g
$(NH_4)_2SO_4$	0.45	g
$CaCl_2$ x 2 H_2O	0.06	g
$MgSO_4$ x 7 H_2O	0.09	g
Indigocarmine	5.00	mg
$NaHCO_3$	6.40	g
Disodium succinate	5.00	g
Yeast extract	5.00	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	600.00	ml

1. Dissolve ingredients (except bicarbonate, succinate, yeast extract, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 100% CO_2 gas atmosphere. Add the bicarbonate and equilibrate the medium with the CO_2 gas to pH 6.8. Distribute under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add succinate, yeast extract, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

2. Note: Supplementing the medium with 1.50 g/l agar stimulates growth of strains after resuscitation from ampoules.

For DSM 10503, DSM 10504, DSM 10505: Reduce amount of yeast extract to 2.00 g/l.

For DSM 11001, DSM 11004, DSM 11016: Omit yeast extract and succinate and supplement medium with 5.00 g/l each of Trypticase peptone and Na-(DL)-lactate.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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667: DESULFALLAS THERMOSAPOVORANS MEDIUM

Solution A	973.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	5.00	ml
Solution E	1.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution D is autoclaved under 100% N_2 gas. Solution E is prepared under 80% N_2 and 20% CO_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.0 - 7.2.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	1.00	g
NaCl	8.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.50	ml
Selenite-tungstate solution	1.00	ml
Na-acetate	0.50	g
Na-butyrate	2.20	g
Yeast extract	0.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	970.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

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Solution D

Na_2S x 9 H_2O	0.20	g
Distilled water	5.00	ml

Solution E

Na-dithionite solution (5% w/v)	1.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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Na-dithionite solution (5% w/v) (from medium 829)

$NaHCO_3$	50.00	g
$Na_2S_2O_4$	50.00	g
Distilled water	1000.00	ml

Dissolve $NaHCO_3$ in water and make the solution anoxic by sparging with 80% N_2 and 20% CO_2 gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.

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668. FLEXIBACTER MEDIUM

Tryptone (Difco 0123)	1.00	g
Vitamin-free casamino acids	1.00	g
Na-glutamate	5.00	g
Na-glycerophosphate	0.10	g
Vitamin B_{12}	1.00	µg
Ho-le trace element solution (see below)	1.00	ml
Agar (if needed)	15.00	g
Filtered sea water to	1000.00	ml

Trace element solution Ho-le:

H_3BO_3	2.85	g
$MnCl_2$ x 4 H_2O	1.80	g
$FeSO_4$	1.36	g
Na-tartrate	1.77	g
$CuCl_2$ x 2 H_2O	26.90	mg
$ZnCl_2$	20.80	mg
$CoCl_2$ x 6 H_2O	40.40	mg
Na_2MoO_4 x 2 H_2O	25.20	mg
Distilled water	1000.00	ml

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669. CYTOPHAGA MEDIUM

Yeast extract	10.0	g
NH_4Cl	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
K_2HPO_4	0.2	g
$FeCl_3$	1.0	mg
NaCl	20.0	g
Agar	3.0	g
Tap water	1000.0	ml

Adjust pH to 7.5.

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670: ACIDIFERROBACTER (MS) MEDIUM

$(NH_4)_2SO_4$	2.00	g
$MgSO_4$ x 7 H_2O	0.25	g
K_2HPO_4	0.10	g
KCl	0.10	g
$FeSO_4$ x 7 H_2O (40% w/v in 0.2 N H_2SO_4)	50.00	ml
Distilled water	950.00	ml

1. Dissolve ingredients except ferrous sulfate and adjust pH to 2.2 with 4 N H_2SO_4. Sterilize medium by autoclaving for 15 min at 121°C. Before use, add $FeSO_4$ x 7 H_2O from a 40% (w/v) stock solution prepared with 0.2 N H_2SO_4 and filter-sterilized under 100% N_2 gas atmosphere.

2. Note: Incubate statically without shaking.

For DSM 9463: Adjust pH to 3.5 with 1 N H_2SO_4 prior to autoclaving and omit $FeSO_4$ x 7 H_2O from the medium. Before use, add 5.00 g/l of powdered sulfur sterilized by steaming in a boiling water bath for 3 hours on each of 3 successive days.

For DSM 9466: Adjust pH to 3.0 with 1 N H_2SO_4 prior to autoclaving and omit $FeSO_4$ x 7 H_2O from the medium. Before use, add 5.00 g/l of powdered sulfur sterilized by steaming in a boiling water bath for 3 hours on each of 3 successive days and 0.20 g/l of yeast extract (from a sterile stock solution).

For DSM 9467: Adjust pH to 3.0 with 1 N H_2SO_4 prior to autoclaving and omit $FeSO_4$ x 7 H_2O from the medium. Before use, add 2.00 g/l D-glucose and 0.1 g/l yeast extract from filter-sterilized stock solutions.

For DSM 9468: Adjust pH to 1.6 with 4 N H_2SO_4 prior to autoclaving.

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671: THERMOANAEROBACTER (BA) MEDIUM

NH_4Cl	1.00	g
NaCl	0.10	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.05	g
K_2HPO_4 x 3 H_2O	0.40	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.75	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Cellobiose	4.00	g
Cellulose, Avicel SIGMA (optional)	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, cellobiose, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cellobiose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of cellobiose and vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

2. Note: Some strains can be adapted to cellulose as substrate using 2.00 g/l Avicel microcrystalline cellulose (SIGMA).

For DSM 29083, DSM 25963: Use 4.00 g/l D-xylose (sterilized separately) instead of cellobiose.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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672. HALF STRENGTH NUTRIENT BROTH OR AGAR

Difco 0003 or Difco 0001

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673. THERMOCOCCUS PROFUNDUS MEDIUM

Prepare medium 266 with the following modification: reduce the yeast extract concentration to 1 g/l, add 5 g/l peptone and reduce the NaCl concentration to 25 g/l. Prepare the medium under N_2 as described in medium 266. The final pH should be adjusted to pH 7.2.

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674. MEDIUM FOR ACIDURIC, THERMOPHILIC BACILLUS STRAINS

Solution A:

$(NH_4)_2SO_4$	0.20	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
KH_2PO_4	3.00	g
Yeast extract	1.00	g
Tryptone (Bacto)	1.00	g
Glucose	1.00	g
Starch	1.00	g
Distilled water	500.00	ml

Adjust pH to 4.3.

Solution B:

Agar	20.00	g
Distilled water	500.00	ml

Solutions A and B are sterilized separately. After cooling to approximately 45°C solution A is poured into solution B. Mix thoroughly.

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675. BACILLUS THERMANTARCTICUS MEDIUM

Yeast extract	6.0	g
NaCl	3.0	g
Soil extract (see medium 80)	500.0	ml
Distilled water	500.0	ml

Adjust pH between 5.6 - 5.8.

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677: CLOSTRIDIUM (P-2) MEDIUM

K_2HPO_4	3.00	g
KH_2PO_4	2.00	g
NH_4Cl	2.00	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.05	g
Yeast extract	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	5.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except glucose, cysteine and sulfide. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

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678: COPROTHERMOBACTER (CP) MEDIUM

NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.40	g
K_2HPO_4 x 3 H_2O	0.40	g
Modified Wolin's mineral solution	10.00	ml
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	8.40	g
Gelatine	3.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, gelatine and sulfide), then sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and equilibrate the medium with the CO_2 gas to achieve a pH of 7.0. Dispense the medium under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add gelatine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas. Prior to inoculation check pH of complete medium and adjust to 7.0, if necessary.

For DSM 9219: Use 5.0 g/l of D-glucose as the substrate replacing gelatine.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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680. TRYPTONE YEAST EXTRACT AGAR (TYG)

Tryptone	3.0	g
Yeast extract	5.0	g
Glucose	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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681. TGA-MEDIUM

Tryptone (Bacto)	10.0	g
Glucose	2.0	g
NaCl	5.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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683: TISSIERELLA (AMO.1) MEDIUM

NaCl	5.80	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.27	g
KH_2PO_4	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract (OXOID)	10.00	g
Trypticase peptone (BD BBL)	0.50	g
L-Serine	2.00	g
L-Threonine	2.00	g
N-methylhydantoin (SIGMA)	5.64	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, cysteine and vitamins) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Adjust pH to 8.3 with 10 N NaOH, add bicarbonate and cysteine and dissolve. Switch gas phase to 80% N_2 and 20% CO_2 gas mixture and flush only the head space of the medium vessel. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave 15 min at 121°C. Add vitamins to the autoclaved medium from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 8.3, if necessary.

2. Note: If creatinine is used as the substrate replacing N-methylhydantoin, prepare separately an anoxic, filter sterilized stock solution of creatinine under 100% N_2 gas atmosphere.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg

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Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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684: SYNTROPHOBACTER (MPOB) MEDIUM

Na_2HPO_4 x 2 H_2O	0.53	g
KH_2PO_4	0.41	g
NH_4Cl	0.30	g
$CaCl_2$ x 2 H_2O	0.11	g
$MgCl_2$ x 6 H_2O	0.10	g
NaCl	0.30	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na_2-fumarate	3.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, fumarate and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding vitamins, fumarate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of vitamins and fumarate are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2. After inoculation pressurize culture vials to 0.7 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture.

2. Note: A white precipitate forms in this medium after autoclaving, which has however no negative effect on growth.

For DSM 14055: Replace Na_2-fumarate with 2.50 g/l Na-pyruvate added after autoclaving from a sterile anoxic solution prepared under 100% N_2 gas atmosphere.

For DSM 14823: Replace Na_2-fumarate with 5.00 g/l glucose. Sterilize glucose separately by filtration under 100% N_2 gas atmosphere. Reduce amount of Na_2CO_3 to 1.00 g/l and adjust pH to 6.5 - 6.8.

For DSM 17232: Replace Na_2-fumarate with 2.00 ml/l methanol added after autoclaving from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere.

For DSM 23957: Replace Na_2-fumarate with glycerol 2.3 g/L; anaerobic

For DSM 27333: Replace Na_2-fumarate with 2.30 g/l glycerol added after autoclaving from a sterile anoxic solution prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 7.5.

For DSM 29728: Adjust pH of complete medium to 6.8.

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For DSM 103119: Replace Na_2-fumarate with 3.2 g/l sucrose added after autoclaving from a sterile anoxic solution prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 7.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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685: ANAEROBRANCA MEDIUM

KH_2PO_4	0.50	g
Na_2HPO_4 x 2 H_2O	3.90	g
KCl	0.50	g
Yeast extract	5.00	g
Modified Wolin's mineral solution	5.00	ml
Na_2-fumarate	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.15	g
Na_2S x 9 H_2O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except fumarate, vitamins, cysteine and sulfide), adjust pH to 8.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add fumarate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of fumarate and vitamins are sterilized by filtration. Adjust pH of complete medium to 8.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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688: PSEUDOTHERMOTOGA SUBTERRANEA MEDIUM

NaCl	12.00	g
$MgSO_4$ x 7 H_2O	0.50	g
PIPES (SIGMA)	3.40	g
KCl	2.00	g
NH_4Cl	0.10	g
$CaCl_2$ x 2 H_2O	0.03	g
K_2HPO_4	0.02	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.50	g
Peptone (meat, peptic)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins and sulfide), adjust pH to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add sulfide from a sterile anoxic stock solution autoclaved under100% N_2 gas. Vitamins are prepared under 100 N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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689: ACE MEDIUM

KCl	0.33	g
$MgCl_2$ x 6 H_2O	6.00	g
$MgSO_4$ x 7 H_2O	1.00	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Yeast extract (Difco)	1.00	g
Resazurin	1.00	mg
L-Cysteine HCl x H_2O	0.50	g
$NaHCO_3$	2.00	g
Glucose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, bicarbonate, glucose and vitamins, then sparge medium with 100% N_2 gas to make it anoxic. Add cysteine and sodium bicarbonate, then adjust pH to 7.5. Dispense medium in anoxic Hungate-type tubes under 100% N_2 gas and autoclave. A precipitate forms which will redissolve slowly over about 3 days. Before use inject separately sterile glucose solution (20% w/v) and vitamins prepared under 100% N_2 gas atmosphere. Vitamins should be sterilized by filtration. Check medium pH and adjust pH to 7.6, if necessary.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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690: DMA MEDIUM

NH_4Cl	0.50	g
KH_2PO_4	0.85	g
K_2HPO_4	0.80	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$MgSO_4$ x 7 H_2O	0.25	g
$NaHCO_3$	3.00	g
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except magnesium sulfate, bicarbonate, glucose, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add magnesium sulfate, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust

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final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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691: CreDm1 MEDIUM

KH_2PO_4	1.40	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$NaHCO_3$	1.50	g
Na_2-DL-malate	1.60	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except vitamins, bicarbonate and malate. Adjust pH to 6.5, dispense medium in culture vessels and autoclave. Prior to inoculation add vitamins, bicarbonate and malate from stock solutions sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.9.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg

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691: CreDm1 MEDIUM

Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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692: METHANOSALSUM WeN5 MEDIUM

NaCl	15.00	g
$NaHCO_3$	10.00	g
KH_2PO_4	0.30	g
NH_4Cl	0.50	g
Modified Wolin's mineral solution	10.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
Methanol (50% v/v)	10.00	ml
Na_2S x 9 H_2O	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, methanol and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonate and adjust pH to 9.0 - 9.2, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add methanol (50% v/v solution) and sulfide from anoxic stock solutions autoclaved under 100% N_2 gas. Adjust pH of complete medium to 9.2 - 9.4.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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693. COLUMBIA BLOOD MEDIUM

Columbia agar base supplemented with 5% defibrinated sheep blood. Rehydrate pellets of freeze-dried ampoules in liquid medium 545, 215 or 1 (see below).

ATTENTION: organisms, for which only M 693 is mentioned in the catalogue entry, **do not grow** in one of the media listed below! Those are meant to rehydrate the freeze-dried pellets, not for cultivation.

545. TRYPTONE SOYA BROTH (TSB)

Peptone from casein	17.0	g
Peptone from soymeal	3.0	g
D(+)-Glucose	2.5	g
NaCl	5.0	g
K_2HPO_4	2.5	g
Distilled water	1000.0	ml

Adjust pH to 7.3.

The same as Caso Bouillon (Merck or Oxoid, Germany).

215. BHI MEDIUM

Brain Heart Infusion (Difco)	37.0	g
Distilled water	1000.0	ml

1. NUTRIENT BROTH

Peptone	5.0	g
Meat extract	3.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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694. MEDIUM E-2

$NaNH_4HPO_4$ x 4 H_2O	3.500	g
K_2HPO_4 x 3 H_2O	7.500	g
KH_2PO_4	3.700	g
$MgSO_4$ x 7 H_2O (100 mM)	10.000	ml
Thiamine	0.001	%
Tap water	1000.000	ml

Adjust pH to 7.0. n-Octane is evaporated in a desiccator as sole source of carbon.

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695. MEDIUM FOR ERYTHROBACTER LONGUS

Peptone (BBL 11910)	2.0	g
Soytone (Difco)	1.0	g
Yeast extract	1.0	g
Proteose peptone no.3 (Difco)	1.0	g
5% Fe(III) citrate	2.0	ml
Artificial seawater	700.0	ml
Distilled water	300.0	ml
pH 7.5		

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696. STREPTOCOCCUS SUIS MEDIUM

Peptone	10.00	g
Meat extract	8.00	g
Yeast extract	3.00	g
KH_2PO_4	2.50	g
K_2HPO_4	2.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$MnSO_4$ x 4 H_2O	0.05	g
Glucose	5.00	g
Lactose	5.00	g
Cysteine-HCl x H_2O	0.50	g
Distilled water	1000.00	ml
pH 6.8		

After sterilization add 5% bovine serum.

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697. TODD-HEWITT MEDIUM

Meat infusion	10.0	g
Casein peptone	20.0	g
Dextrose	2.0	g
$NaHCO_3$	2.0	g
NaCl	2.0	g
Na_2HPO_4	0.4	g
Distilled water	1000.0	ml
pH 7.8		

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698. DOEPEL MEDIUM

Glucose	5.0	g
Casein hydrolysate	8.0	g
Yeast extract	8.0	g
K_2HPO_4	2.0	g
$MgSO_4$	0.3	g
Distilled water	1000.0	ml
pH 7.2		

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699. TRIPLE-SUGAR-IRON MEDIUM (Difco)

Bacto beef extract	3.000	g
Bacto yeast extract	3.000	g
Bacto peptone	15.000	g
Dextrose	1.000	g
Lactose	10.000	g
Sucrose	10.000	g
$FeSO_4$	0.200	g
NaCl	5.000	g
$Na_2S_2O_3$	0.300	g
Phenol red	0.024	g
Agar (if required)	12.000	g
Distilled water	1000.000	ml

pH 7.4

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700. ALKALIPHILIC SPIROCHAETE MEDIUM

Na_2CO_3	10.0	g
$NaHCO_3$	15.0	g
NaCl	10.0	g
K_2HPO_4	0.2	g
NH_4Cl	1.0	g
KCl	0.2	g
Na_2S x 9 H_2O	1.0	g
Yeast extract	0.5	g
Sucrose	5.0	g
Vitamin solution (see medium 141)	10.0	ml
Trace element solution (see medium 141)	1.0	ml
Distilled water	1000.0	ml

Prepare the medium under N_2, omitting Na_2CO_3, Na HCO_3, Na_2S, sucrose and vitamin solution.
Boil the medium and cool under nitrogen, add the Na_2CO_3 and $NaHCO_3$ and adjust the pH to 9.7 with 6N NaOH (about 15 ml). Sterilize by autoclaving and add the Na_2S (neutralized), vitamins and sucrose from sterile stock solutions.

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701. HALOANAEROBACTER CHITINOVORANS MEDIUM

NH_4Cl	1.000	g
$MgCl_2$ x 6 H_2O	7.000	g
$MgSO_4$ x 7 H_2O	9.600	g
$CaCl_2$ x 2 H_2O	0.500	g
KCl	3.800	g
K_2HPO_4 x 3 H_2O	0.400	g
NaCl	10.000	g
$NaHCO_3$	3.000	g
Na_2CO_3	1.000	g
Na_2S x 9 H_2O	0.500	g
Yeast extract	1.000	g
L-Cysteine	0.500	g
Resazurin	0.001	g
Substrate (glucose, chitin etc.)	5.000	g
Trace element solution SL 6 (see medium 141)	1.000	ml
Distilled water	1000.000	ml

pH 7.2

Prepare the medium without the Na_2S, Na_2CO_3, $NaHCO_3$, L-cysteine and substrate. Boil the medium and cool under a stream of nitrogen. Add the Na_2CO_3 and $NaHCO_3$. Adjust the pH to 7.2. If chitin is used as the substrate it must be added before autoclaving. Autoclave the medium and add the Na_2S (from a sterile, neutralized stock solution prepared under nitrogen), L-cysteine (from a stock solution prepared under nitrogen), and the glucose or N-acetylglucosamine (if used as substrate) from sterile stock solution prepared under nitrogen. Check the final pH.

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702. HALOCELLA CELLULOLYTICA MEDIUM

Use medium 591 with the following modifications:

NaCl	150.0	g
Yeast extract	2.0	g
$MgCl_2$ x 6 H_2O	3.3	g
$NaHCO_3$	2.5	g
Microcrystalline cellulose or cellobiose	5.0	g
Distilled water	1000.0	ml

Omit the peptone. Prepare the medium without $NaHCO_3$, Na_2S, cellobiose or vitamins, boil and cool under a stream of nitrogen. Add the $NaHCO_3$ and autoclave the medium. Na_2S, cellobiose and vitamins are added from sterile, anaerobic stock solutions just before inoculating. When medium is prepared using microcrystalline cellulose instead of cellobiose as a substrate the microcrystalline cellulose must be added to the medium before autoclaving at the appropiate concentration. The final pH is 7.0.

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703. SEMISOLID BRUCELLA BROTH

Bacto peptone	10.0	g
Casein peptone	10.0	g
Yeast extract	2.0	g
D-Glucose	1.0	g
NaCl	5.0	g
Agar	1.6	g
Distilled water	1000.0	ml

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704: BUTYRIVIBRIO SP. MEDIUM

Mineral solution	75.00	ml
Clarified rumen fluid	150.00	ml
K_2HPO_4	0.30	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	2.00	g
Volatile fatty acid mixture	3.10	ml
Haemin solution (0.05% w/v)	2.00	ml
Glycerol	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
D-Glucose	1.00	g
Maltose	1.00	g
Cellobiose	1.00	g
Starch (soluble)	1.00	g
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	770.00	ml

Dissolve ingredients (except carbonate, carbohydrates, cysteine and sulfide) and sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add the carbonate and equilibrate the medium with the CO_2 gas to pH 6.8. Distribute under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose, maltose, cellobiose, starch, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Cellobiose should be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

Mineral solution (from medium 330)

KH_2PO_4	6.00	g
NaCl	12.00	g
$(NH_4)_2SO_4$	6.00	g
$CaCl_2$ x 2 H_2O	1.60	g
$MgSO_4$ x 7 H_2O	2.50	g
Distilled water	1000.00	ml

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then

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stored frozen at -20°C.

Volatile fatty acid mixture (from medium 330)

Acetic acid	548.50	ml
Propionic acid	193.50	ml
Butyric acid	129.00	ml
n-Valeric acid	32.25	ml
iso-Butyric acid	32.25	ml
DL-2-Methylbutyric acid	32.25	ml
iso-Valeric acid	32.25	ml

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml
Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

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705: BACILLUS INFERNUS MEDIUM

Ferric citrate monohydrate	5.30	g
NaCl	5.80	g
NH_4Cl	1.00	g
K_2HPO_4 x 3 H_2O	0.40	g
$MgCl_2$ x 6 H_2O	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Trace element solution	10.00	ml
Na-acetate	0.80	g
Na-lactate	1.20	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.50	g
Distilled water	1000.00	ml

1. First, dissolve ferric citrate in water by heating and adjust to pH 7.5 with NaOH, then add other medium ingredients except acetate, lactate, and coenzyme M. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, lactate, and coenzyme M from sterile anoxic stock solutions prepared under 100% N_2 gas.

2. Prior to inoculation the pH of the complete medium should be checked and adjusted to 7.5, if necessary.

Trace element solution (from medium 705)

Na_2-EDTA x 2 H_2O	0.50	g
$CoCl_2$ x 6 H_2O	0.15	g
$MnCl_2$ x 4 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	0.10	g
$ZnCl_2$	0.10	g
AlCl3 x 6 H_2O	40.00	mg
Na_2WO_4 x 2 H_2O	40.00	mg
Na_2SeO_3 x 5 H_2O	30.00	mg
$NiCl_2$ x 6 H_2O	20.00	mg
$CuCl_2$ x 2 H_2O	20.00	mg
H_3BO_3	10.00	mg
Na_2MoO_4 x 2 H_2O	10.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7 using 2 N NaOH, then dissolve remaining compounds.

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708: DESULFORHABDUS AMNIGENA MEDIUM

Na_2HPO_4 x 2 H_2O	0.53	g
KH_2PO_4	0.41	g
NH_4Cl	0.30	g
$CaCl_2$ x 2 H_2O	0.11	g
$MgCl_2$ x 6 H_2O	0.10	g
Na_2SO_4	2.80	g
NaCl	0.30	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na-propionate	2.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins, propionate and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, propionate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. The pH of the complete medium should be 7.2 - 7.6.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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709: ACIDIMICROBIUM MEDIUM

$MgSO_4$ x 7 H_2O	0.50	g
$(NH_4)_2SO_4$	0.40	g
K_2HPO_4	0.20	g
KCl	0.10	g
$FeSO_4$ x 7 H_2O	0.01	g
Yeast extract	0.25	g
Distilled water	1000.00	ml

1. Dissolve ingredients except yeast extract and adjust pH to 2.0 with H_2SO_4. After autoclaving add yeast extract from a sterile stock solution.

2. Note: For autotrophic growth omit yeast extract and add to the medium 13.90 g/l $FeSO_4$ x 7 H_2O. Adjust medium pH to 1.7 with H_2SO_4 prior to autoclaving.

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710: THERMOANAEROBACTER (KoKo) MEDIUM

Tryptone (BD Bacto)	1.00	g
Peptone (meat)	1.00	g
Yeast extract (BD Bacto)	1.00	g
K_2HPO_4	1.60	g
NaH_2PO_4 x 2 H_2O	1.00	g
NH_4Cl	0.50	g
$MgSO_4$ x 6 H_2O	0.16	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.06	g
$NaHCO_3$	1.00	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

2. Note: Supplementation of medium with 10.00 g/l MOPS buffer (pH 6.9 - 7.0; 10% (w/v) anoxic stock solution) may enhance the buffer capacity of the medium.

For DSM 12299: Omit D-glucose.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

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Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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711: SPOROBACTER MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Yeast extract	0.50	g
Na-acetate x 3 H_2O	0.50	g
Trace element solution SL-10	1.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
3,4,5-Trimethoxycinnamic acid (TMC)	1.19	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, TMC and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins, TMC, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The vitamin solution should be sterilized by filtration. The stock solution of TMC (6% w/v) should be neutralized with NaOH prior to sterilization by filtration. Adjust pH of complete medium to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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712: PSEUDOBUTYRIVIBRIO MEDIUM

K_2HPO_4	0.23	g
KH_2PO_4	0.23	g
NaCl	0.45	g
$(NH_4)_2SO_4$	0.45	g
$CaCl_2$ x 2 H_2O	0.06	g
$MgSO_4$ x 7 H_2O	0.09	g
Yeast extract	5.00	g
Indigocarmine	5.00	mg
$NaHCO_3$	6.40	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, glucose, cysteine and sulfide), then sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add the bicarbonate and equilibrate the medium with the CO_2 gas to pH 6.8. Distribute medium under CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add vitamins, glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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713: METHANOHALOPHILUS EUHALOBIUS MEDIUM

NH_4Cl	0.50	g
NaCl	60.00	g
$MgCl_2 \times 6\ H_2O$	2.40	g
KCl	1.00	g
KH_2PO_4	0.40	g
Na_2-EDTA	0.50	g
Yeast extract	2.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Trimethylamine-HCl	10.00	g
Wolin's vitamin solution	5.00	ml
$CaCl_2 \times 2\ H_2O$	2.00	g
L-Cysteine HCl x H_2O	0.50	g
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, trimethylamine, vitamins, calcium chloride, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add trimethylamine, calcium chloride, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g

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Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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714. GEO-MEDIUM

Yeast extract	1.0	g
Glucose	1.0	g
Starch, soluble	1.0	g
$CaCO_3$	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.2

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715. COLUMBIA AGAR

Special mixture of peptones	23.0	g
Starch	1.0	g
NaCl	5.0	g
Agar	10.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 - 7.2.

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716: AMMONIFEX DEGENSII MEDIUM

K_2HPO_4 x 3 H_2O	0.30	g
KH_2PO_4	0.22	g
$(NH_4)_2SO_4$	0.22	g
NaCl	1.00	g
$MgSO_4$ x 7 H_2O	0.09	g
$CaCl_2$ x 2 H_2O	0.06	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	0.20	ml
Modified Wolin's mineral solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
KNO_3	1.00	g
Na_2CO_3	1.50	g
DL-Dithiothreitol	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except nitrate, carbonate, and dithiothreitol), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic serum vials (e.g., 10 ml medium in 25 ml Balch-type tubes) and autoclave. Before use, add nitrate from a sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Before inoculation add dithiothreitol from an anoxic stock solution sterilized by filtration under 100% N_2 gas and adjust the pH of the medium to 7.2.

2. After growth has started pressurize vials to 0.5 bar overpressure with a sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g

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Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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717: DESULFITOBACTERIUM (PCE) MEDIUM

$(NH_4)H_2PO_4$	2.88	g
$MgSO_4$ x 7 H_2O	0.10	g
$Ca(NO_3)_2$ x 4 H_2O	0.05	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.10	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-L-lactate	5.00	g
Na_2-fumarate	3.20	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, lactate, fumarate, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add lactate, fumarate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of fumarate and vitamins should be sterilized by filtration. The pH of the complete medium should be at 7.0 - 7.2.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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718: PETROTOGA MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 7 H_2O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
D-Glucose	5.00	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose, yeast extract, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 6.5 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose, yeast extract, vitamins, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Prior to inoculation adjust pH of complete medium to 6.5 - 7.7, if necessary.

For DSM 10691: Supplement medium with 1.00 g/l Trypticase peptone and increase amount of yeast extract to 1 g/l.

For DSM 13781: Supplement medium with 5.00 g/l $Na_2S_2O_3$ x 5 H_2O and 5.00 g/l Trypticase peptone added from anoxic stock solutions sterilized by filtration. Omit D-Glucose. Adjust amount of yeast extract to 2.00 g/l.

For DSM 13782: Supplement medium with 5.00 g/l $Na_2S_2O_3$ x 5 H_2O and 1.00 g/l Trypticase peptone added from anoxic stock solutions sterilized by filtration. Increase amount of yeast extract to 1.00 g/l.

For DSM 14811: Supplement medium with 2.00 g/l $Na_2S_2O_3$ x 5 H_2O added from a sterile anoxic stock solution sterilized by filtration.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g

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NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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719: ANAEROFILUM MEDIUM

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Trace element solution SL-10	1.00	ml
Yeast extract	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
D-Glucose	5.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, glucose, cysteine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Fatty acid mixture (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml
Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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720: DESULFITOBACTERIUM HAFNIENSE MEDIUM

NH_4Cl	1.00	g
NaCl	0.10	g
$MgCl_2 \times 6\ H_2O$	0.10	g
$CaCl_2 \times 2\ H_2O$	0.05	g
$K_2HPO_4 \times 3\ H_2O$	0.40	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.60	g
Na-pyruvate	2.50	g
$Na_2S_2O_3 \times 5\ H_2O$	1.25	g
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, pyruvate, thiosulfate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate and adjust pH to 7.0 - 7.2, then dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to use add pyruvate, thiosulfate, vitamins, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of pyruvate and vitamins are sterilized by filtration.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA $\times 2\ H_2O$	5.20	g
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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722: OCEANIDESULFOVIBRIO MEDIUM

Na_2SO_4	3.00	g
KCl	0.30	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
NaCl	50.00	g
$MgCl_2$ x 6 H_2O	3.30	g
$MgSO_4$ x 7 H_2O	1.60	g
Yeast extract	0.10	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-DL-lactate	2.50	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.20	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, lactate, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add lactate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be 7.0 - 7.2.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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726. RHIZOMONAS SUBERIFACIENS MEDIUM

Enzymatic hydrolysate of casein (Sigma)		
(or Casitone from Difco)	5.0	g
Glucose	2.5	g
K_2HPO_4 x 3 H_2O	1.3	g
KNO_3	0.5	g
$MgSO_4$ x 7 H_2O	0.5	g
$Ca(NO_3)_2$ x 4 H_2O	60.0	mg
Purified agar (e.g. Difco Agar Noble)	11.0	g
Distilled water	1000.0	ml

pH 7.2

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728: DESULFOBACCA MEDIUM

Na_2HPO_4 x 2 H_2O	0.53	g
KH_2PO_4	0.41	g
NH_4Cl	0.30	g
$MgCl_2$ x 6 H_2O	0.10	g
NaCl	0.30	g
Na_2SO_4	3.00	g
Na-acetate	1.64	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
$CaCl_2$ x 2 H_2O	0.11	g
Seven vitamins solution	0.50	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, calcium chloride, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be 7.5 - 7.7.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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729. MYX AGAR

Na_2-glutamate	5.0	g
Yeast extract (Difco)	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
Agar, if necessary	15.0	g
Glucose	2.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2. Autoclave glucose separately.

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730: FERROGLOBUS PLACIDUS MEDIUM

Solution A	950.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml

1. Sparge solution A with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials to 10% volume (e.g., 10 ml medium in 100 ml bottle) and autoclave. Solution B is autoclaved separately after sparging with 80% N_2 and 20% CO_2 gas mixture. Solution C and D are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution E is autoclaved under 100% N_2 gas atmosphere. To complete the medium add appropriate amounts of solutions B to E to the sterile solution A in the sequence as indicated.

2. Adjust pH of complete medium to 7.2, if necessary.

3. Note: Cultures are very sensitive to the nitrite produced during growth. Thus, incubate this strain ony a few hours at one time (8-12 h) and thereafter check growth by microscopy. Immediately stop the incubation at 85°C, when the medium begins to show some turbidity.

Solution A

NaCl	18.00	g
$MgCl_2$ x 6 H_2O	4.30	g
$CaCl_2$ x 2 H_2O	0.14	g
KCl	0.34	g
K_2HPO_4	0.14	g
NH_4Cl	0.24	g
KNO_3	1.00	g
Modified Wolin's mineral solution	10.00	ml
Distilled water	940.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

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Solution D

Na-pyruvate	1.00	g
Distilled water	10.00	ml

Solution E

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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731: THERMOSYNTROPHA MEDIUM

K_2HPO_4	0.30	g
KCl	0.30	g
NaCl	0.50	g
NH_4Cl	1.00	g
$MgCl_2 \times 6\ H_2O$	0.30	g
$CaCl_2 \times 2\ H_2O$	0.05	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	3.00	g
Na_2CO_3	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl $\times H_2O$	0.15	g
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, carbonate, vitamins, cysteine and sulfide. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dissolve carbonate and bicarbonate and adjust pH of medium to 8.5 using HCl. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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732: DEHALOBACTER RESTRICTUS MEDIUM (TCE)

Solution A	870.00	ml
Solution B	100.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	2.00	ml
Solution F	10.00	ml
Solution G	15.00	ml

1. Sparge solution A with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials (e.g., 9 ml medium in 50 ml bottle). Pressurize closed bottles with 80% H_2 and 20% CO_2 gas mixture to 0.5 bar overpressure and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C, D and F are autoclave under 100% N_2 gas atmosphere. Solution E is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Prepare solution G by filling 13.5 ml hexadecane into a 50 ml serum bottle, then sparge with 100% N_2 gas to make it anoxic and autoclave. Add 1.50 ml anoxic autoclaved tetrachloroethene to the sterile anoxic hexadecane solution by syringe. To complete the medium add appropriate amounts of solutions B to F to the sterile solution A in the sequence as indicated. The pH of the medium before inoculation should be at 7.2.

2. Add solution G only after inoculation of the medium!

Solution A

K_2HPO_4	0.65	g
$NaH_2PO_4 \times H_2O$	0.17	g
Peptone (BD Bacto)	0.10	g
Na-acetate	0.46	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	870.00	ml

Solution B

$(NH_4)_2CO_3$	0.27	g
$NaHCO_3$	3.73	g
Distilled water	100.00	ml

Solution C

$CaCl_2 \times 2\ H_2O$	0.11	g
$MgCl_2 \times 6\ H_2O$	0.10	g
Distilled water	10.00	ml

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Solution D

Trace element solution	1.00	ml

Solution E

Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml

Solution F

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Solution G

Hexadecane	13.50	ml
Tetrachloroethene	1.50	ml

Trace element solution (from medium 732)

Na_2-EDTA	0.50	g
$FeCl_2$ x 4 H_2O	2.00	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
AlCl3	10.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add remaining compounds.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg

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Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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733. SULFITOBACTER PONTIACUS MEDIUM

Na-acetate	20.00	mM
HEPES	8.00	g
K_2HPO_4	1.00	g
NH_4Cl	0.50	g
Yeast extract	1.00	g
Peptone	0.50	g
NaCl	15.00	g
$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution (see medium 141)	1.00	ml
Biotin	0.10	g
Distilled water	1000.00	ml

Prepare the HEPES, NaCl, K_2HPO_4 and NH_4Cl as a basal salt solution, adjusting the pH to 7.5-7.8 with NaOH before sterilisation. All other components are added from sterile stock solutions.

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734: METHANOBREVIBACTER CURVATUS MEDIUM

Final pH: 7.2
Final volume: 1003 ml

Clarified rumen fluid	400.00	ml
NaCl	1.00	g
KCl	0.50	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
Na_2SO_4	0.15	g
Casamino acids (BD Bacto)	0.50	g
Yeast extract (OXOID)	0.50	g
Nutrient broth (BD Difco)	2.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
MOPS (SIGMA)	2.10	g
Seven vitamins solution	1.00	ml
DL-Dithiothreitol (DTT)	0.16	g
Distilled water	600.00	ml

Dissolve ingredients (except carbonate, MOPS buffer, vitamins and DTT), bring medium to the boil, then cool to room temperature under 80% H_2 and 20% CO_2 gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add MOPS buffer adjusted to pH 7.2, vitamins and DTT from anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.2, if necessary.

For DSM 115569: Supplement medium after autoclaving with 20 ml/l methanol (50% v/v) added from a sterile anoxic stock solution prepared under 100% N_2.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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735. MALT EXTRACT GLUCOSE AGAR

Malt extract	10.0	g
Glucose	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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736. RICH MEDIUM

Bacto peptone (Difco)	10.00	g
Yeast extract	5.00	g
Casamino acids (Difco)	5.00	g
Meat extract (Difco)	2.00	g
Malt extract (Difco)	5.00	g
Glycerol	2.00	g
$MgSO_4$ x 7 H_2O	1.00	g
Tween 80	0.05	g
Agar	20.00	g
Distilled water	1000.00	ml

Final pH is 7.2.

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737. DPM MEDIUM

KH_2PO_4	1.000	g
$MgSO_4$ x 7 H_2O	0.100	g
$CaCl_2$ x 2 H_2O	0.010	g
Na-propionate	1.200	g
Chelated iron solution (see below)	1.800	ml
Trace element solution (see below)	1.000	ml
Distilled water	1000.000	ml
Chelated iron solution:		
$FeSO_4$ x 7 H_2O	5.540	g
Na_2-EDTA	7.560	g
Distilled water	1000.000	ml
Trace element solution:		
$CoCl_2$	0.025	g
$CuSO_4$ x 5 H_2O	0.080	g
H_3BO_3	2.860	g
$MnCl_2$ x 4 H_2O	1.810	g
Na_2MoO_4 x 2 H_2O	0.025	g
$ZnSO_4$ x 7 H_2O	0.220	g
Distilled water	1000.000	ml

The pH of the final solution is adjusted to 6.8.

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738. BPHD Medium

NH_4Cl	1.00	g
Na_2HPO_4 x 2 H_2O	0.42	g
$Na_2H_2PO_4$ x H_2O	0.18	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
$FeSO_4$ x 7 H_2O	0.04	g
Vitamin solution (see medium 141)	10.00	ml
Trace element solution (see medium 141)	10.00	ml
Cresol or phenol	1.00	mM*
Distilled water	1000.00	ml

Adjust pH to 6.5. Add cresol/phenol after autoclaving.
* (e.g.: 20 ml/l of a 4.7 g/l phenol-solution)

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739: DESULFONEMA ISHIMOTOI MEDIUM

Solution A	952.00	ml
Solution B	10.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	20.00	ml
Solution F	10.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic and reach a pH of around 6, then distribute under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Solutions B and C are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions D and F are autoclaved separately under 100% N_2 gas. Solution E is prepared under 80% N_2 and 20% CO_2 gas gas atmosphere. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.0. After combining solutions the medium should equilibrate overnight and a white precipitate should be apparent.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N_2 and filter-sterilized) just before inoculation may stimulate growth at the beginning. For transfers use 5 - 10% (v/v) inoculum.

Solution A

NaCl	25.00	g
$MgCl_2$ x 6 H_2O	5.60	g
$MgSO_4$ x 7 H_2O	6.80	g
$CaCl_2$ x 2 H_2O	1.40	g
KCl	0.72	g
KH_2PO_4	0.14	g
NH_4Cl	0.25	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na-acetate x 3 H_2O	2.50	g
Disodium succinate	0.10	g
Isobutyric acid	0.19	ml
Distilled water	10.00	ml

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Adjust to pH 7.0 with 1 N NaOH.

Solution C

Wolin's vitamin solution (10x)	1.00	ml

Solution D

$AlK(SO_4)_2 \times 12\ H_2O$	0.48	g
Distilled water	10.00	ml

Solution E

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution F

$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
$Na_2SeO_3 \times 5\ H_2O$	3.00	mg
$Na_2WO_4 \times 2\ H_2O$	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg

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739: DESULFONEMA ISHIMOTOI MEDIUM

Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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740: FERVIDOBACTERIUM TF(A) MEDIUM

NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.16	g
K_2HPO_4	1.60	g
Na_2HPO_4 x H_2O	1.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.06	g
D-Glucose	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except calcium chloride, glucose, vitamins, cysteine and sulfide. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. The pH of the complete medium should be 6.8 - 7.0.

For DSM 17883: Add a whiff of sterile sulfur to each vessel with autoclaved medium and adjust pH of complete medium to 7.5. For sterilization steam sulfur for 3 hours on each of 3 successive days.

For DSM 21710: Add a whiff of sterile sulfur to each vessel with autoclaved medium and adjust pH of complete medium to 6.5. For sterilization steam sulfur for 3 hours on each of 3 successive days.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g

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Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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742: DESULFONATRONOVIBRIO MEDIUM

NaCl	10.00	g
Na_2SO_4	3.00	g
Na_2HPO_4	0.20	g
KCl	0.20	g
NH_4Cl	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	15.00	g
Na_2CO_3	10.00	g
Yeast extract	1.50	g
Na-formate	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, carbonate, yeast extract, vitamins, formate, and sulfide. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then add bicarbonate and carbonate and adjust pH to 9.5. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins, formate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. The pH of the complete medium should be at 9.5 - 9.7.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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743. HYDROGENOBACTER ACIDOPHILUS MEDIUM

Sulfur	5.00	g
$(NH_4)_2SO_4$	1.00	g
K_2HPO_4	1.00	g
NaCl	1.00	g
$MgSO_4$ x 7 H_2O	0.30	g
$FeSO_4$ x 7 H_2O	1.00	mg
$CaCl_2$	1.00	mg
Trace element solution (see medium 533)	0.50	ml
Distilled water	1000.00	ml

Adjust pH to 3.0 with HCl. Steam medium for 3 hours on each of 3 successive days. Incubate under a gas phase H_2:O_2:CO_2 = 7:1:1 with an overpressure of one atmosphere at 65°C.

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744. HYDROGENOBACTER HALOPHILUS MEDIUM

K_2HPO_4	2.5	g
KH_2PO_4	0.5	g
$(NH_4)_2SO_4$	2.0	g
NaCl	29.3	g
$MgSO_4$ x 7 H_2O	0.2	g
$CaCl_2$	10.0	mg
$FeSO_4$ x 7 H_2O	10.0	mg
Trace element solution (see medium 533)	0.5	ml
Distilled water	1000.0	ml

Adjust pH to 6.8-7.0.

Incubate at 37°C under a gas phase of H_2:O_2:CO_2 = 8:1:1.

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745. RHODOBIUM MEDIUM

KH_2PO_4	1.00	g
$(NH_4)_2SO_4$	1.00	g
NaCl	50.20	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-8	1.00	ml
Na-DL-malate	3.60	g
Yeast extract	1.00	g
$Na_2S_2O_3$ x 5 H_2O	0.50	g
Distilled water	1000.00	ml

Trace element solution SL-8:

Na_2-EDTA	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	62.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	17.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoSO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

pH 6.8

Boil the medium under a stream of nitrogen gas for few minutes and distribute in screw-capped bottles or tubes. Autoclave at 121°C for 15 minutes.

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746. RHODOVULUM STRICTUM MEDIUM

Solution 1:

$(NH_4)_2SO_4$	1.00	g
NaCl	8.20	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-8 (see medium 745)	1.00	ml
Na-DL-malate	3.60	g
Yeast extract	1.00	g
$Na_2S_2O_3$ x 5 H_2O	0.50	g
Distilled water	500.00	ml

Solution 2:

Potassium phosphate buffer (pH 7.8) 20mM	500.00	ml

Boil both solutions under a stream of nitrogen gas for few minutes and autoclave separately under anaerobic conditions at 121°C for 15 minutes. Mix aseptically after autoclaving under a stream of sterile nitrogen gas and adjust final pH to 7.8. Distribute in screw-capped bottles or tubes.

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747. DSIC MEDIUM (modified)

Solution A:

NaCl	125.00	g
NH_4Cl	0.50	g
KH_2PO_4	0.60	g
K_2SO_4	2.50	g
Na-acetate	2.00	g
$Na_2S_2O_3$ x 5 H_2O	0.10	g
Yeast extract	0.75	g
Vitamin B_{12} (10 mg/100ml H_2O)	1.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
MOPS buffer	2.10	g
Distilled water	960.00	ml

The acronym MOPS stands for the organic buffer N-morpholinopropanesulfonic acid at pH 7.0. The pH of solution A is 7.0. Boil the medium under N_2 gas and distribute about 13 ml in 15 ml Hungate tubes. Autoclave at 121°C for 15 minutes.

Solution B:

$MgCl_2$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	0.20	g
Distilled water	70.00	ml

pH 7.0.

Boil medium under N_2 gas and autoclave in a srew-capped bottle at 121°C for 15 minutes.

Solution C:

$NaHCO_3$	1.00	g
Distilled water	12.00	ml

Filter sterilize this solution.

After cooling inject 1.0 ml of solution B and 0.2 ml of solution C in each tube of solution A. The pH of the complete medium is 7.0-7.1.

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748. CENS MEDIUM

K_2HPO_4	0.9	g
KH_2PO_4	0.6	g
NH_4Cl	1.0	g
EDTA	5.0	mg
$MgSO_4$ x 7 H_2O	0.2	g
$CaCl_2$ x 2 H_2O	75.0	mg
True Blue Trace elements (see below)	1.0	ml
Chelated Iron solution (see below)	2.0	ml
Vitamin B_{12}	20.0	µg
Biotin	15.0	µg
$Na_2S_2O_3$ x 5 H_2O	0.5	g
Soytone (Difco 0436)	4.0	g
Na-pyruvate (filter-sterilized)	2.2	g
Distilled water	1000.0	ml

True Blue Trace Elements:

EDTA	2.5	g
$MnCl_2$ x 4 H_2O	0.2	g
H_3BO_3	0.1	g
Na_2MoO_4 x 2 H_2O	0.1	g
$ZnCl_2$	50.0	mg
$NiCl_2$ x 6 H_2O	50.0	mg
$CoCl_2$ x 2 H_2O	20.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
Na_2SeO_3	5.0	mg
$NaVO_3$ x n H_2O (meta)	5.0	mg
Distilled water	250.0	ml

Dissolve chemicals one at a time in approximately 200.0 ml distilled water. Bring final volume to 250.0 ml with additional water.

Chelated Iron solution:

$FeCl_2$ x 4 H_2O	1.0	g
EDTA	2.0	g
HCl (conc.)	3.0	ml
Distilled water	900.0	ml

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749: THERMODESULFOVIBRIO YELLOWSTONII MEDIUM

Na_2SO_4	4.00	g
Na_2HPO_4	0.20	g
NH_4Cl	0.25	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.50	g
Na-DL-lactate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.30	g
Wolin's vitamin solution (10x)	1.00	ml
Na-thioglycolate	0.20	g
L-Ascorbic acid	0.20	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, vitamins, thioglycolate and ascorbic acid, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, thioglycolate and ascorbic acid from anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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750. MEDIUM FOR MARINE METHYLOTROPHS

Beef extract	7.0	g
Peptone	10.0	g
NaCl	25.0	g
K_2HPO_4	1.0	g
$(NH_4)_2SO_4$	1.0	g
Agar	20.0	g
Distilled water	1000.0	ml
pH 7.0		

After sterilisation, cool to 50°C and add 10 ml methanol.

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751. SC MEDIUM

Corn meal agar	17.0	g
Peptone from soy meal	8.0	g
K_2HPO_4	1.0	g
KH_2PO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Hemin (0.1% in 0.05N NaOH)	15.0	ml
Distilled water	1000.0	ml

Adjust pH to 6.6. Autoclave, cool to 50°C and add the following filter-sterilized components:

20% bovine serum albumin, fraction V	10.0	ml
10% glucose	5.0	ml
10% cysteine (free base)	10.0	ml

For liquid medium, omit corn meal agar and change the phosphate concentration to KH_2PO_4, 1.5 g and K_2HPO_4, 0.5 g.

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752. HALOMONAS PANTELLERIENSE

Yeast extract	10.0	g
Na_2-citrate	3.0	g
KCl	2.0	g
$MgSO_4$ x 7 H_2O	1.0	g
NaCl	100.0	g
Na_2CO_3	2-3	g
$MnCl_2$ x 4 H_2O (0.36g/l)	1.0	ml
$FeSO_4$ x 7 H_2O (50g/l)	1.0	ml
Distilled water	1000.0	ml

Liquid medium may be prepared by adding all components together. Agar (2%) medium should be prepared without Na_2CO_3 and sterilised by autoclaving. Add 20-30 ml of a sterile 10% Na_2CO_3 solution to the autoclaved medium containing agar so that the final pH is 9.0.

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753. MY MEDIUM

Yeast extract (Difco 0127)	10.0	g
Maltose	20.0	g
KH_2PO_4	2.0	g
NaCl	5.0	g
Distilled water	1000.0	ml

Adjust pH for final pH 6.0. Autoclave at 121°C for 15 minutes.

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754. INORGANIC SALTS-MALTOSE MEDIUM

Peptone	2.00	g
Yeast extract	4.00	g
Inorganic salt solution (see below)	980.00	ml

Adjust pH to 7.5 with KOH and autoclave at 121°C for 15 minutes. After sterilization add 20 ml of a 25% maltose (filter-sterilized) solution.

Inorganic salt solution:

$CaCl_2$ x 2 H_2O	1.29	g
NaCl	43.80	g
$MgSO_4$ x 7 H_2O	49.37	g
Distilled water	1000.00	ml

Add the salts in the order listed to avoid the formation of a precipitate. When agar medium is desired the concentration should be 0.75%.

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755. HALOBACILLUS MEDIUM

NaCl	100.0	g
$MgSO_4$ x 7 H_2O	5.0	g
Peptone, casein digest	5.0	g
Yeast extract	3.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5.

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756. HETEROTROPHIC NITROBACTER MEDIUM

Yeast extract	1.50	g
Peptone	1.50	g
Na-pyruvate	0.55	g
Trace element solution	1.00	ml
Stock solution	100.00	ml
Distilled water	899.00	ml

Adjust pH to 7.4 with NaOH or KOH.

Stock solution:

$CaCO_3$	0.07	g
NaCl	5.00	g
$MgSO_4$ x 7 H_2O	0.50	g
KH_2PO_4	1.50	g
Distilled water	1000.00	ml

Trace element solution:

$MnSO_4$ x H_2O	33.80	mg
H_3BO_3	49.40	mg
$ZnSO_4$ x 7 H_2O	43.10	mg
$(NH_4)_6Mo_7O_{24}$	37.10	mg
$FeSO_4$ x 7 H_2O	97.30	mg
$CuSO_4$ x 5 H_2O	25.00	mg
Distilled water	1000.00	ml

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760. MARINE THERMOCOCCUS MEDIUM

Prepare liquid Bacto Marine Broth (see medium 514) and filter through normal filter paper. An iron sediment will collect in the filter. Boil the medium in a water bath and cool under an anaerobic gas mixture (N_2 for DSM 2476, DSM 5473, DSM 5474, DSM 9503, DSM 10343, DSM 10394, DSM 10395, DSM 11113, DSM 11836, DSM 12593, DSM 12819, DSM 12820, DSM 12597, DSM 12767, DSM 14981, DSM 15226, DSM 16538, DSM 17994, DSM 17882, DSM 22688, DSM 27149

or
N_2:CO_2 80:20 for DSM 5262).

The final pH of the medium should be pH 5.8 for DSM 2476,
pH 6.0 for DSM 12767,
pH 6.5 for DSM 5262, DSM 10394, DSM 10395, DSM 12593 and DSM 16538,
pH 7.0 for DSM 11836, DSM 14981, DSM 15226, DSM 17882 DSM 17994 DSM 22688, DSM 27149, DSM 27260 DSM 27261
pH 7.2 for DSM 9503, DSM 10343, DSM 11113, and DSM 12819, and
pH 7.5 for DSM 5473, DSM 5474, DSM 12820, DSM 12597,
.
Dispense the medium into Hungate tubes or serum bottles containing finely divided sulphur (0.5% w/v), seal the tubes or bottles under the same anaerobic gas used when cooling the medium. Sterilise the medium at 100°C for 3 hours on 3 consecutive days. Reduce the medium by adding 10% neutralised sodium sulphide to a final concentration of 0.05 %. The medium should not give a heavy black precipitate, if it does the iron sediment was not adequately removed by filtering in the initial stages and the medium should be made again, making sure that the iron is removed by filtering.

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762. HALOMONAS DESIDERATA MEDIUM

Glucose	5.0	g
$MgCl_2$ x 6 H_2O	0.2	g
KH_2PO_4	1.0	g
KNO_3	2.0	g
Na_2CO_3	5.4	g
$NaHCO_3$	4.2	g
Distilled water	1000.0	ml

Prepare the medium without Na_2CO_3 and $NaHCO_3$ in 900 ml distilled water and autoclave. Prepare the Na_2CO_3 and $NaHCO_3$ in 100 ml water, sterilise separately and add to the cooled medium. Final pH 9.5-10.0.

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763. BOSEA THIOOXIDANS MEDIUM

Na_2HPO_4	4.0	g
KH_2PO_4	1.5	g
$MgCl_2$	0.1	g
Na-glutamate	0.5	g
Yeast extract	0.1	g
$Na_2S_2O_3$ x 5 H_2O	5.0	g
Na-succinate	5.0	g
Distilled water	1000.0	ml

Final pH 7.5-8.5.

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764. HALOANAEROBIUM LACUSROSEUS MEDIUM

Use medium 761, but add 1 g/l yeast extract and increase the NaCl concentration to 200 g/l. The glucose is added from a sterile stock solution (see below).
Prepare the medium and boil, cool under N_2:CO_2 (80:20 v/v). Dispense medium into Hungate tubes or serum bottles under N_2:CO2 (80:20 v/v), autoclave. To the sterile medium add from sterile stock solutions 2% Na_2S x 9 H_2O (0.1ml/10ml), 10% $NaHCO_3$ (0.5ml/10ml), and 1M glucose (0.25ml/10ml). Final pH 7.0.

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766. THERMOCOCCUS CHITINOPHAGUS MEDIUM

$(NH_4)_2SO_4$	0.500	g
$NaHCO_3$	0.200	g
KH_2PO_4	0.500	g
Na_2WO_4 x 2 H_2O	0.150	mg
Na_2SeO_4	0.150	mg
$(NH_4)_2Ni(SO_4)_2$	0.300	mg
Chitin stock solution (see below)	4.000	g
Na_2S x 9 H_2O	0.500	g
Trace elements SL-6 (see medium 141)	15.000	ml
Resazurin	0.001	g
Synthetic seawater (see medium 600)	485.000	ml
Distilled water	500.000	ml

The chitin solution is prepared as follows: mix 20 g chitin (practical grade from crab shells) with 200 ml of 37% HCl (pre-cooled to 4°C) and stir for 1 hr at 4°C. Pour the suspension into 1 litre of distilled water (pre-cooled to 4°C) and filter through filter paper (Schleicher and Schüll Nr. 311853). Wash the residue five times with 500 ml distilled water and resuspend in 1 litre of distilled water. Neutralise the suspension with 10 ml of 5M KOH (final pH 6.5). Filter and wash with 3 litres of distilled water to remove KCl.

Prepare the medium without Na_2S x 9 H_2O and $NaHCO_3$, boil and cool under N_2. Add the $NaHCO_3$ to the cooled medium and adjust the pH to 6.7. Dispense into Hungate tubes or serum bottles under N_2 and autoclave. Reduce the medium before use from a sterile stock solution of neutralised Na_2S x 9 H_2O.

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767. ERYTHROMICROBIUM AND ROSEOCOCCUS MEDIUM

NH_4Cl	0.30	g
K_2HPO_4	0.30	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.05	g
KCl	0.30	g
Na-acetate	1.00	g
Yeast extract	1.00	g
Bactopeptone	1.00	g
Trace elements SL-6 (see medium 27)	1.00	ml
Vitamin B_{12}	20.00	µg
Distilled water	1000.00	ml

Final pH 7.5-7.8

Add vitamin B_{12} from a filter sterilsed stock solution.

For **DSM 8509** add 15g /l NaCl.

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768. M17 MEDIUM FOR FILOMICROBIUM FUSIFORME

Na-acetate	1.0	g
KNO_3	1.0	g
Hutner's salts (see below)	20.0	ml
Vitamin solution (see below)	5.0	ml
Artificial seawater (see below)	500.0	ml
Distilled water	475.0	ml

Final pH 7.2.

Artificial sea water:

NaCl	23.48	g
Na_2SO_4	3.92	g
$NaHCO_3$	0.19	g
KCl	0.66	g
KBr	0.10	g
$MgCl_2$ x 6 H_2O	5.00	g
$CaCl_2$ x 2 H_2O	1.10	g
H_3BO_3	0.03	g
$SrCl_2$	0.02	g
NaF	3.00	mg
Distilled water	1000.000	ml

Add $NaHCO_3$ from a filter-sterilized stock solution when the medium has cooled.

Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

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Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

Vitamin solution (double conc.):

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Distilled water	1000.00	ml

Store in the dark and cold (5°C).

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769: CLOSTRIDIUM VINCENTII MEDIUM

Sea Salt (SIGMA)	12.00	g
NH_4NO_3	0.10	g
Yeast extract (BD Bacto)	1.00	g
Trypticase peptone (BD BBL)	0.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Na-phosphate buffer (0.2 M, pH 6.5)	20.00	ml
Lactose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	980.00	ml

1. Dissolve ingredients (except bicarbonate, phosphate-buffer, lactose, vitamins, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add phosphate-buffer, lactose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

2. Note: Sea salts can be replaced by 300.0 ml/l of filtered aged sea water.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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770: SULFOPHOBOCOCCUS ZILLIGII MEDIUM

Solution A	970.00	ml
Solution B	10.00	ml
Solution C	10.00	ml
Solution D	5.00	ml
Solution E	10.00	ml

1. Dissolve ingredients of solution A except carbonate, sparge with 100% N_2 gas for 30 - 45 min to make it anoxic, then add carbonate and adjust pH to 9.0 - 9.3. Dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 30 ml medium per 100 ml bottle) and autoclave. Solutions B and C are autoclaved separately under 100% N_2 gas atmosphere. Solutions D and E are prepared under 100% N_2 gas and sterilized by filtration. To complete the medium add appropriate amounts of solutions B to E to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.5.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning.

3. For transfers use 10% (v/v) inoculum.

Solution A

Na_2-EDTA	32.00	mg
$CaCl_2$ x 2 H_2O	66.00	mg
$MgSO_4$ x 7 H_2O	31.00	mg
KCl	31.00	mg
$ZnCl_2$ (0.1% w/v)	2.10	ml
$MnSO_4$ x H_2O (0.1% w/v)	2.30	ml
$Na_2B_4O_7$ x 10 H_2O (0.1% w/v)	1.80	ml
Glycine	1.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	230.00	mg
Distilled water	965.00	ml

Solution B

Yeast extract	1.00	g
Distilled water	10.00	ml

Solution C

Trypticase peptone (BD BBL)	1.00	g
Distilled water	10.00	ml

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Solution D

$FeSO_4$ x 7 H_2O	50.00	mg
H_2SO_4 (0.1 N)	5.00	ml

Solution E

Dithiothreitol (DTT)	150.00	mg
Distilled water	10.00	ml

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771: SULFUROSPIRILLUM BARNESII MEDIUM

K_2HPO_4	225.00	mg
KH_2PO_4	225.00	mg
NaCl	460.00	mg
$(NH_4)_2SO_4$	225.00	mg
$MgSO_4$ x 7 H_2O	117.00	mg
Yeast extract	1.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.50	g
Na-DL-lactate	2.25	g
$NaNO_3$	1.70	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.10	g
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, lactate, nitrate, vitamins, cysteine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add lactate, nitrate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be 7.2 - 7.4.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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772. PARACOCCUS ALCALIPHILUS MEDIUM

$(NH_4)_2SO_4$	3.0	g
KH_2PO_4	1.4	g
Na_2HPO_4	3.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Fe-citrate	30.0	mg
$CaCl_2$ x 2 H_2O	30.0	mg
$MnCl_2$ x 4 H_2O	5.0	mg
$ZnSO_4$ x 7 H_2O	5.0	mg
$CuSO_4$ x 2 H_2O	0.5	mg
Thiamine-HCl x 2 H_2O	0.4	mg
Distilled water	1000.0	ml

Thiamine hydrochloride may be replaced by 2 g/l yeast extract. The pH of the medium is adjusted to pH 9.0 with filter sterilised 10% Na_2CO_3. 10 ml sterile methanol (filter sterilised, or autoclaved in crimp top sealed bottles) is added to the sterilised medium.

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773. PARACOCCUS KOCURII MEDIUM

NaH_2PO_4 x 2 H_2O	1.71	g
K_2HPO_4	1.41	g
$(NH_4)_2SO_4$	1.00	g
Tetramethyl ammonium chloride	1.00	g
Thiamine-HCl x 2 H_2O	0.50	mg
Hutner' salts (see medium 590)	20.00	ml
Distilled water	1000.00	ml

Final pH 6.8-7.0

The tetramethyl ammonium chloride and thiamine hydrochloride are added from filter sterilised stock solutions.

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774. MEDIUM FOR PARACOCCUS AMINOPHILUS AND P. AMINOVORANS

Peptone	5.0	g
Yeast extract	5.0	g
Glucose	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml

pH 7.0

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775. R - CW MEDIUM

Trypticase	5.0	g
Tryptone	5.0	g
Yeast extract	5.0	g
KH_2PO_4	5.0	g
Na-acetate	5.0	g
$(NH_4)_2$ citrate	2.0	g
$MgSO_4$ x 7 H_2O	0.5	g
$MnSO_4$ x H_2O	0.2	g
Tween 80	1.0	ml
Cheese whey	1000.0	ml

Adjust pH to 5.5.

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776. MICROLUNATUS MEDIUM

Glucose	0.5	g
Peptone	0.5	g
Yeast extract	0.5	g
Na-glutamate	0.5	g
KH_2PO_4	0.5	g
$(NH_4)_2SO_4$	0.1	g
$MgSO_4$ x 7 H_2O	0.1	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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778: THERMOTERRABACTERIUM MEDIUM

KH_2PO_4	0.33	g
NH_4Cl	0.33	g
KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	0.20	ml
Yeast extract	1.00	g
Na_2-9,10-anthraquinone-2,6-disulfonate (TCI A0308)	8.25	g
$NaHCO_3$	2.50	g
$CaCl_2$ x 2 H_2O	0.33	g
Glycerol	2.60	ml
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, calcium chloride, glycerol and vitamins), boil medium for some minutes to dissolve the anthraquinone, then cool under 80% N_2 and 20% CO_2 gas atmosphere to room temperature. Add solid bicarbonate and adjust pH to 6.8, dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, glycerol and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

For DSM 16624: Replace bicarbonate with 2.5 g/l carbonate and glycerol with 10.00 g/l Trypticase peptone. Adjust pH of the complete medium to 7.5 - 8.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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779. CAPNOCYTOPHAGA II MEDIUM

Lab-lemco meat extract (Oxoid)	2.4	g
Proteose peptone no.3 (Difco)	10.0	g
Yeast extract (Difco, Bacto)	5.0	g
Na_2HPO_4	4.0	g
Glucose	1.5	g
Starch, soluble	0.5	g
Cysteine-HCl x H_2O	0.5	g
Agar, if necessary	15.0	g
Horse blood	50.0	ml
Distilled water	1000.0	ml

Adjust pH to 7.6 - 7.8.
Add the horse blood to the heat sterilized medium after being cooled to 50°C. Incubate under air plus 5% CO_2 or anaerobically under 95% N_2 plus 5% CO_2.

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780. MIDDLEBROOK MEDIUM WITH MYCOBACTIN

Reconstitute the contents of one vial of 2 mg Mycobactine J with 2 ml absolute ethanol (analytical grade). Shake carefully so that the product is completely dissolved. Pour the contents of the bottle into 1 litre of medium 645 kept melted in a water bath at +56°C, taking care to pour out all of the product. Rinse out the vial that contained the Mycobactine J with an additional 2 ml of absolute ethanol and pour into the culture medium. Mix the Mycobactine J alcohol solution and the culture medium very thoroughly.

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781. DUNG EXTRACT AGAR

Dung extract	100.00	ml
Malt extract	5.00	g
$MgSO_4$ x 7 H_2O	0.50	g
$Ca(NO_3)_2$ x 4 H_2O	0.72	g
K_2HPO_4	0.25	g
Peptone	0.10	g
Agar	15.00	g
Distilled water	900.00	ml

Dung extract: Boil an average sized piece of horse dung (fresh or frozen) for two hours in 150 ml water using a water bath; filter and use immediately.

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782. OM-2 MEDIUM

NH_4-oxalate	15.00	g
$NaHCO_3$	10.00	g
NaH_2PO_4 x 2 H_2O	10.00	g
NaCl	0.70	g
KCl	0.57	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.01	g
$Na_2S_2O_3$ x 5 H_2O	1.00	g
Distilled water	1000.00	ml

pH 6.8-7.0

The first three salts must be dissolved in the indicated sequence. After autoclaving for 20 min at 121°C the pH of the medium shifted to 8.5-9.0. Solid medium contained 1.5 to 2.0% (w/v) of Bacto-Agar (Difco).

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783. HYDROGENOTHERMUS HIRSCHII MEDIUM

$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_2O	5.50	g
KCl	0.65	g
NaBr	0.10	g
$NaHCO_3$	2.00	g
NH_4Cl	0.15	g
K_2HPO_4	0.15	g
$CaCl_2$ x 2 H_2O	0.50	g
Trace element solution (see medium 141)	10.00	ml
Sulfur, powdered	0.50	g
Distilled water	1000.00	ml

Prepare the medium aerobically and adjust pH to 7.0 using H_2SO_4. Fill 20 ml medium into 100 ml serum bottles under H_2/CO_2 (80/20) gas and seal with a rubber stopper. Steam medium for 3 hours on each of 3 successive days. Before use add 100 µl of a sterile $CaCO_3$ solution (10%). Give an atmosphere of H2/CO2 with an overpressure of two atmospheres and 20 ml filter-sterilized air. Incubate under shaking at 60°C.

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784: NATRONIELLA MEDIUM

KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
NH_4Cl	1.00	g
KCl	0.20	g
NaCl	16.00	g
Trace element solution SL-11	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	68.00	g
$NaHCO_3$	38.00	g
Ethanol absolute	5.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, bicarbonate, ethanol, vitamins and sulfide. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonates and sulfide while gassing the head space only. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add ethanol and vitamins from sterile anoxic stock solution prepared under 100% N_2 gas. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 9.5 - 10.0, if necessary.

For DSM 24923: Omit ethanol and add to the autoclaved medium 2.5 g/l $Na_2S_2O_3$ x 5 H_2O from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Increase amount of yeast extract to 2.00 g/l.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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785. BOGORIELLA MEDIUM

Peptone	5.0	g
Yeast extract	5.0	g
Glucose	10.0	g
KH_2PO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
NaCl	40.0	g
Na_2CO_3	10.0	g
Distilled water	1000.0	ml

Adjust pH to 9.6

Note: sterilize dissolved sodium carbonate and sodium chloride separately and mix with the other sterile components.

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786: DETHIOSULFOVIBRIO PEPTIDOVORANS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	3.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
Na-acetate	0.50	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	5.00	g
NaCl	30.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, thiosulfate, cysteine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. The pH of the complete medium should be 7.2 - 7.4.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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788: CALORAMATOR MEDIUM

KH_2PO_4	0.41	g
Na_2HPO_4 x 2 H_2O	0.52	g
NH_4Cl	2.40	g
$MgCl_2$ x 6 H_2O	0.40	g
Yeast extract	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.20	g
Na_2CO_3	1.50	g
D-Glucose	5.00	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except calcium chloride, carbonate, glucose, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Prior to use adjust pH of complete medium to 7.2 - 7.4, if necessary.

For DSM 12679: Omit yeast extract and supplement medium with 2.40 g/l NaCl.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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788: CALORAMATOR MEDIUM

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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789. DILUTE POTATO MEDIUM

Potato Decoction (see below)	100.00	ml
Peptone	0.05	g
Glucose	1.00	g
$Ca(NO_3)_2$ x 4 H_2O	0.05	g
Na_2HPO_4	0.12	g
Distilled water	900.00	ml

Adjust medium for final pH 6.8. Autoclave at 121°C for 15 min.

Potato decoction:

Diced potato	20.00	g
Distilled water	1000.00	ml

Boil for 30 min; filter out potato and return volume of liquid to 1000 ml.

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790. PEPTONE-YEAST MEDIUM with MgSO4

Peptone	10.0	g
Yeast extract, dehydrated	1.0	g
$MgSO_4$ x 7 H_2O	2.0	g
$(NH_4)_2SO_4$	2.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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791. PORPHYROBACTER TEPIDARIUS MEDIUM

Sodium glutamate	0.50	g/l
Sodium succinate	0.50	g/l
Sodium acetate	0.50	g/l
Yeast extract	0.50	g/l
Casamino acids	0.50	g/l
$(NH_4)_2SO_4$	0.50	g/l
Sodium thiosulphate	0.50	g/l
Basal salts solution	5.00	ml
Phosphate solution	5.00	ml
Vitamin solution (see medium 87a)	1.00	ml
Distilled water	989.00	ml
Final pH 7.5		

Add the vitamin solution, aseptically to the cooled, autoclaved medium.

Basal salts solution

Tri sodium EDTA	4.53	g/l
$FeSO_4$ x 7 H_2O	1.11	g/l
$MgSO_4$ x 7 H_2O	24.65	g/l
$CaCl_2$ x 2 H_2O	2.94	g/l
NaCl	23.40	g/l
$MnSO_4$ x 4 H_2O	112.00	mg/l
$ZnSO_4$ x 7 H_2O	29.00	mg/l
$Co(NO_3)_2$ x 6 H_2O	29.00	mg/l
$CuSO_4$ x 5 H_2O	25.00	mg/l
Na_2Mo_4 x 2 H_2O	24.00	mg/l
H_3BO_3	31.00	mg/l
Distilled water	1000.00	ml

Phosphate solution

KH_2PO_4	75.00	g/l
K_2HPO_4	78.00	g/l
Distilled water	1000.00	ml

Vitamin solution: See next page.

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Vitamin solution

Nicotinic acid	100.00	mg
Thiamine	100.00	mg
Biotin	5.00	mg
p-aminobenzoic acid	50.00	mg
Vitamin B_{12}	1.00	mg
Calcium pantothenate	50.00	mg
Pyridoxine hydrochloride	50.00	mg
Folic acid	50.00	mg
Tri-sodium EDTA	200.00	mg
Distilled water	100.00	ml

Filter sterilize.

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792: PYROLOBUS FUMARII MEDIUM

Solution A	989.00	ml
Solution B	10.00	ml
Solution C	1.00	ml
Solution D	10.00	ml

1. Sparge solution A with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials (e.g., 20 ml medium in 100 ml serum bottles) and autoclave. Solutions B and D are autoclaved separately under 100% N_2 gas atmosphere and solution C is prepared under 100% N_2 gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B, C and D are added to solution A. Adjust pH of the complete medium to 5.8 using a sterile anoxic stock solution of 5% (w/v) Na_2CO_3, if necessary.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

3. Note: Solution A has to be prepared freshly. It cannot be stored for a long period of time.

Solution A

NaCl	13.85	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
$CaCl_2$ x 2 H_2O	0.38	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
KI (0.01% w/v)	0.50	ml
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Double distilled water	980.00	ml

Solution B

KNO_3	1.00	g
KH_2PO_4	0.50	g
Distilled water	10.00	ml

Solution C

Seven vitamins solution	1.00	ml

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Solution D

Na_2S x 9 H_2O	0.15	g
Distilled water	10.00	ml

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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793: MOORELLA GLYCERINI MEDIUM

KH_2PO_4	0.33	g
NH_4Cl	0.33	g
KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Yeast extract	0.50	g
Glycerol (87%)	3.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins, and sulfide, then sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Dissolve carbonate and adjust pH to 6.7, then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration.

For DSM 111068: Omit glycerol and supplement medium after autoclaving with 2.50 g/l Na -lactate and 0.65 g/l Na_2SO_3 added from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. The solution of Na_2SO_3 should be sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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793: MOORELLA GLYCERINI MEDIUM

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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794: PSEUDOTHERMOTOGA HYPOGEA MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
Na-acetate	0.50	g
NaCl	10.00	g
Yeast extract (BD Bacto)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
$NaHCO_3$	2.00	g
D-Xylose	3.00	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, bicarbonate, xylose, thiosulfate and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve cysteine, adjust pH to 6.5, then dispense medium under 80% N_2 and 20% CO_2 gas mixture into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add thiosulfate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Xylose is prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g

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Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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795: STETTERIA MEDIUM

Synthetic seawater (2 x conc.)	500.00	ml
$NaHCO_3$	0.16	g
KH_2PO_4	0.50	g
Modified Wolin's mineral solution	15.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	3.00	ml
Peptone (BD Bacto)	2.00	g
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered), powdered	10.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	500.00	ml

Dissolve ingredients except sulfur and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle) that contain already the appropriate amount of sulfur. After closing pressurize bottles to 2 bar overpressure using 80% H_2 and 20% CO_2 gas mixture, than heat at 100°C for 1.5 hours on each of 3 successive days. Before use add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.0, if necessary. Incubate without shaking.

For DSM 25542, DSM 25543: Use medium without adding 80% H_2 and 20% CO_2 gas mixture before sterilization and adjust pH of complete medium to 6.8.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Synthetic seawater (2 x conc.) (from medium 795)

NaCl	55.40	g
SrCl2 x 6 H_2O	0.03	g
$MgSO_4$ x 7 H_2O	14.00	g
KI	0.10	mg
$MgCl_2$ x 6 H_2O	11.00	g
Na_3-citrate	20.00	mg
KCl	1.30	g
$CaCl_2$ x 2 H_2O	1.50	g
NaBr	0.20	g
H_3BO_3	0.06	g
Distilled water	1000.00	ml

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796: ARCHAEOGLOBUS VENEFICUS MEDIUM

NaCl	18.00	g
KCl	0.33	g
$MgCl_2$ x 6 H_2O	7.15	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4 x 3 H_2O	0.18	g
Trace element solution	1.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Na_2SO_3	1.00	g
Na-acetate	1.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, sulfite, acetate and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Thereafter, add and dissolve bicarbonate and equilibrate the medium with the 80% N_2 and 20% CO_2 gas mixture to achieve a medium pH of 7.0. Dispense medium under same gas atmosphere into serum vials (e.g., 25 ml per 100 ml bottle) and autoclave. After sterilization add sulfite, acetate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The stock solution of sulfite should be freshly prepared and sterilized by filtration. Prior to inoculation check pH of complete medium and adjust to 7.0, if necessary.

2. After inoculation, pressurize bottles to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Trace element solution

$MnCl_2$ x 4 H_2O	5.80	g
NaCl	10.00	g
$(NH_4)_2Ni(SO_4)_2$	2.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.00	g
$CuSO_4$ x 5 H_2O	0.10	g
H_3BO_3	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
Na_2WO_4 x 2 H_2O	0.10	g

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Na_2SeO_3 x 5 H_2O	0.10	g
Distilled water	1000.00	ml

Adjust pH to 1.0 with HCl.

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798. TINDALLIA MEDIUM

Na_2CO_3	8.0	g
KCl	0.2	g
NaCl	10.0	g
NH_4Cl	0.5	g
K_2HPO_4	0.2	g
Yeast extract	4.0	g
Trace elements SL-10 (see medium 320)	1.0	ml
Vitamin solution (see medium 141)	1.0	ml
Na_2S x 9 H_2O	0.5	g
Distilled water	1000.0	ml

pH 9.0

Prepare the medium anaerobically without the vitamins. Boil the medium, cool under N_2 and dispense under N_2 and autoclave the medium. Add the vitamin solution to the cooled, autoclaved medium.

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800. ANAEROBIOSPIRILLUM THOMASII MEDIUM

Use Wilkins Chalgrene Anaerobe Broth (Oxoid CM 643) and prepare it anaerobically under 80% N_2 + 20% CO_2 gas. After autoclaving and cooling to room temperature add per 10 ml the following sterile, anaerobic solutions:

5% (w/v) $NaHCO_3$ (flushed with N_2 + CO_2)	0.5	ml
Fildes Enrichment (BBL 20810) (under N_2)	1.0	ml
3% (w/v) cysteine-HCl x H_2O (under N_2)	0.1	ml
3% (w/v) Na_2S x 9 H_2O (under N_2)	0.1	ml

The pH of the completed medium should be 6.8 - 7.0. For growth an agar plates, Columbia sheep blood agar plates (BBL) may be used and incubated under an anaerobic atmosphere containig 5 - 10% CO_2.

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801. MALTEXTRACT/CHARCOAL MEDIUM

Malt extract	30.0	g
Charcoal (medical)	3.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Sterilize at 121°C for 10 min.

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802. GRACILIBACILLUS MEDIUM

NaCl	100.0	g/l
$MgSO_4$ x 7 H_2O	20.0	g/l
KCl	2.0	g/l
NaBr	0.1	g/l
Yeast extract (Difco)	2.0	g/l
Trypticase peptone (BBL)	2.0	g/l
Tris-HCl	12.0	g/l
Trace metals solution	2.0	ml
Distilled water	998.0	ml

Adjust pH to 7.8 (with NaOH) before autoclaving. To the sterile medium, from sterile stock solutions, add 10 ml KH_2PO_4 (50 g/l), 5 ml $CaCl_2$ (100 g/l), 2 ml $FeCl_2$/$MnCl_2$ ($FeCl_2$ x 4 H_2O, 20 g/l and $MnCl_2$ x 4 H_2O, 20 g/l), and glucose to a final concentration of 2 g/l.

Trace metals solution:

HCl (32%)	10.0	ml
$FeCl_2$ x 4 H_2O	2.0	g
$CoCl_2$ x 6 H_2O	250.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
$ZnCl_2$	70.0	mg
H_3BO_3	6.0	mg
Na_2MoO_4 x 2 H_2O	40.0	mg
$NiCl_2$ x 6 H_2O	70.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$AlCl_3$ x 6 H_2O	60.0	mg
Na_2WO_4 x 2 H_2O	6.0	mg
Distilled water	to 1000.0	ml

Dissolve salts in the HCl in the oder given above, then make up the solution to 1 litre with distilled water.

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803. SPHAEROTILUS-LEPTOTHRIX MEDIUM

Yeast extract	1.00	g
Peptone	1.50	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$	0.05	g
Ferric ammonium citrate	0.50	g
$MnSO_4$ x H_2O	0.05	g
$FeCl_3$ x 6 H_2O	0.01	g
Agar	20.00	g
Tap water	1000.00	ml

pH 7.1.

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804. HAEMOPHILUS MEDIUM

Mueller-Hinton broth	21.0	g
Yeast extract	5.0	g
Distilled water	1000.0	ml

For *Haemophilus* spp., add 15 mg NAD and 15 mg hemine per L (X + V factors).

NAD stock solution: dissolve 1 mg NAD per ml dist. water, filter sterilize.
Hemine stock solution: dissolve 50 mg hemine in 1 ml 1 M NaOH, make up to 25 ml with dist. water, filter sterilize.

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805. METHYLOBACTERIUM THIOCYANATUM MEDIUM

KSCN	0.25	g
$MgSO_4$ x 7 H_2O	0.10	g
K_2HPO_4	1.50	g
Na_2HPO_4 x 2 H_2O	7.90	g
Glucose	4.50	g
2% $FeSO_4$ x 7 H_2O	1.00	ml
Distilled water	1000.00	ml

To solid media add 15 g/l agar. Sterilise medium at 115°C for 10 minutes.

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807. HALOANAEROBIUM ALCALIPHILUM MEDIUM

$MgSO_4$ x 7 H_2O	17.00	g
NaCl	100.00	g
Solution A	50.00	ml
Solution B	50.00	ml
Trace element solution (see medium 320)	10.00	ml
Resazurin	1.00	mg
Yeast extract	10.00	g
Trypticase	10.00	g
Glucose	5.00	g
Na_2S x 9 H_2O	0.25	g
$NaHCO_3$	4.10	g
Cysteine-HCl x H_2O	0.50	g
Distilled water	890.00	ml

pH 7.0

Solution A

K_2HPO_4	6.00	g/l

Solution B

KH_2PO_4	6.00	g
$(NH_4)SO_4$	6.00	g
NaCl	12.00	g
$MgSO_4$ x 7 H_2O	2.60	g
NH_4Cl	2.50	g
$CaCl_2$ x 2 H_2O	0.28	g
K_2HPO_4	0.28	g

Prepare the medium without yeast extract, glucose, $NaHCO_3$, cysteine hydrochloride and Na_2S x 9 H_2O. Boil and then cool under a gas atmosphere of N_2:CO_2 (80:20 v/v) and add the $NaHCO_3$ and cysteine hydrochloride. Autoclave the medium and add from sterile stock solutions yeast extract, trypticase, glucose, and Na_2S x 9 H_2O. The final pH should be 7.0.

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809. ALCANIVORAX BORKUMENSIS MEDIUM

NaCl	23.00	g
$MgSO_4$ x 7 H_2O	5.80	g
$MgCl_2$ x 2 H_2O	6.16	g
$CaCl_2$ x 2 H_2O	1.47	g
Na_2HPO_4 x 7 H_2O	0.89	g
$NaNO_3$	5.00	g
$FeSO_4$ x 7 H_2O	0.03	g
Sodium pyruvate	10.00	g
Distilled water	1000.00	ml

pH 7.0 - 7.5

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810. SKIM MILK/GLUCOSE AGAR

Solution A:

Skim milk	40.0	g
Water	500.0	ml

Solution B:

Glucose	20.0	g
Agar	20.0	g
Water	500.0	ml

Autoclave separately, mix and adjust pH to 6.8

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812. SULFOBACILLUS DISULFIDOOXIDANS MEDIUM

$(NH_4)_2SO_4$	3.0	g
KCl	0.1	g
KH_2PO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.5	g
$Ca(NO_3)_2$ x 4 H_2O	0.1	g
Yeast extract	0.1	g
Distilled water	1000.0	ml

Adjust pH to 2.25 prior to autoclaving. Before use add per liter 10 ml of a filter sterilized 10% (w/v) glutathione solution.

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813: DESULFONATRONUM MEDIUM

KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
NH_4Cl	1.00	g
KCl	0.20	g
Na_2SO_4	5.00	g
NaCl	10.00	g
Trace element solution SL-10	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	3.50	g
Na_2S x 9 H_2O	0.50	g
Na-formate	4.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve the ingredients (except carbonate, sulfide, formate and vitamins) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonate and sulfide, then adjust pH to 8.8 - 9.0, dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add formate and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N_2 gas.

For DSM 26308: Replace formate with 2.0 g/l Na-L-lactate and adjust pH of the complete medium to 9.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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814: METHANOBACTERIUM SUBTERRANEUM MEDIUM

NH_4Cl	0.40	g
$MgCl_2$ x 6 H_2O	0.03	g
NaCl	0.45	g
K_2HPO_4	0.50	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	3.00	ml
Modified Wolin's mineral solution	10.00	ml
Tryptone (BD Bacto)	1.00	g
Na-formate	3.20	g
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 8.0 - 8.5.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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815: DESULFOHALOTOMACULUM MEDIUM

Na_2SO_4	3.00	g
$CaCl_2$ x 2 H_2O	6.00	g
NaCl	40.00	g
KCl	2.00	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	8.00	g
SrCl2 x 6 H_2O	0.10	g
Trace element solution SL-11	1.00	ml
MOPS	3.00	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Iron powder (or nails)	100.00	g
Na-L-lactate	3.60	g
Na_2CO_3	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except iron, lactate and carbonate, adjust pH to 6.0 with NaOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials containing 1 - 2 g iron powder or an iron nail. Autoclave for 30 min at 105°C. Before use add lactate from a sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Final medium pH should be 7.2.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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816: SPOROTOMACULUM MEDIUM

Solution A	920.00	ml
Solution B	1.00	ml
Solution C	1.00	ml
Solution D	50.00	ml
Solution E	10.00	ml
Solution F	5.00	ml
Solution G	5.00	ml
Solution H	10.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B, C, F, G, and H are autoclaved separately under 100% N_2 gas. Solution D is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere and autoclaved. Solution E is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be checked and adjusted to 7.6 with a sterile anoxic stock solution of Na_2CO_3 (5% w/v), if necessary.

Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.30	g
KCl	0.50	g
NaCl	1.00	g
$CaCl_2$ x 2 H_2O	0.15	g
$MgCl_2$ x 6 H_2O	0.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B

Trace element solution SL-10	1.00	ml

Solution C

Selenite-tungstate solution	1.00	ml

Solution D

Na_2CO_3	2.50	g
Distilled water	50.00	ml

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Solution E

3-Hydroxybenzoic acid	2.80	g
Distilled water	10.00	ml

Neutralized with NaOH.

Solution F

Yeast extract	0.50	g
Distilled water	5.00	ml

Solution G

Trypticase (BD BBL)	0.50	g
Distilled water	5.00	ml

Solution H

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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817: THERMOSPHAERA MEDIUM

$MgCl_2$ x 6 H_2O	2.20	g
NaCl	0.90	g
KCl	17.00	mg
NH_4Cl	12.00	mg
$CaCl_2$ x 2 H_2O (0.1% w/v)	7.00	ml
K_2HPO_4 x 3 H_2O (0.1% w/v)	7.00	ml
$FeCl_3$ (0.01% w/v in 0.2 N HCl)	0.50	ml
Yeast extract (BD Bacto)	1.00	g
Peptone (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 6.5.

2. After inoculation pressurize tubes or bottles (heavy walled) to 2 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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818: CHRYSIOGENES MEDIUM

NaCl	1.20	g
KCl	0.30	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
Na_2SO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	0.60	g
KNO_3	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na-acetate	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, nitrate, vitamins and acetate. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, adjust pH to 7.0 - 7.4, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add nitrate, vitamins and acetate from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 7.4 - 7.8, if necessary.

For DSM 16346: Supplement medium with 0.80 g/l yeast extract and replace Na-acetate with 1.10 g/l Na-L-lactate.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0

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and bring volume to 1000 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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819: SEDIMINISPIROCHAETA SMARAGDINAE MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2 \times 6\ H_2O$	0.20	g
$CaCl_2 \times 2\ H_2O$	0.10	g
KCl	0.20	g
NaCl	50.00	g
Yeast extract	5.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2CO_3	1.00	g
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, carbonate and sulfide), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Then, add cysteine, distribute medium under 80% N_2 and 20% CO_2 gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Check pH of complete medium and adjust to pH 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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822. FRR-6, FRR-8 MEDIUM

Yeast extract	1.0	g
Proteose peptone (Difco No.3)	1.0	g
Casamino acids (Difco)	1.0	g
Glucose	1.0	g
NaCl	1.0	g
Na pyruvate	0.5	g
$MgSO_4$ x 7 H_2O	0.1	g
K_2HPO_4	0.6	g
Distilled water	1000.0	ml

Final pH 7.2.

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823. HALOBACULUM GOMORRENSE MEDIUM

NaCl	125.0	g
$MgCl_2$ x 6 H_2O	160.0	g
K_2SO_4	5.0	g
$CaCl_2$ x 2 H_2O	0.1	g
Yeast extract (Difco)	1.0	g
Casamino acids (Difco)	1.0	g
Starch	2.0	g
Distilled water	1000.0	ml

pH 7.0

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824: TISSIERELLA CREATINOPHILA MEDIUM

Na-formate	2.72	g
KH_2PO_4	0.20	g
NaCl	0.50	g
KCl	1.00	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.25	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Creatine	3.80	g
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins and sulfide. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.5 - 8.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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825: METHANOBACTERIUM II MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1011 ml

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Modified Wolin's mineral solution	10.00	ml
Na-acetate	0.50	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, vitamins, cysteine and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 7.0, then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. After inoculation pressurize cultivation vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 24406: Supplement medium with 0.5 g/l DL-dithiothreitol (DTT), 20 ml/l methanol (50% v/v), and 0.5 g/l 2-mercaptoethanesulfonate (coenzyme M) added to the autoclaved medium from sterile anoxic stock solutions. The stock solution of DTT has to be sterilized by filtration. Omit Na_2S x 9 H_2O and L-cysteine HCl x H_2O. Adjust the pH of the final medium to 6.5.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g

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NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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826: GEOBACTER SULFURREDUCENS MEDIUM

NH_4Cl	1.50	g
Na_2HPO_4	0.60	g
KCl	0.10	g
Na-acetate	0.82	g
Modified Wolin's mineral solution	10.00	ml
$NaHCO_3$	2.50	g
Na_2-fumarate	8.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, fumarate and vitamins), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate and equilibrate the medium with 80% N_2 and 20% CO_2 gas mixture to reach a pH of 6.8, then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add fumarate and vitamins from anoxic stock solutions prepared under 100% N_2 gas atmosphere and sterilized by filtration. Check medium pH and adjust to 6.8, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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827: THERMOANAEROBACTER SULFUROPHILUS MEDIUM

NH_4Cl	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Na_2CO_3	1.00	g
Yeast extract	0.50	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, carbonate, yeast extract, glucose, vitamins, and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Heat the medium vials to 90-100°C for 1 hour on each of 3 successive days to sterilize the medium. Add yeast extract, glucose, vitamins (sterilized by filtration), and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

For DSM 16547: Supplement medium with 3.00 g/l Trypticase peptone.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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828. NEVSKIA MEDIUM

Potassium phosphate buffer, 1M, pH 7	27.50	ml
$MgSO_4$ x 7 H_2O	0.05	g
$CaCl_2$ x 2 H_2O	14.00	mg
Na-lactate	0.56	g
Trace element solution SL-9 (see below)	0.50	ml
Seven vitamins solution (see medium 503)	0.50	ml
Agar, if necessary	15.00	g
Distilled water	972.50	ml

Adjust the pH to 7.0 - 7.2.
Filter sterilize the vitamin solution and add to the autoclaved medium.

SL-9:
Prepare SL-10 (see medium 320) with 12.8 g/l nitrilotriacetic acid (NTA) and 2 g/l $FeCl_2$ x 4 H_2O. Adjust pH to 6.0.

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829: DESULFUROBACTERIUM MEDIUM

Sea Salt (SIGMA)	30.00	g
NH_4Cl	1.00	g
KH_2PO_4	0.35	g
MES [2-(N-morpholino) ethane sulfonic acid]	1.95	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Na_2CO_3	0.50	g
Seven vitamins solution	1.00	ml
Growth-stimulating factors	1.00	ml
Na-dithionite solution (5% w/v)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, carbonate, vitamins, growth-stimulating factors and Na-dithionite), boil medium for 1 min, then cool to room temperature under 80% H_2 and 20% CO_2 gas atmosphere. Adjust pH to 6.0 and dispense under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large head space. Autoclave at a temperature of **110°C** for 20 min! Add vitamins and growth-stimulating factors from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate and Na-dithionite from sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins and Na-dithionite are sterilized by filtration. Adjust pH of the complete medium to 6.0, if necessary.

2. After inoculation use 1 bar overpressure of sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 16661, DSM 21157: Omit growth-stimulating factors and pressurize vials after inoculation to 2 bar with sterile 80% H_2 and 20% CO_2 gas mixture.

Growth-stimulating factors (from medium 829)

Isobutyric acid	5.00	g
Valeric acid	5.00	g
2-Methylbutyric acid	5.00	g
3-Methylbutyric acid	5.00	g
Caproic acid	2.00	g
Succinic acid	6.00	g
Distilled water	1000.00	ml

Dissolve ingredients, adjust pH to 9.0 with NaOH, then autoclave under 100% N_2 gas.

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Na-dithionite solution (5% w/v) (from medium 829)

$NaHCO_3$	50.00	g
$Na_2S_2O_4$	50.00	g
Distilled water	1000.00	ml

Dissolve $NaHCO_3$ in water and make the solution anoxic by sparging with 80% N_2 and 20% CO_2 gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.

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830: R2A MEDIUM

Yeast extract	0.50	g
Proteose peptone (Difco no. 3)	0.50	g
Casamino acids	0.50	g
Glucose	0.50	g
Starch (soluble)	0.50	g
Na-pyruvate	0.30	g
K_2HPO_4	0.30	g
$MgSO_4$ x 7 H_2O	0.05	g
Agar	15.00	g
Distilled water	1000.00	ml

Final pH 7.2; adjust with crystalline K_2HPO_4 or KH_2PO_4 before adding agar. Add agar, heat medium to boiling to dissolve agar, and autoclave for 15 min at 121°C.

For DSM 2406: Rehydrate and grow freeze-dried cells from ampoule in liquid medium 54 or 830 first. Subsequent subcultivation may be carried out in liquid medium or on agar plates.

For DSM 4136: add 1% NaCl

For DSM 6696: Rehydate and grow freeze dried cells from ampoule in liquid medium 830. Subsequent subcultivation may be carried out in liquid medium or on agar plates, or in medium 457 with styrol

For DSM 6708: Rehydrate and grow lyophylisate in liquid medium 830. Subsequently, subculturing may be carried out in liquid medium or on agar plates, on medium 830, 464 or 465 as indicated

For DSM 6780: Reactivate freeze dried culture in medium 830 (liquid medium and agar plate) and incubate for up to 14 days

For DSM 11199: Aerobic; reactivate lyophilized cells in liquid medium 830, incubate for at least 10 days

For DSM 12184: liquid

For DSM 12445: Rehydrate and grow freeze-dried cells from the ampoule in medium 830, subsequent subculturing may be carried out in medium 457 with carbon source or in medium 830 or 464.

For DSM 13665: With or without 10 mg/l 2,4,6-trichlorophenol and 20 mg/l pentachlorophenol

For DSM 14655: Rehydrate and grow lyophilized cells from the ampoule in 5 ml liquid broth 1. Subsequent subculturing may be carried out in liquid medium or on agar plates

For DSM 14692: Aerobic, rehydrate and grow lyophilized cells in liquid medium. Subsequent subculturing may be carried out on agar plates also.

For DSM 14804: Only one passage beginning with mineral medium with hexane is possible

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For DSM 14805: Aerobic; reactivate freeze dried cells in liquid medium.

For DSM 15083: Add 1% methanol; aerobe

For DSM 15124: Aerobic. Rehydrate and grow lyophilized cells from ampoules in liquid broth 830. Subsequent subculturing may be carried out in liquid medium or on agar plates.

For DSM 15892: Cultivate in liquid medium, only thin colonies on agar plate

For DSM 16361: Omit Agar. Adjust pH of final medium to 6.0.

For DSM 16616: Rehydrate and grow lyophilized cells from the ampoule in 5 ml liquid broth. Subsequent subculturing may be carried out in liquid medium or on agar plates.

For DSM 16845: Rehydrate and grow freeze dried cells from ampoule in liquid medium 830. Subsequent subsculturing may be carried out in medium 465 with TM or on agar plates, too.

For DSM 16968: Plus $MnSO_4$ (10mg/l)

For DSM 16969, DSM 18135, DSM 21316, DSM 21346, DSM 21568: Plus $MnSO_4$

For DSM 16976, DSM 29786, DSM 29790, DSM 29794: PH 9.0

For DSM 17513: Rehydrate and grow freeze-dried cells from ampoule in liquid medium 830 first. Subsequent subcultivation may be carried out in liquid medium or on agar plates and in medium 457 with 3-chlorosalicylate.

For DSM 17870: Rehydrate and grow lyophilized cells from the ampoule in 5 ml liquid broth 830. Subsequent subculturing may be carried out in liquid medium or on agar plates

For DSM 17871: Plus $MnSO_4$ (10 mg/l)

For DSM 17997: Adjust pH to 10 by adding 200 ml 0,5M Na_2CO_3

For DSM 17998: Adjust pH to 10 by adding 200ml 0.5M sodium carbonate [Na_2CO_3]

For DSM 17999: Add 0.5 M Na_2CO_3, pH 10.0

For DSM 18042, DSM 18043, DSM 18044, DSM 18045, DSM 45021: Plus 10% NaCl

For DSM 18094, DSM 19250: Preferably liquid medium

For DSM 18203: Plus 2g/l methanol

For DSM 18221: Keep agar surface moist with a film of liquid medium

For DSM 18504, DSM 18629: adjust pH to 5.5

For DSM 18603: 10-fold diluted and supplemented with 0.05% glucose

For DSM 18644: Growth is better on solid media if the surface is covered with a layer of liquid medium

For DSM 18693: Dilute the medium 10 fold and adjust the pH to 5.0-5.5

For DSM 18704: Dilute the medium 10 fold and adjust the pH to 4.5-5.0

For DSM 18758: Dilute the medium 1:10, adjust the pH to 5.5

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For DSM 18855: Microaerobic (candle jar)

For DSM 19016, DSM 19017, DSM 19018: Reactive in M. 1712; aerobic

For DSM 19019: Reactivate in M. 1712; aerobic

For DSM 19020: Reactive in M. 1712; aerob

For DSM 19021: Microaerobic, growing at 2-4% oxygen in atmosphere

For DSM 19096: (one-fifth-strength)

For DSM 19249: Preferably liquid medium

For DSM 19309: (dilute 1:5)

For DSM 19717, DSM 19724: With 5 g/l NaCl

For DSM 19937: PH: 9.5

For DSM 21036: Dilute medium 1:1 with distilled water

For DSM 21240: With NaCl (20g/l) and MgCl2x 6H2O (3g/l)

For DSM 21362: 1 drop of 10x Wolin Vitamine Solution (see medium 141) and 10 microliter trace element solution SL-10 (see medium 320) for better growth in liquid

For DSM 21439: 1:2 diluted

For DSM 21490: 1:5 diluted, with cysteine and pyruvate (0.2 g/l each); keep agar surface moist

For DSM 21594: At 20 °C or 28 °C; (this medium is recommended for the routine cultivation of the strain)

For DSM 21599, DSM 21701: diluted 1:1

For DSM 21636: At half concentration

For DSM 21646: Keep agar surface moist and incubation atmosphere humid

For DSM 21648: Slowly growing, keep agar surface moist

For DSM 21670: With 10 mg/L $MnSO_4$, adjust to pH 9 with Na-sesquicarbonate

For DSM 21675: Adjust pH to 7.5

For DSM 21697: PH 7.2

For DSM 21712: Diluted 1:2

For DSM 21788, DSM 21937, DSM 21938, DSM 23562: PH 7

For DSM 21791: PH 6.2

For DSM 21895: Keep agar surface moist during the whole incubation period

For DSM 21942: Plus Na-sesquicarbonate solution (see medium 31); pH 9.0

For DSM 21953, DSM 21954: half concentrated and prepared with 75% seawater

For DSM 21994: Dilute R2A medium 1:5 and add Na-pyruvate and cysteine-HCl x H_2O, 2 g

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per L each

For DSM 22177, DSM 23415: half strengh

For DSM 22274, DSM 22275, DSM 22383, DSM 22384, DSM 22899, DSM 22900, DSM 23227, DSM 23324, DSM 23450, DSM 23456, DSM 23459: PH 7.0

For DSM 22342: Plus 10 mg/l $MnSO_4$

For DSM 22349: Diluted 1:5. Add Na-pyruvate and cysteine-HCl x H_2O, 0,2 g per L each

For DSM 22385: PH 6.0 - 8.0

For DSM 22386: PH 6.5 - 8.0

For DSM 22405: Plus 10 mg/L $MnSO_4$

For DSM 22464: Dilute the medium 20 fold and add 0.05% fructose, adjust pH 4.0 - 5.0

For DSM 22489: 20-fold diluted and supplied with 0.05% glucose or fructose, pH 5.0 - 5.8

For DSM 22513: PH 7.3

For DSM 22869: Plus 10 µg/ml $MnSO_4$

For DSM 23054: PH 7.0 - 8.0

For DSM 23119: Adjust the pH of the medium to 6.0

For DSM 23128, DSM 23136, DSM 23137, DSM 23138: Adjust pH to 5.0 - 5.6 with HCl. Solidify with 5g/l agar

For DSM 23228: Plus 10 mg/L $MnSO_4$; pH 8.5

For DSM 23480: Half strength substrate concentration

For DSM 23489, DSM 23491: Plus 10 mg/L $MnSO_4$; pH 7.0

For DSM 23490: Plus 3% NaCl plus 10 mg/L $MnSO_4$; pH 7.0

For DSM 23630: (leave out phosphate and pyruvate. Dilute 1:10 and adjust pH to 4.5 - 5.0 with alginic acid (or MES)); fill tubes for anaerobes (Hungate) half with medium, close tightly. Do not shake.

For DSM 23641: PH 5.5, use gelrite instead of agar

For DSM 23886: Dilute the medium 10 fold, add 0.02% glucose and adjust the pH to 5.0 - 5.5

For DSM 23906: Plus 0.1% Tween 80

For DSM 24297: 1:10 diluted, pH 4.5, without pyruvate, without phosphate

For DSM 24448: With 1mg/l riboflavine

For DSM 24462: Plus 100 µl Anaerobiersalze (salt solution of DSMZ Medium 104)

For DSM 25158: Subculturing is recommended to be carried out on agar plates because growth in liquid medium is weak

For DSM 25262: Plus 1.0 g/l NaCl

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For DSM 25320: Plus 0,1% Tween 80

For DSM 25321: Plus Tween 80

For DSM 25461: With 5 ml vitamin solution, see e.g. medium 461

For DSM 26743: plus 0.5 g/l mannitol

For DSM 26776: PH 6.0

For DSM 27573: Keep agar surface moist during incubation. No visible growth in liquid media

For DSM 27864: Plus vitamin solution (2ml/L); see medium 141

For DSM 27942: Liquid media require heavy inoculum

For DSM 28057, DSM 29854: PH 8.0

For DSM 28206: pH 5.5

For DSM 28215: pH 5,5

For DSM 28344: Rehydrate and grow lyophilized cells from the ampoule in 5 ml liquid broth. Subsequent subculturing may be carried out in liquid medium or on agar plates

For DSM 29249: 20% R2A; 20-37°C, pH 6.0-8.0 optimum

For DSM 29289: 1:10 diluted

For DSM 29414, DSM 45618, DSM 29840: PH 5.0

For DSM 45540: pH 5,3

For DSM 45816: pH 6.5

For DSM 100059: Only 25% strenth of the origianl medium 830

For DSM 100467, DSM 100468, DSM 100482: plus 30 g/l NaCl

For DSM 100496, DSM 100572: Plus 30 g/l NaCl

For DSM 100788: PH=9.0

For DSM 102969: Plus 1% methanol

For DSM 103219: add 0.1% methylammoniumchloride

For DSM 103337: Slow growth, poor growth in liquid media

For DSM 103476: Twofold concentrated; incubation period 6-8 d

For DSM 104092, DSM 104093: Add 0.06% sodium pyruvate

For DSM 105494: Plus 0,1% Methylamine

For DSM 105890: One-tenth-strength

For DSM 106290, DSM 103404, DSM 106523: pH 10, plus 2% NaCl

For DSM 106492: PH 6.6

For DSM 106737, DSM 106674, DSM 106675, DSM 108426: PH 8.5

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For DSM 108208: braucht pH 8.5, wächst nicht auf pH 7.2

For DSM 108248: 1/10 diluted

For DSM 108317: braucht pH 8.5, wächst nicht auf pH 7.2 nach LN

For DSM 108346: pH 8.5

For DSM 109470, DSM 109471: PH 5.9

For DSM 109757: cover agar with liquid medium 830

For DSM 110366: Only 20 mg/L of medium 830

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831. PEPTON-CORN MEAL MEDIUM

Starch	10.0	g
NaCl	5.0	g
$CaCl_2$	0.5	g
Pepton	5.0	g
Corn meal Agar (Oxoid CM103)	17.0	g
Distilled water	1000.0	ml

pH 7.2.

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833: DEHALOSPIRILLUM MEDIUM

Solution A	892.00	ml
Solution B	10.00	ml
Solution C	2.00	ml
Solution D	35.00	ml
Solution E	20.00	ml
Solution F	40.00	ml
Solution G	3.00	ml
Solution H	1.00	ml

1. Sparge solution A with 100% N_2 gas for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B and H are autoclaved separately under 100% N_2 gas. Solutions C, E, F and G are prepared under 100% N_2 gas and sterilized by filtration. Solution D is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.3 - 7.6, if necessary.

2. Note: Before inoculation, the medium must be completely reduced, i.e. resazurin must be colorless. The addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) can help to reduce the medium.

Solution A

Na_2SO_4	0.70	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	0.25	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	2.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	890.00	ml

Solution B

Potassium phosphate buffer (0.1 M, pH 7.5)	10.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

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Seven vitamins solution	1.00	ml

Solution D

Na_2CO_3	1.75	g
Distilled water	35.00	ml

Solution E

Na-pyruvate	4.50	g
Distilled water	20.00	ml

Solution F

Na_2-fumarate	6.40	g
Distilled water	40.00	ml

Solution G

$FeSO_4$ x 7 H_2O	30.00	mg
H_2SO_4 (0.1 N)	3.00	ml

Solution H

L-Cysteine HCl x H_2O	50.00	mg
Distilled water	1.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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834: COPROTHERMOBACTER PLATENSIS MEDIUM

Meat peptone (BD Bacto)	5.00	g
Tryptone (BD Bacto)	5.00	g
Yeast extract	10.00	g
KH_2PO_4	0.30	g
NaCl	0.60	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.08	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins and cysteine), boil medium for 1 min, then cool to room temperature under 100% N_2 gas atmosphere. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation, add vitamins (sterilized by filtration) and cysteine from sterile anoxic stock solution prepared under 100% N_2 gas. Adjust pH of complete medium to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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835: DESULFUROMONAS CHLOROETHENICA MEDIUM

Solution A	921.00	ml
Solution B	35.00	ml
Solution C	25.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 -45 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions D and F are autoclaved under 100% N_2 gas atmosphere. Solutions C and E are prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0 - 7.2.

Solution A

NH_4Cl	0.40	g
KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.30	g
NaCl	0.40	g
$CaCl_2$ x 2 H_2O	10.00	mg
Trace element solution SL-10	1.00	ml
Na-acetate	0.41	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B

Na_2CO_3	1.75	g
Distilled water	35.00	ml

Solution C

Na_2-fumarate	3.20	g
Distilled water	25.00	ml

Solution D

L-Cysteine HCl x H_2O	0.25	g
Distilled water	10.00	ml

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Solution E

Wolin's vitamin solution (10x)	1.00	ml

Solution F

Na_2S x 9 H_2O	0.25	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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836: DESULFITOBACTERIUM PCP-1 MEDIUM

KH_2PO_4	0.27	g
K_2HPO_4	0.35	g
NH_4Cl	0.53	g
$MgCl_2$ x 6 H_2O	0.10	g
$FeCl_2$ x 4 H_2O (0.1% w/v in 0.2 N HCl)	2.00	ml
$CaCl_2$ x 2 H_2O	0.07	g
Trace element solution SL-10	1.00	ml
Yeast extract	1.00	g
Na-pyruvate	6.05	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.50	g
Na_2SO_3	0.63	g
Na_2S x 9 H_2O	0.05	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, sulfite and sulfide), adjust pH of solution to 7.0 and sparge with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to use add sulfite and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The stock solution of sulfite should by freshly prepared and sterilized by filtration. Prior to use check pH of complete medium and adjust to 7.5, if necessary.

For DSM 13498: Supplement medium with 3.00 g/l Na-formate.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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837: DESULFUROMONAS PALMITATIS MEDIUM

Solution A	880.00	ml
Solution B	50.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	50.00	ml
Solution F	1.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 100% N_2 gas atmosphere. Solutions C and F are prepared under 80% N_2 and 20% CO_2 gas atmosphere. Solutions D and E are prepared under 100% N_2 gas atmosphere. Solution C is autoclaved and solutions D, E and F are sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 6.9, if necessary.

2. Note: For transfers use 5 - 10% (v/v) inoculum.

Solution A

NaCl	20.80	g
KCl	0.77	g
NH_4Cl	1.00	g
KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.02	g
Na-acetate x 3 H_2O	1.36	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	860.00	ml

Solution B

$MgCl_2$ x 6 H_2O	10.60	g
$CaCl_2$ x 2 H_2O	1.50	g
Distilled water	50.00	ml

Solution C

Na_2CO_3	1.00	g
Distilled water	20.00	ml

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Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2-fumarate	8.00	g
Distilled water	50.00	ml

Solution F

Na-dithionite solution (5% w/v)	1.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg

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Distilled water	1000.00	ml

Na-dithionite solution (5% w/v) (from medium 829)

$NaHCO_3$	50.00	g
$Na_2S_2O_4$	50.00	g
Distilled water	1000.00	ml

Dissolve $NaHCO_3$ in water and make the solution anoxic by sparging with 80% N_2 and 20% CO_2 gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.

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838: GEOANAEROBACTER MEDIUM

Fe(III) citrate (19% Fe)	10.00	g
KH_2PO_4	0.60	g
NH_4Cl	0.30	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.50	g
Na-acetate	0.80	g
Na-ascorbate	0.80	g
Distilled water	1000.00	ml

First dissolve ferric citrate by heating the water under continuous stirring. After cooling to room temperature adjust the pH to 6.0, then add and dissolve the remaining ingredients, except carbonate, acetate and ascorbate. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add acetate and ascorbate from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to inoculation check the medium pH and adjust to 6.8, if necessary.

For DSM 9736: Supplement medium with 1.00 g/l NaCl. After autoclaving supplement medium with 1.00 ml/l of seven vitamins solution (see medium 503) and replace Na -ascorbate with 0.15 g/ of L-cysteine-HCl x H_2O added from a sterile anoxic stock solution prepared under 100% N_2 gas.

For DSM 15288, DSM 16228: Omit Na-acetate and Na-ascorbate. Supplement medium after autoclaving with 10.00 ml/l of a Wolin's vitamin solution (see medium 141), 1.00 g/l Na-DL-lactate and 1.00 g/l yeast extract added from sterile anoxic stock solutions prepared under 100% N_2 gas.

For DSM 16401: Supplement medium with 20.00 g/l NaCl and omit Na-ascorbate. After autoclaving supplement medium with 10.00 ml/l of a sterile anoxic Wolin's vitamin solution (see medium 141).

For DSM 19350, DSM 22248, DSM 24905, DSM 26175: Prepare medium without Na -ascorbate.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg

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H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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840. SERPULINA MURDOCHII MEDIUM

Trypticase Soy Agar (BBL 11043) with 5% defibrinated sheep blood or Columbia Agar Plates with 5% sheep blood (BBL 4354005).

Cultivation in liquid medium:

Prepare Trypticase Soy Broth (BBL 11774) under 80% N_2 + 20% CO_2 gas mixture in Hungate tubes (5 ml per tube) or in 100 ml serum bottles (10 ml per bottle).
After autoclaving and cooling add (per tube/per bottle):

0.30 ml/0.60 ml sterile 10% w/v $NaHCO_3$ solution
0.35 ml/0.70 ml sterile bovine serum
(heat inactivated at 56°C for 45 min)
0.05 ml/0.10 ml sterile 30% w/v glucose solution
0.5 ml/5.0 ml air

Incubate inoculated tubes in slanted position.

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841. MSV-THIOTRIX-MEDIUM

Solution I:

$(NH_4)_2SO_4$	0.500	g
$MgSO_4$ x 7 H_2O	0.100	g
$CaCl_2$ x 2 H_2O	0.050	g
K_2HPO_4	0.110	g
KH_2PO_4	0.085	g
$FeCl_3$ x 6 H_2O	0.002	g
Na_2-EDTA	0.003	g
Na-acetate	0.546	g
$Na_2S_2O_3$ x 5 H_2O	0.400	g
Distilled water	991.000	ml

Autoclave for 20 min. at 121°C.

Solution II:
$NaHCO_3$ 2.625 g per 50 ml distilled water

Autoclave for 20 min. at 121°C and add 8 ml/l sterile solution II shortly, before inoculation of bacterial culture.

Solution III:
H_3P-vitamine solution (see medium 428)

Filter sterilize vitamine solution and add 1 ml/l medium.

Solution IV:
10% inositol (1g/10 ml distilled water)

Filter sterilize solution IV and add 0.1 ml/l medium.
Final pH of appr. 7.4 should be controlled sterile.

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842. HALOANAEROBACTER SALINARIUS MEDIUM

NaCl	150.00	g
$MgCl_2$ x 6 H_2O	15.00	g
KCl	3.00	g
NH_4Cl	0.50	g
KH_2PO_4	0.35	g
$CaCl_2$ x 2 H_2O	0.05	g
Yeast extract	0.10	g
Na_2S x 9 H_2O	0.50	g
$NaHCO_3$	2.00	g
Glucose	1.20	g
Resazurin	1.00	mg
Trace element solution SL12 (see medium 28)	1.00	ml
Selenite-tungstate solution (see below)	1.00	ml
Vitamin solution 7 (see below)	1.00	ml
Distilled water	997.00	ml

Final pH 7.2 - 7.4

Prepare the medium without yeast extract, Na_2S x 9 H_2O, $NaHCO_3$, glucose and vitamin solution (prepared as anaerobic, sterile stock solutions). Boil the medium and cool under anaerobic gas mixture of N_2:CO_2 (80:20). Dispense medium into Hungate tubes or serum bottles using the same gas mixture. Autoclave. Add the remaining components to the medium once it has cooled and check the final pH.

Selenite-tungstate solution:

Na_2SeO_3 x 5 H_2O	6.00	mg
Na_2WO_4 x 2 H_2O	8.00	mg
NaOH	0.40	g
Distilled water	1000.00	ml

Vitamin solution 7:

Biotin	10.00	mg
p-Aminobenzoic acid	50.00	mg
Vitamin B_{12}	50.00	mg
Thiamine	100.00	mg
Distilled water	1000.00	ml

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843: HELMUTKOENIGIA MEDIUM

NaCl	1.00	g
KCl	0.50	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
Na_2SO_4	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Sugar mix	30.00	ml
Yeast autolysate	50.00	ml
Seven vitamins solution	1.00	ml
Co-factors solution	20.00	ml
DL-Dithiothreitol (DTT)	0.40	g
Distilled water	900.00	ml

1. Dissolve ingredients (except carbonate, sugar mix, yeast autolysate, vitamins, co-factors, and DTT), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume (e.g., 4.5 ml per Hungate-type tube) and autoclave. After sterilization add carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere and sugar mix, yeast autolysate, vitamins, co-factors and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and sterilized by filtration. Check pH of complete medium and adjust to 7.2 - 7.4, if necessary.

2. Note: Basal medium and yeast autolysate have to be prepared freshly and cannot be stored for longer periods.

Sugar mix (from medium 843)

Cellobiose	56.70	g
Sucrose	56.70	g
Maltose x H_2O	60.00	g
Trehalose	63.30	g
D-Xylose	25.00	g
Distilled water	1000.00	ml

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Yeast autolysate

Yeast extract (dry baker's yeast, SIGMA YSC-1)	280.00	g
Distilled water	1000.00	ml

Suspend dry baker's yeast (SIGMA YSC-1) in distilled water and allow cells to autolyse for 48 h at 55°C. Thereafter, cell debris are removed by centrifugation (3.700 x g for 30 min) and the amber-colored supernatant is stored in aliquots at -20°C.

Co-factors solution

Thiamine pyrophosphate	2.50	g
Pyridoxal hydrochloride	0.25	g
Pyridoxal phosphate	0.25	g
Ca-folinate	0.05	g
ß-NAD	0.05	g
Coenzyme A	0.05	g
FAD	0.05	g
Haemin (dissolved separately in 10 mM NaOH)	65.00	mg
Nicotinamide	25.00	mg
Folic acid	2.50	mg
Riboflavin	0.50	mg
Distilled water	1000.00	ml

Adjust pH to 7.0 with 1 N NaOH against indicator paper, filter sterilize and store under 100% N_2 gas atmosphere.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g

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Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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844: BACTERIOVORAX MEDIUM

Proteose peptone no. 3 (BD Bacto)	1.00	g
Yeast extract (BD Bacto)	0.30	g
$MgCl_2$ x 6 H_2O	0.60	g
$CaCl_2$ x 2 H_2O	0.30	g
Agar (Bacto) (optional)	12.00	g
Distilled water	1000.00	ml

Dissolve ingredients except magnesium chloride and calcium chloride. Add magnesium chloride and calcium chloride after autoclaving from sterile stock solutions of 5% (w/v) each. For agar plates add 1.2% Bacto agar

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845: SPIRILLUM WINOGRADSKYI MEDIUM

Peptone (BD Bacto)	5.00	g
Disodium succinate	1.00	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_2O	1.00	g
$FeCl_3$ x 6 H_2O	2.00	mg
$MnSO_4$ x H_2O	2.00	mg
Agar (BD Bacto)	1.00	g
Trace element solution SL-11	1.00	ml
Seven vitamins solution	1.00	ml
Catalase (2000 U/ml)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except trace elements, vitamins and catalase, adjust pH to 7.2 - 7.4, bring medium to the boil and cool to room temperature under 100% N_2 gas atmosphere. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. To complete the medium add trace elements, vitamins and catalase from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Vitamins and catalase should be sterilized by filtration. After inoculation, inject sterile air into the culture vessel up to about 20% of the total gas volume (i.e., 4 parts N_2 and 1 part air).

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg

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845: SPIRILLUM WINOGRADSKYI MEDIUM

D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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846: AMINOMONAS MEDIUM

NH_4Cl	0.30	g
K_2HPO_4	0.20	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.15	g
KCl	0.50	g
NaCl	1.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
L-Serine	1.05	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, serine, vitamins, cysteine, and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add serine, vitamins, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The vitamin solution should be sterilized by filtration. Adjust the pH of the complete medium to 7.2, if necessary.

For DSM 12260: Replace L-Serine with 1.74 g/l L-Arginine x HCl.

For DSM 27871: Adjust the pH of the complete medium to 7.8.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg

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Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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847: MACROMONAS MEDIUM

$(NH_4)_2SO_4$	0.30	g
$MgSO_4$ x 7 H_2O	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract (OXOID)	0.20	g
Agar (BD Bacto)	1.00	g
Seven vitamins solution	1.00	ml
Trace element solution SL-11	1.00	ml
$Na_2S_2O_3$ x 5 H_2O	1.00	g
Disodium succinate	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except vitamins, trace elements, thiosulfate and succinate. Adjust pH to 7.2 - 7.4 and bring medium to the boil, distribute into culture vessels and autoclave. To complete the medium add vitamins, trace elements, thiosulfate and succinate from stock solutions sterilized by filtration.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous

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chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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848. RAE MEDIUM

Glucose	40.00	g
Peptone	10.00	g
Yeast extract	10.00	g
Citric acid x H_2O	1.50	g
Na_2HPO_4 x 2 H_2O	3.38	g
Glacial acetic acid	10.00	ml
Absolute ethanol	10.00	ml
Distilled water	980.00	ml

Final pH ca. 3.8 (pH is not adjusted)
Both liquid and solid media are prepared by autoclaving the medium without the addition of glacial acetic acid and absolute ethanol. Glacial acetic acid and absolute ethanol are sterilised either by filtration (use Teflon filters) or they may be autoclaved in completely closed screw cap glass bottles sealed with a Teflon coated septum. The preparation of solid media employs a double layer system similar to that described for *Acetobacter europaeus* medium (see medium 631). Substitute the components listed above and prepare a lower, 0.5% agar layer, which is then overlaid with a 1.0% agar layer as described in medium 631. Incubate in closed containers to keep the humidity high (> 90%).

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849. AE MEDIUM

Glucose	5.0	g
Yeast extract	3.0	g
Peptone	4.0	g
Absolute ethanol	30.0	ml
Glacial acetic acid	30.0	ml
Agar	9.0	g
Distilled water	940.0	ml

Both liquid and solid media are prepared by autoclaving the medium without the addition of glacial acetic acid and absolute ethanol. Glacial acetic acid and absolute ethanol are sterilised either by filtration (use Teflon filters) or they may be autoclaved in completely closed screw cap glass bottles sealed with a Teflon coated septum. A double layer system das not appear to be essential for the growth of this organism, however, plates should be incubated in sealed containers to keep the humidity high (> 90%).

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850. ALTERNATIVE ACETOBACTER INTERMEDIUS MEDIUM

Glucose	5.0	g
Yeast extract	3.0	g
Agar	12.0	g
Distilled water	1000.0	ml

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851. GLUCOSE SULFIDE MEDIUM

Glucose	0.15	g
Yeast extract	1.00	g
$(NH_4)_2SO_4$	0.50	g
$CaCO_3$	0.10	g
$Ca(NO_3)_2$	0.10	g
KCl	0.05	g
K_2HPO_4	0.05	g
$MgSO_4$ x 7 H_2O	0.05	g
Na_2S x 9 H_2O	0.20	g
Vitamin solution (see medium 131)	10.00	ml
Distilled water	990.00	ml

Adjust pH to 7.3.

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852. GYP GLUCOSE-YEAST-PEPTONE MEDIUM

Glucose	20	g
Yeast extract	10	g
Peptone (tryptic)	10	g
Na-acetate	10	g
Salt solution (see below)	5	ml
Distilled water	1000	ml

Salt solution:

$MgSO_4$ x 7 H_2O	40	g
$MnSO_4$ x 4 H_2O	2	g
$FeSO_4$ x 7 H_2O	2	g
NaCl	2	g
Distilled water	1000	ml

For solid media add 10 g/l bacteriological agar. Adjust to pH 6.8. After sterilization of the medium add filter sterilized salt solution.

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853: FUSIBACTER MEDIUM

Final pH: 7.2 - 7.4
Final volume: 1010 ml

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	3.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	1.00	g
NaCl	30.00	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Na-acetate x 3 H_2O	0.50	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$Na_2S_2O_3$ x 5 H_2O	3.16	g
L-Cysteine HCl x H_2O	0.50	g
Na_2CO_3	1.50	g
D-Glucose	3.60	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients, except thiosulfate, cysteine, carbonate, glucose, and sulfide, then sparge medium for 30 - 45 min with 80% N_2 and 20% CO_2 gas mixture to make it anoxic. Add thiosulfate and cysteine, then adjust pH to 7.0 and dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After autoclaving, add glucose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Adjust the pH of the complete medium to 7.2 - 7.4, if necessary.

For DSM 112048: Omit D-Glucose.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g

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$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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854: HIPPEA MEDIUM

NH_4Cl	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
NaCl	25.00	g
MOPS buffer (SIGMA)	3.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.10	g
Na-acetate	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, vitamins, and sulfide), adjust pH to 5.5 - 6.0, and sparge the medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense the medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already an appropriate amount of sulfur powder. Autoclave 20 min at **110°C**. Prior to use add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins are sterilized by filtration. Adjust the pH of the complete medium to 6.0 - 6.2, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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855: THAUERA AROMATICA MEDIUM (ANAEROB)

Solution A	952.00	ml
Solution B	10.00	ml
Solution C	10.00	ml
Solution D	30.00	ml
Solution E	1.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solutions B and C are autoclaved separately under 100% N_2 gas. Solution D is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution E is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions B to E are added to the sterile, cooled solution A in appropriate amounts in the sequence as indicated. Adjust pH of final medium to 7.2, if necessary.

2. For aerobic growth prepare medium under air. Omit carbonate and KNO_3. 3,5-Dihydroxybenzoic acid or Na-benzoate as substrate can be replaced with 10 mM Na -malate. Adjust medium pH to 7.2.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Distilled water	950.00	ml

Solution B

3,5-Dihydroxybenzoic acid **or** Na-benzoate	0.60	g
Distilled water	10.00	ml

Solution C

KNO_3	0.60	g
Distilled water	10.00	ml

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Solution D

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution E

Wolin's vitamin solution (10x)	1.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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856. STARCH NITRATE MEDIUM

Starch	20.0	g
K_2HPO_4	1.0	g
KNO_3	2.0	g
$MgSO_4$	0.5	g
$CaCO_3$	3.0	g
NaCl	100.0	g
Agar	20.0	g
Trace salt solution (see below)	1.0	ml
Distilled water	1000.0	ml

Trace salt solution:

$FeSO_4$ x 7 H_2O	0.1	g
$MnCl_2$ x 4 H_2O	0.1	g
$ZnSO_4$ x 7 H_2O	0.1	g
Distilled water	100.0	ml

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858. ANAEROBIC CHOLESTEROL MEDIUM

Solution A:

$MgSO_4$ x 7 H_2O	0.10	g
NH_4Cl	0.10	g
$CaCl_2$	0.02	g
$NaNO_3$	0.16	g
Distilled water	200.00	ml

Solution B:

KH_2PO_4	1.00	g
Distilled water	100.00	ml

Solution C:
Trace elements SL-10 (see medium 320)

Solution D:
Vitamin solution (see medium 461)

Solution E:
Selenite-tungstate solution (see medium 385)

Solution F:

$NaHCO_3$	8.40	g
Distilled water	100.00	ml

Prepare solution A, distribute approbiate amounts of cholesterol (e.g. 5 mg for 10 ml medium) and solution A in anaerobic tubes, and autoclave anaerobically under 80% N_2 + 20% CO_2. Solutions B and F are also autoclaved under these conditions. Solution C, D and E may be prepared aerobically. After cooling, add to 9 ml solution A: 500 µl sol. B, 500 µl sol. F, 25 µl (one drop) sol. C and E, each, and 50 µl sol. D under anaerobic conditions. Adjust pH to 7.0±0.2.

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859. LACTOBACILLUS MEDIUM IV

Tryptone	10.00	g
Meat extract	3.00	g
Yeast extract	5.00	g
Glucose	4.00	g
Maltose	4.00	g
Na-acetate x 3 H_2O	5.00	g
$(NH_4)_2$ citrate	2.00	g
K_2HPO_4 x 3 H_2O	2.60	g
$MgSO_4$ x 7 H_2O	0.10	g
$MnSO_4$ x 4 H_2O	0.05	g
Cysteine-HCl x H_2O	0.50	g
Tween 80	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 6.3

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860: ANAEROCOLUMNA MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Yeast extract	1.00	g
Trace element solution SL-10	1.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
D-Fructose	4.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, fructose, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fructose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.0 - 7.2, if necessary.

For DSM 12504: Replace fructose with 1.0 g/l syringic acid.

For DSM 13105, DSM 19740: Replace fructose with 2.5 g/l $Na_2S_2O_3$ x 5 H_2O and adjust pH of complete medium to 7.4.

For DSM 13106, DSM 23801: Replace fructose with 2.0 g/l D-glucose and adjust pH of complete medium to 7.4.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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861: DESULFOFRIGUS MEDIUM

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	1.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 - 45 min), then dispensed under the same gas atmosphere into serum vials (use at least bottles of 50 ml volume filled with 30 ml medium) and autoclaved. Solutions B and F are prepared under 80% N_2 and 20% CO_2 gas atmosphere and sterilized. Sterile solutions C, D and E are prepared under 100% N_2 gas. Solutions D and F are sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0 - 7.2.

2. Note: For transfer of cultures use at least 10% (v/v) inoculum.

For DSM 12341: Replace Na-DL-lactate with 1.50 g/l Na-acetate.

For DSM 12344: Replace Na-DL-lactate with 1.50 g/l Na-propionate.

Solution A

NaCl	20.00	g
Na_2SO_4	4.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
$MgCl_2$ x 6 H_2O	3.00	g
$CaCl_2$ x 2 H_2O	0.15	g
KBr	0.09	g
KCl	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

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Solution C

Na-DL-lactate	2.50	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	10.00	ml

Solution F

Na-dithionite solution (5% w/v)	1.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
$Na_2SeO_3 \times 5\ H_2O$	3.00	mg
$Na_2WO_4 \times 2\ H_2O$	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg

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Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Na-dithionite solution (5% w/v) (from medium 829)

$NaHCO_3$	50.00	g
$Na_2S_2O_4$	50.00	g
Distilled water	1000.00	ml

Dissolve $NaHCO_3$ in water and make the solution anoxic by sparging with 80% N_2 and 20% CO_2 gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.

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864: HALOTHIOBACILLUS MEDIUM

NaCl	29.00	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_2O	1.50	g
$CaCl_2$ x 2 H_2O	0.42	g
KCl	0.70	g
Trace element solution SL-11	1.00	ml
Bromothymol blue (0.1% w/v)	4.00	ml
K_2HPO_4	0.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients except hydrogenphosphate and thiosulfate, adjust pH to 6.5, then dispense medium into culture vessels and autoclave. Add hydrogenphosphate and thiosulfate from sterile stock solutions. Adjust pH of complete medium to 6.5 - 7.0.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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866: DESULFOFALX MEDIUM

KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
KCl	0.20	g
NH_4Cl	1.00	g
Na_2SO_4	5.00	g
NaCl	5.00	g
Trace element solution SL-10	1.00	ml
Yeast extract	1.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.50	g
$NaHCO_3$	8.00	g
Na-formate	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve the ingredients (except carbonate, bicarbonate, vitamins, formate and sulfide,) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonate and bicarbonate, adjust pH to 8.8 - 9.0, then dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add vitamins (sterilized by filtration), formate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas.

For DSM 16529: Replace Na-formate with 2.5 g/l Na-L-lactate as substrate.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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869: ANAEROBIC TRYPTIC SOY BROTH MEDIUM

Trypticase soy broth (BD BBL)	5.50	g
Resazurin (0.1% w/v)	0.50	ml
D-Glucose	2.00	g
Distilled water	1000.00	ml

Dissolve dehydrated TSB medium and resazurin, bring to the boil and cool to room temperature while sparging with 100% N_2 gas. Adjust pH to 5.5, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose from a separately sterilized anoxic stock solution.

For DSM 12992: Adjust pH of complete medium to 6.5.

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870: SYNTROPHOTHERMUS MEDIUM

KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
Modified Wolin's mineral solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na-crotonate solution (1 M)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
L-Cysteine HCl x H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, crotonate, vitamins, sulfide and cysteine, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add crotonate, vitamins, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Sterilize vitamins by filtration. Adjust pH of complete medium to 7.0, if necessary.

For DSM 12681: Replace Na-crotonate with 2.20 g/l Na-butyrate.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Na-crotonate solution (1 M) (from medium 870)

Crotonic acid (ALDRICH 113018)	86.00	g
NaOH (10 N)	100.00	ml
Distilled water	900.00	ml

Dissolve crotonic acid in 800 ml distilled water, add around 100 ml of 10 N NaOH and adjust pH to around 7. Then add water to reach a volume of 1000 ml. Sterilize by filtration under 100% N_2 gas atmosphere.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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871. GYM + SEAWATER

Glucose	4.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
$CaCO_3$	2.0	g
Agar	15.0	g
Sea water	1000.0	ml

pH should be between 7.0 - 7.4.

Seawater:
32 g seasalt in 1000 ml distilled water.

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872: PAPILLIBACTER MEDIUM

NH_4Cl	0.30	g
K_2HPO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.50	g
NaCl	1.00	g
Yeast extract (BD Bacto)	5.00	g
Trypticase peptone (BD BBL)	5.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.00	g
trans-Cinnamic acid	0.75	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, cinnamic acid, sulfide and cysteine, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cinnamic acid, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Adjust pH of complete medium to 7.2 - 7.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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873: THERMOANAEROVIBRIO MEDIUM

NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$NaHCO_3$	0.80	g
Yeast extract (OXOID)	0.25	g
Peptone (BD Bacto)	0.25	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$MgCl_2 \times 6\ H_2O$	0.33	g
$CaCl_2 \times 2\ H_2O$	0.22	g
Na_2CO_3	1.50	g
D-Glucose	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients, except magnesium chloride, calcium chloride, carbonate, vitamins, glucose and sulfide, then sparge medium for 30 - 45 min with 80% N_2 and 20% CO_2 gas mixture to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving, add magnesium chloride, calcium chloride, vitamins, glucose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.3, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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874: FERROPLASMA ACIDIPHILUM MEDIUM

Solution A	930.00	ml
Solution B	50.00	ml
Solution C	10.00	ml
Solution D	10.00	ml

Add solutions B and C to solution A, adjust pH of the combined solution to 1.6 - 1.8 with H_2SO_4, then sterilize using membrane filter and dispense in sterile culture vessels. Prior to use, add the appropriate volume of solution D sterilized by autoclaving.

For DSM 16651: Supplement medium prior to inoculation with 2.0 g/l potassium tetrathionate added from a stock solution sterilized by filtration. Incubate without agitation.

For DSM 18409: Replace trace elements with 1.00 ml/l of trace element solution SL-10 (see medium 320). Supplement medium prior to inoculation with 1.50 g/l potassium tetrathionate added from a stock solution sterilized by filtration.

Solution A

$MgSO_4$ x 7 H_2O	0.40	g
$(NH_4)_2SO_4$	0.20	g
KCl	0.10	g
K_2HPO_4	0.10	g
Distilled water	930.00	ml

Solution B

$FeSO_4$ x 7 H_2O	25.00	g
H_2SO_4 (1 N)	40.00	ml
Distilled water	10.00	ml

Solution C

Trace element solution	10.00	ml

Solution D

Yeast extract (OXOID)	0.20	g
Distilled water	10.00	ml

Trace element solution (from medium 158)

$FeCl_3$ x 6 H_2O	1.93	g
$MnCl_2$ x 4 H_2O	0.18	g

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874: FERROPLASMA ACIDIPHILUM MEDIUM

$Na_2B_4O_7$ x 10 H_2O	0.45	g
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 5 H_2O	3.80	mg
$CoSO_4$ x 7 H_2O	2.00	mg
Distilled water	1000.00	ml

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875: DESULFACINUM HYDROTHERMALE MEDIUM

Solution A	950.00	ml
Solution B	1.00	ml
Solution C	30.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 - 45 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solutions B, E and F are autoclaved separately under 100% N_2 gas atmosphere. Solution C is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0 - 7.3.

Solution A

NaCl	26.00	g
$MgCl_2$ x 6 H_2O	5.60	g
$CaCl_2$ x 2 H_2O	1.40	g
$MgSO_4$ x 7 H_2O	6.80	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
KCl	0.72	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Trace element solution SL-10	1.00	ml

Solution C

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution D

Seven vitamins solution	1.00	ml

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Solution E

Na-DL-lactate	2.50	g
Distilled water	10.00	ml

Solution F

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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876: DESULFOTHERMUS NAPHTHAE MEDIUM

Na_2SO_4	4.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	9.80	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na-caproate	0.50	g
Na-butyrate	0.30	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.40	g
Distilled water	1000.00	ml

1. Dissolve all ingredients except carbonate, caproate, butyrate, vitamins and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add caproate, butyrate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.9, if necessary.

2. Note: Growth can be stimulated by supplementing the medium with 1% (v/v) of fatty acid mixture (see medium 119).

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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877. LUEDEMANN MEDIUM (LUEDEMANN; 1968)

Yeast extract	0.5	g
Malt extract broth	1.5	g
Soluble starch	1.0	g
Glucose	1.0	g
$CaCO_3$	0.2	g
NaCl	0.5	g
Agar	1.5	g
Distilled water	100.0	ml

pH 8.6. Sterilize at 121°C for 15 min at 1 atm.

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878. THERMUS 162 MEDIUM

Yeast extract	1.00	g
Tryptone	1.00	g
Agar	28.00	g
Nitrilotriacetic acid	100.00	mg
$CaSO_4$ x 2 H_2O	40.00	mg
$MgCl_2$ x 6 H_2O	200.00	mg
0.01 M Fe citrate	0.50	ml
Trace element solution (see below)	0.50	ml
Phosphate buffer (see below)	100.00	ml
Distilled water	900.00	ml

Adjust pH to 7.2 with NaOH. Autoclave at 121°C for 15 min. Autoclave the phosphate buffer separately and then add to the medium.

Phosphate buffer:

KH_2PO_4	5.44	g
Na_2HPO_4 x 12 H_2O	43.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2

Trace element solution:

H_2SO_4	0.50	ml
$MnSO_4$ x H_2O	2.28	g
$ZnSO_4$ x 7 H_2O	0.50	g
H_3BO_3	0.50	g
$CuSO_4$ x 5 H_2O	25.00	mg
Na_2MoO_4 x 2 H_2O	25.00	mg
$CoCl_2$ x 6 H_2O	45.00	mg
Distilled water	1000.00	ml

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879: CLOSTRIDIUM LJUNGDAHLII MEDIUM

Final pH: 5.9
Final volume: 1011 ml

NH_4Cl	1.00	g
KCl	0.10	g
$MgSO_4$ x 7 H_2O	0.20	g
NaCl	0.80	g
KH_2PO_4	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
Yeast extract	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
D-Fructose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, fructose, vitamins and reducing agents), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic and adjust pH to 5.5. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 15 min. Add fructose, vitamins (sterilized by filtration), cysteine and sulfide from sterile stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Adjust pH of complete medium to 5.9!

For DSM 13641: Replace fructose with 5.00 g/l sucrose added to the autoclaved medium from a sterile anoxic stock solution and increase amount of $NaHCO_3$ to 2.50 g/l. Adjust pH of complete medium to 6.9!

For DSM 115981: Omit Yeast extract.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g

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$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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880: THERMOACETOGENIUM MEDIUM

KH_2PO_4	0.30	g
NaCl	0.60	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.08	g
NH_4Cl	1.00	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$KHCO_3$	3.50	g
L-Cysteine HCl x H_2O	0.60	g
Methanol (50% v/v)	6.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, cysteine, methanol, vitamins and sulfide), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then add solid bicarbonate and cysteine and equilibrate the medium with the gas mixture to reach a pH of 7.0. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. To the autoclaved medium add methanol (50% v/v solution), vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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881: DENITROVIBRIO MEDIUM

Na_2SO_4	0.02	g
KH_2PO_4	1.00	g
NH_4Cl	0.25	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Na-acetate	1.64	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na_2-fumarate	6.40	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, fumarate, vitamins and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fumarate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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882: LEPTOSPIRILLUM (HH) MEDIUM

Final pH: 2.3
Final volume: 1001 ml

Solution A	950.00	ml
Solution B	50.00	ml
Solution C	1.00	ml

1. Sterilize solution A and solution C by autoclaving at 121°C for 20 min and solution B by autoclaving at 112°C for 30 min. Prior to use mix solutions A and B and add trace elements solution. The final pH of the medium should be 2.3.

2. Note: At the beginning of growth incubate statically without shaking.

For DSM 14647, DSM 29444, DSM 29468, DSM 27195, DSM 583: Adjust the pH of the complete medium to 2.0.

Solution A

$(NH_4)_2SO_4$	132.00	mg
$MgCl_2$ x 6 H_2O	53.00	mg
KH_2PO_4	27.00	mg
$CaCl_2$ x 2 H_2O	147.00	mg
Distilled water	950.00	ml

Adjust pH to 2.3 with 10 N H_2SO_4.

Solution B

$FeSO_4$ x 7 H_2O	20.00	g
H_2SO_4 (0.25 N)	50.00	ml

The pH of this solution should be 1.2.

Solution C

Trace element solution	1.00	ml

Trace element solution

$MnCl_2$ x 4 H_2O	76.00	mg
$ZnCl_2$	68.00	mg
$CoCl_2$ x 6 H_2O	64.00	mg
H_3BO_3	31.00	mg

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Na_2MoO_4	10.00	mg
$CuCl_2$ x 2 H_2O	67.00	mg
Distilled water	1000.00	ml

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883: CALDIVIRGA MEDIUM

Trisodium citrate x 2 H_2O	2.94	g
$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Yeast extract (OXOID)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, yeast extract, vitamins and sulfide, then adjust the pH to 3.5 with 4 N H_2SO_4. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Autoclave at **105°C** for 20 min. Prior to inoculation add yeast extract, vitamins (sterilized by filtration) and sulfide from anoxic stock solutions prepared under 100% N_2 gas atmosphere. The final pH of the complete medium should be around 4.0.

2. After inoculation, pressurize the cultivation vessels to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg

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883: CALDIVIRGA MEDIUM

Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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884. TWEEN 80-AGAR

Solution A:

Peptone	10.0	g
NaCl	5.0	g
$CaCl_2$ x 2 H_2O	0.1	g
Agar	15.0	g
Destilled water	900.0	ml

Solution B:

Tween 80	10.0	g
Destilled water	100.0	ml

Autoclave solutions A and B separately and combine after cooling to approximately 50°C.

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887. OS MEDIUM

NaCl	256.00	mg
KH_2PO_4	1.70	mg
$CaCl_2$ x 2 H_2O	0.80	mg
H_3BO_3	10.30	mg
Na_2SO_4	23.00	mg
$NaNO_3$	0.30	mg
KCl	15.00	mg
$FeCl_3$ x 6 H_2O	0.10	mg
$MnSO_4$ x 4 H_2O	0.06	mg
$NaHCO_3$	1.00	g
Trace element solution (see medium 141)	10.00	ml
$Na_2S_2O_3$ x 5 H_2O	1.00	g
H_2O	1000.00	ml

Bubble the medium 30 minutes with N_2 gas. Adjust pH to 7.0 with H_2SO_4. Fill 10 ml medium into 100 ml Serum bottles and seal with a rubber stopper. Prepare $NaHCO_3$ and $Na_2S_2O_3$ as separate sterile stock solution and add to sterile medium after autoclaving. Give an atmosphere of $N_2/H_2/O_2$ (94/3/3) at 300 kPa. Incubate at 80°C.

Modification for DSM 23557 – add 10 mM Tris-HCl, change pH to 7.5

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888. AQUASPIRILLUM MEDIUM

$(NH_4)_2SO_4$	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
$CaCl_2$ x 6 H_2O	30.0	mg
$Na_2H_2PO_4$	10.0	mg
Sodium succinate	1.00	g
Casamino acids (DIFCO)	1.5	g
Trace element solution (see medium 1369)	1.0	ml
Agar (DIFCO)	0.5	g
Distilled water	1000.0	ml
pH 7.5		

Add after autoclaving sterile solutions of

$Na_2S_2O_3$ x 5 H_2O (10% solution)	1.0	ml
vitamin solution (see medium 1478)	1.0	ml

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889. ISS1 MEDIUM

NaCl	20.00	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_20	5.50	g
KCl	0.65	g
NaBr	0.10	g
$NaHCO_3$	2.00	g
NH_4Cl	0.30	g
K_2HPO_4	0.20	g
$CaCl_2$ x 2 H_2O	0.50	g
Yeast extract	0.20	g
Trace element solution (see medium 141)	10.00	ml
Sulfur,powdered	5.00	g
Distilled water	ad 1000.00	ml

Adjust pH to 7.0 using H_2SO_4.
Prepare the medium under H_2 gas and fill 20 ml medium into 100 ml serum bottles and seal with a rubber stopper. Steam medium for 3 hours on each of 3 successive days.
Give an atmosphere of $H_2/CO_2/O_2$ (78/20/2) with an overpressure of two atmospheres.
Incubate under shaking at 85°C.

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890. VM1 MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	12.60	g
$NaHCO_3$	0.16	g
K_2HPO_4	0.20	g
NH_4Cl	0.30	g
Na_2SO_4	3.24	g
$CaCl_2$ x 2 H_2O	2.38	g
KCl	0.56	g
Sulfur, powdered	0.50	g
Trace element solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Adjust pH to 7.0 using H_2SO_4.

Prepare the medium under H_2 gas and fill 20 ml medium into 100 ml serum bottles and seal with a rubber stopper. Steam medium for 3 hours on each of 3 successive days. Give an atmosphere of $H_2/CO_2/O_2$ (78/20/2) with an overpressure of two atmospheres. Incubate under shaking at 65°C.

For *heterotrophic growth* add yeast extract 1.0 g, peptone 5.0 g and starch 2.0 g to above medium. Incubate at 65°C in gas phase of 80% N_2 and 20% air.

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891. SME MEDIUM MODIFIED FOR IS7

NaCl	30.00	g
$MgSO_4$ x 7 H_2O	0.70	g
$MgCl_2$ x 6 H_20	0.55	g
KCl	0.65	g
NaBr	0.10	g
$NaHCO_3$	2.00	g
NH_4Cl	0.15	g
K_2HPO_4	0.15	g
$CaCl_2$ x 2 H_2O	0.50	g
Sulfur, powdered	0.50	g
Trace element solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Adjust pH to 7.0 using H_2SO_4.
Prepare the medium under H_2 gas and fill 20 ml medium into 100 ml serum bottles and seal with a rubber stopper. Steam medium for 3 hours on each of 3 successive days.
Give an atmosphere of $H_2/CO_2/O_2$ (78/20/2) with an overpressure of two atmospheres.
Incubate at 70°C without shaking.

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892: METHANOCALCULUS PUMILUS MEDIUM

KH_2PO_4	0.40	g
NH_4Cl	0.90	g
$MgCl_2$ x 6 H_2O	0.36	g
NaCl	10.00	g
Na-acetate	0.50	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve carbonate, adjust pH to 7.2 then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 7.3 - 7.5, if necessary.

2. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 1.5 - 2 bar overpressure.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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893. TS AGAR

Tryptone	5.0	g
Beef extract	3.0	g
Glucose	1.0	g
Sucrose	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0

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894. BENNETT'S MODIFIED MEDIUM (P. AGRAWAL, UNPUBLISHED)

Lab lemco	10.0	g
Yeast extract	2.0	g
Glucose	10.0	g
Starch	100.0	mg
Tryptose	2.0	g
$CaCO_3$	100.0	mg
$CoCl_2$	traces	-
Ferric ammonium	traces	-
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 and sterilise at 121°C for 15 minutes.

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895: ANAEROBRANCA GOTTSCHALKII MEDIUM

$(NH_4)_2SO_4$	1.00	g
NH_4Cl	0.40	g
$Na_2S_2O_3$ x 5 H_2O	0.10	g
K_2HPO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.05	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Yeast extract (BD Bacto)	0.25	g
Tryptone (BD Bacto)	0.25	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2CO_3	3.00	g
Starch (soluble)	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve all ingredients except cysteine, carbonate, starch and vitamins. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, add cysteine and adjust pH to 7.5, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add starch and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 9.3 - 9.5, if necessary.

For DSM 14826: Omit starch from the medium, increase the concentration of Tryptone to 2.00 g/l and supplement medium with 4.00 g/l $Na_2S_2O_3$ x 5 H_2O added to the medium from a sterile anoxic stock solution prepared under 100% N_2 gas.

For DSM 14828: Omit starch, add 5.00 g/l D-glucose and increase the concentration of Tryptone to 2.00 g/l. Adjust pH of complete medium to 9.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g

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$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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896: SOLIDESULFOVIBRIO MEDIUM

KH_2PO_4	0.20	g
NH_4Cl	0.06	g
Fe(III) quinate solution 0.01 M	2.00	ml
Modified Wolin's mineral solution	4.00	ml
Na_2-fumarate	0.58	g
Na-pyruvate	0.44	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.05	g
Distilled water	1000.00	ml

Dissolve ingredients except vitamins and cysteine, adjust pH to 7.2 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Fe(III) quinate solution 0.01 M (from medium 380)

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$FeCl_3$ x 6 H_2O	4.50	g
Quinic acid	1.90	g
Distilled water	1000.00	ml

Sterilize by filtration under 100% N_2 gas atmosphere.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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897: IGNICOCCUS MEDIUM

NaCl	13.65	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
KH_2PO_4	0.50	g
$(NH_4)_2SO_4$	0.25	g
$CaCl_2$ x 2 H_2O	0.38	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
KI (0.01% w/v)	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	5.00	g
Meat extract	1.00	g
Na_2S x 9 H_2O	0.20	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, meat extract and sulfide, adjust pH to 5.0 - 5.5 with 2 N H_2SO_4 and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials already containing the appropriate amount of sulfur, only to 30% of their volume to allow for a large head space. For sterilization heat cultivation vessels in boiling water for 1 -2 hours on each of 3 successive days. Add meat extract and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere.

2. After inoculation pressurize the vessels with sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.

For DSM 18386: Prepare medium without meat extract.

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898: ANOXYBACILLUS MEDIUM

KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
KCl	0.20	g
NH_4Cl	1.00	g
NaCl	5.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.76	g
$NaHCO_3$	10.00	g
Yeast extract	0.50	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonates, yeast extract, glucose, vitamins and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add carbonate and hydrogencarbonate, adjust pH to 9.0 - 9.5, dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 9.5 - 9.7.

For DSM 16504: Omit glucose and add to the autoclaved medium 2.70 g/l betaine and 0.65 g/l Na_2SO_3 from filter-sterilized anoxic stock solutions prepared under 100% N_2 gas.

For DSM 18346: Omit glucose, increase amount of yeast extract to 2.00 g/l. Adjust pH of complete medium to 8.5.

For DSM 18978: Adjust pH of complete medium to 9.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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899: CALDANAEROBACTER MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.20	g
NaCl	0.60	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
D-Glucose	4.00	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.45	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, glucose, thiosulfate, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, thiosulfate (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 7.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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900: ACETOBACTERIUM TUNDRAE MEDIUM

KCl	0.33	g
$MgCl_2 \times 6\ H_2O$	0.52	g
$CaCl_2 \times 2\ H_2O$	0.30	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
Trace element solution SL-10	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
D-Fructose	5.00	g
Seven vitamins solution	1.00	ml
L-Cysteine HCl $\times H_2O$	0.30	g
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, fructose, vitamins and reducing agents. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fructose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 7.0.

For DSM 8238: Replace D-fructose with 4.00 g/l Na-(DL)-lactate as the substrate. Adjust pH of complete medium to 7.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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901: ACIDOLOBUS ACETICUS MEDIUM

NH_4Cl	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Yeast extract	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, yeast extract, vitamins and sulfide. Adjust pH to 3.5 with H_2SO_4 and sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of sulfur. Heat vessels containing medium to 90°C for 1 hour on each of 3 succesive days. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The sulfide stock solution (3% w/v) should be neutralized before use (see medium 29). The pH of the complete medium should be at 3.5 - 3.8.

For DSM 16705: Reduce the amount of yeast extract to 0.10 g/l and supplement medium after sterilization with 2.00 g/l D-glucose added from a sterile anoxic stock solution.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.

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902: CARCBOXYDIBRACHIUM MEDIUM

NaCl	20.00	g
KCl	0.70	g
$MgSO_4$ x 7 H_2O	3.90	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	0.30	g
Na_2HPO_4	0.15	g
Na_2SiO_3	0.03	g
Yeast extract	0.05	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	0.50	g
Na-pyruvate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.45	g
Na_2S x 9 H_2O	0.45	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, pyruvate, vitamins and reducing agents, then adjust pH of solution to 7.0 with NaOH. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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903: CALDANAEROBIUS MEDIUM

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Trypticase peptone (BD BBL)	0.25	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Sucrose	5.00	g
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, sucrose, vitamins and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sucrose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g

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Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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904: MARINITOGA MEDIUM

Sea Salt (SIGMA)	30.00	g
PIPES (SIGMA)	6.00	g
Yeast extract	1.00	g
Tryptone (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	2.50	g
Na_2S x 9 H_2O	0.50	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except glucose, sulfide and cysteine, adjust pH to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (to 30% of volume) and autoclave. Add glucose, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas.

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905: METHANOCALCULUS HALOTOLERANS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
KCl	0.17	g
NaCl	50.00	g
Na-acetate	0.50	g
Yeast extract (OXOID)	0.50	g
Trypticase (BD BBL)	0.50	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
L-Cysteine HCl x H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.60	g
$MgCl_2$ x 6 H_2O	3.20	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, cysteine, calcium chloride, magnesium chloride and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve solid bicarbonate and cysteine, then adjust pH to 7.5 with 10 N KOH. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (to 30% of volume) and autoclave. Add calcium chloride, magnesium chloride and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The pH of the complete medium should be between 7.2 and 7.6.

2. After inoculation pressurize vessels with sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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906: DETHIOSULFOVIBRIO MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KH_2PO_4	1.00	g
Na_3-citrate x 5 H_2O	3.00	g
Yeast extract (OXOID)	5.00	g
Peptone (BD Bacto)	2.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.20	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except calcium chloride, thiosulfate and sulfide. Sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, thiosulfate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The pH of the complete medium should be at 6.7 - 6.8.

For DSM 112078: Adjust pH of complete medium to 7.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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907. PSEUDOMONAS AGAR F

Tryptone	10.0	g
Proteose peptone	10.0	g
K_2HPO_4	1.5	g
$MgSO_4$	1.5	g
Glycerol	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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908. MEDIUM FOR CHLORATE RESPIRERS

Solution A:

NH_4Cl	0.25	g
NaH_2PO_4	0.60	g
KCl	0.10	g
$NaHCO_3$	2.50	g
$NaClO_3$	1.00	g
Na-Acetate	1.36	g
Distilled water	1000.00	ml

Solution B:

$MgSO_4$	30.00	mg
$CaCl_2$ x 2 H_2O	10.00	mg
Distilled water	10.00	ml

Solution C:

Na_2MoO_4	25.00	mg
Na_2WO_4 x 2 H_2O	25.00	mg
Distilled water	1000.00	ml

Solution D: Trace element solution SL-10

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Solution E: Vitamins

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

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Stir for some hours, filter sterilize the solution.

continued

Autoclave solutions A, B and C separately and anaerobically under 80% N_2 + 20% CO_2 atmosphere. Combine 1000 ml solution A, 10 ml solution B and 10 ml solution C. Add 5 ml vitamin solution E and 1 ml trace element solution D. The pH should be 7.2 without further adjustment. Reactivate ampoules of DSM 13638 in medium 1 under aerobic conditions first, then transfer to anaerobic medium 908.

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909. TREPONEMA DENTICOLA MEDIUM

Solution A:

Brain heart infusion broth	12.50	g
Trypticase (BBL 11921)	10.00	g
Yeast extract	2.50	g
Na-thioglycolate	0.50	g
L-Cysteine x HCl	1.00	g
L-Asparagine	0.25	g
Glucose	2.00	g
Agar	3.00	g
Distilled water	1000.00	ml

Boil for 5 min, cool to 50°C while gassing with 80% N_2 + 20% CO_2. Distribute to anaerobic tubes under the same atmosphere and autoclave.

Solution B:

Thiamine pyrophosphate	6.00	mg
$NaHCO_3$	2.00	g
Distilled water	25.00	ml
Volatile fatty acid solution	20.00	ml

Sterilize by filtration, add 20 ml rabbit serum, gas with 80% N_2 + 20% CO_2.

Add 0.45 ml Sol. B to 10 ml Sol. A. Inoculate and pressurize the tubes with H_2 to 0.5 bar.

Volatile fatty acid solution:

KOH, 0.1 N	100.00	ml
Isobutyric acid	0.50	ml
D,L-2-methylbutyric acid	0.50	ml
Isovaleric acid	0.50	ml
Valeric acid	0.50	ml

For DSM 111712 omit agar from solution A.

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910. MODIFIED CM+YE MEDIUM (B)

Bacto vitamin assay		
casamino acids (Difco)	7.50	g
Bacto yeast extract (Difco)	10.00	g
$MgSO_4$ x 7 H_2O	10.00	g
Na_3Citrate x 2 H_2O	3.00	g
KCl	2.00	g
NaCl	100.00	g
Fe^{2+} solution*	1.00	ml
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.4

Fe^{2+} solution:

$FeSO_4$ x 7 H_2O	4.98	g
Distilled water	100.00	ml

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911. MIDDLEBROCK 7H11 AGAR

Bacto Middelbrook 7H11 agar (see below)	21.0	g
Glycerol	5.0	ml
Distilled water	900.0	ml

Sterilize for 10 min by 121°C cool to 50 - 55°C. Add 100 ml Bacto Middelbrook OADC enrichment Difco (see below) under aseptic conditions and mix thoroughly.

Bacto Middelbrook 7H11 agar (Difco 283810):

Pancreatic digest of casein	1.0	g
$(NH_4)_2SO_4$	0.5	g
KH_2PO_4	1.5	g
Na_2HPO_4	1.5	g
Na-citrate	0.4	g
$MgSO_4$	50.0	mg
L-Glutamic acid	0.5	g
Ferric ammonium citrate	40.0	mg
Pyridoxine	1.0	mg
Biotin	0.5	mg
Malachite green	1.0	mg
Bacto agar	15.0	g
Distilled water	900.0	ml

Final pH at 25°C 6.6 ± 0.2.

Bacto Middelbrook OADC enrichment (Difco 212240):

Oleic acid	50.0	mg
Albumin fraction V, bovine	5.0	g
Dextrose	2.0	g
Catalase (beef)	4.0	mg
NaCl	850.0	mg
Distilled water	100.0	ml

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912: TREPONEMA R8 MEDIUM

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.50	g
KH_2PO_4	0.20	g
K_2HPO_4	0.25	g
NH_4Cl	0.30	g
KCl	0.30	g
$CaCl_2$ x 2 H_2O	15.00	mg
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
MOPS (SIGMA)	2.10	g
D-Glucose	0.90	g
Yeast extract	0.19	g
Peptone (BD Bacto)	0.19	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
Clarified rumen fluid	50.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.40	g
Na_2S x 9 H_2O	0.40	g
Distilled water	950.00	ml

Dissolve ingredients (except bicarbonate, rumen fluid, vitamins, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 7.2, distribute medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add rumen fluid, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Vitamins should be sterilized by filtration. Check pH of complete medium and adjust to 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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913: THERMOTOGA PETROPHILA MEDIUM

Artificial sea water	750.00	ml
$(NH_4)_2SO_4$	1.00	g
KH_2PO_4	0.50	g
Yeast extract	2.00	g
MOPS (SIGMA)	5.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	240.00	ml

Dissolve ingredients except vitamins and sulfide, adjust pH to 7.0, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (to 30% of volume) and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.0, if necessary.

Artificial sea water (from medium 343)

NaCl	27.70	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_2O	5.50	g
KCl	0.65	g
NaBr	0.10	g
H_3BO_3	30.00	mg
SrCl2 x 6 H_2O	15.00	mg
Citric acid	10.00	mg
KI	0.05	mg
$CaCl_2$ x 2 H_2O	2.25	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g

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$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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914. PTYG-MEDIUM

Peptone	5.00	g
Tryptone	5.00	g
Yeast extract	5.00	g
Glucose	10.00	g
$MgSO_4$ x 7 H_2O	0.60	g
$CaCl_2$	0.06	g
Distilled water	1000.00	ml

pH 7.0

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915: BLAUTIA MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$(NH_4)_2SO_4$	0.30	g
NH_4Cl	1.00	g
NaCl	0.61	g
$MgSO_4$ x 7 H_2O	0.12	g
$CaCl_2$ x 2 H_2O	0.08	g
Modified Wolin's mineral solution	10.00	ml
Clarified rumen fluid	100.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	7.50	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	2.00	ml
L-Cysteine HCl x H_2O	0.45	g
Na_2S x 9 H_2O	0.45	g
Distilled water	890.00	ml

Dissolve ingredients (except bicarbonate, glucose, vitamins and reducing agents), then sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Add solid bicarbonate, adjust pH to 6.8 - 7.0 and dispense medium under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The final pH of the medium should be 7.0.

For DSM 14465, DSM 14466: Supplement medium with 0.50 g/l sodium formate.

For DSM 14469: Omit yeast extract from the medium and add 2.00 g/l Trypticase peptone (BD BBL), 2.50 g/l sodium acetate and 2.50 g/l sodium formate.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g

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Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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916: DESULFOFABA HANSENII MEDIUM

Solution A	912.00	ml
Solution B	50.00	ml
Solution C	25.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B and D are autoclaved under 100% N_2 gas atmosphere. Solution C is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions E and F are prepared under 100% N_2 gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. The pH of the complete medium should be 7.0 - 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning.

Solution A

NaCl	20.00	g
KCl	0.67	g
NH_4Cl	0.10	g
KH_2PO_4	0.01	g
$MgSO_4$ x 7 H_2O	0.02	g
Na_2SO_4	1.42	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	910.00	ml

Solution B

$MgCl_2$ x 6 H_2O	10.60	g
$CaCl_2$ x 2 H_2O	1.52	g
Distilled water	50.00	ml

Solution C

Na_2CO_3	1.25	g
Distilled water	25.00	ml

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Solution D

Na-propionate	0.96	g
Distilled water	10.00	ml

Solution E

Wolin's vitamin solution (10x)	1.00	ml

Solution F

$FeCl_2$ x 4 H_2O	0.52	g
Distilled water	10.00	ml

Adjust to pH 2 with HCl.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg

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916: DESULFOFABA HANSENII MEDIUM

Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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917. MODIFIED SEA WATER AGAR

Yeast extract	1.50	g
Peptone	2.50	g
Hexadecane	2.00	g
NaCl	17.70	g
KCl	0.48	g
$MgCl_2$ x 6 H_2O	3.40	g
$MgSO_4$ x 7 H_2O	4.46	g
$CaCl_2$ x 2 H_2O	0.98	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust to pH 7.2. Add agar and autoclave at 121°C for 20 min. A stock solution of calcium chloride should be sterilized separately and added to the medium after autoclaving.

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918. THAUERA MECHERNICHI MEDIUM

Na_2HPO_4	4.20	g
KH_2PO_4	1.50	g
NH_4Cl	0.30	g
$MgSO_4$ x 7 H_20	0.10	g
Na-Acetate	2.93	g
Trace element solution (see below)	2.00	ml
Distilled water	1000.00	ml

pH 7.1

Trace element solution:

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	2.00	g
$CaCl_2$ x 2 H_2O	5.50	g
$MnCl_2$ x 4 H_2O	5.06	g
$FeSO_4$ x 7 H_2O	5.00	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1.10	g
$CuSO_4$ x 5 H_2O	1.57	g
$CoCl_2$ x 6 H_2O	1.61	g
Distilled water	1000.00	ml

pH 6.0

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919. LIMNOBACTER MEDIUM

Yeast extract	0.50	g
Proteose peptone (Difco)	0.50	g
Casamino acids (Difco)	0.50	g
Glucose	0.50	g
Soluble starch	0.50	g
Sodium pyruvate	0.30	g
K_2HPO_4	0.30	g
$MgSO_4$ x 7 H_2O	0.05	g
Distilled water	1000.00	ml

Agar may be added (15.0 g/l) to prepare solid media. Final pH 7.2.

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920. GLUCONACETOBACTER JOHANNAE AND G. AZOTOCAPTANS MEDIUM

Yeast extract	2.70	g
Glucose	2.70	g
Mannitol	1.80	g
MES buffer (Sigma)	4.40	g
K_2HPO_4	4.81	g
KH_2PO_4	0.65	g
Distilled water	1000.00	ml

Agar may be added (15.0-18.0 g/l) to prepare solid media. Final pH 6.7. On revival from the freeze dried pellet growth of DSM 13594 and DSM 13595 appears to be better in liquid media than on plates.

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921. METHYLOSARCINA QUISQUILLARUM AND M. FIBRATA MEDIUM

Solution 1 (10x NMS salts):

KNO_3	10.00	g
$MgSO_4$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	2.00	g
Distilled water	1000.00	ml

Dissolve the ingredients listed above (in that order) in about 700 ml of distilled water, and then make up to 1 litre

Solution 2 (Fe EDTA):

Fe EDTA (Sigma, 03650)	3.80	g
Distilled water	1000.00	ml

Solution 3 (Sodium molybdate):

Na_2MoO_4 x 2 H_2O	0.26	g
Distilled water	1000.00	ml

Trace elements:

EDTA di sodium salt	25.00	mg
$CuSO_4$ x 5 H_2O	100.00	mg
$FeSO_4$ x 7 H_2O	50.00	mg
$ZnSO_4$ x 7 H_2O	40.00	mg
H_3BO_3	1.50	mg
$CoCl_2$ x 6 H_2O	5.00	mg
$MnCl_2$ x 4 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	1.00	mg
Distilled water	100.00	ml

May be stored at 4°C in the dark

Phosphate buffer:

Na_2HPO_4 x 12 H_2O	71.60	g
KH_2PO_4	26.00	g
Distilled water	1000.00	ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Prepare the growth medium as follows:
Dilute 100 ml solution 1 to 1 litre with distilled water and then add 1 ml of solution 3, 1 ml of the trace elements, and 0.1 ml of solution 2. If solid media is required add 1.5% agar. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 50% methane to the gas phase and autoclave at 115°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. When the growth medium is cool 10

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ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.

DSM 111909 may be grown on 1.0% methanol and for solid media use 15g agar per liter.

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923. ALKALIBACTERIUM OLIVAPOVLITICUS

Na glutamate (Sigma)	10.000	g
Yeast extract (Oxoid)	5.000	g
Na_2CO_3	10.000	g
K_2HPO_4	0.150	g
$MgSO_4$ x 7 H_2O	0.025	g
$(NH_4)_2SO_4$	1.000	g
Distilled water	1000.000	ml

We recommend that liquid media be made by autoclaving the Na_2CO_3 separately, otherwise the high pH will cause ammonia to be lost. When making media solidified with agar (20 g/l) the agar should also not be autoclaved with the Na_2CO_3 otherwise the agar will be hydrolysed. When mixing the ingredients for solid media cool all components to about 55°C otherwise hydrolysis of the agar may occur.

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924: METHANOCALCULUS MB MEDIUM

NaCl	10.00	g
$MgCl_2$ x 6 H_2O	1.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.40	g
K_2HPO_4	0.40	g
NH_4Cl	1.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Na-acetate	1.60	g
Na-formate	6.80	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, formate, vitamins and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate, adjust pH to 6.8 - 7.0, dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic serum bottles and autoclave. Add acetate, formate, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The pH of the complete medium should be 7.2.

For DSM 14649: Omit Na-acetate.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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925. ALKALIPHILIC SULPHUR RESPIRING STRAINS MEDIUM

Mineral base:

Na_2CO_3	20.0	g
$NaHCO_3$	10.0	g
NaCl	5.0	g
K_2HPO_4	1.0	g
Distilled water	1000.0	ml

Sterilize at 110°C 20 min in a closed vessel (i.e. a serum tube or bottle). pH after sterilization will about 10.

Trace element solution:

EDTA	5.0	mg
$FeSO_4$ x 7 H_2O	2.0	mg
$ZnSO_4$ x 7 H_2O	100.0	mg
$MnCl_2$ x 4 H_2O	30.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
H_3BO_3	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add:

Trace elements solution	2 ml/l
$MgCl_2$ x 6 H_2O (200.0 g/l)	1 ml/l

(a white colloid will form which will rapidly dissolve after mixing)

Prepare sterile stock solutions of the following

Sodium thiosulfate pentahydrate (2 M - 496 g/l)
KSCN solution (2 M - 194 g/l)
NH_4Cl - (1 M - 53.5 g/l)
KNO_3 - (1 M - 101 g/l)

Growth:
DSM 13531 = ARh1 (KSCN grown):
Growth with thiocyanate : add KSCN up to 15 mM, incubation - 30°C in conical flasks 1/10 liquid to air ratio, statically;
growth is slow, about 1 week

DSM 13532 = ARh2 (KSCN grown):
Growth with 15 mM KSCN: same conditions as for DSM 13531 = ARh1.

Continued on next page

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DSM 13541 = ARh1 (thiosulphate grown)
Growth with thiosulfate (40 mM) and NH_4Cl (5 mM); in conical flasks 1/10 liquid/air ratio on shaker 200 rpm. Heavy sulfur formation is usual and it is necessary to continue cultivation until the sulfur has been utilized.

DSM 13542 = ARh2 (thiosulphate grown)
Growth with thiosulfate (40 mM) and nitrate (10 mM); in conical flasks 1/5 liquid/air ratio on shaker 200 rpm. Growth takes 3-4 days, some sulfur can be formed during initial growth phase.

DSM 13533 = ALRh
Strain ALRh: grows with 40 mM thiosulfate and 5 mM KSCN in conical flasks 1/5 liquid to air ratio at 200 rpm.

DSM 14477 = ALM1
Strain ALM1 grows with 40 mM thiosulphate and 5 mM KNO_3

Thioalkalivibrio versutus AL 2 = DSM 13738, *Tv.nitratus* ALJ 12 = DSM 13741, and *Tv.denitrificans* ALJD = DSM 13742
Use 40 mM thiosulphate, 5 mM KNO_3, reduce the amount of $MgCl_2$ to 0.5 mM

Thioalkalimicrobium aerophilum AL 3 = DSM 13739 and *Tm.sibericum* AL 7 = DSM 13740
Use 80 mM thiosulphate, 5 mM KNO_3, reduce the amount of $MgCl_2$ to 0.5 mM

Thioalkalispira microaerophila, ALEN 1 = DSM 14786. The strain is grown under nitrogen with 1% oxygen in the gas phase. The strain grows slowly with a potential lag phase of several days.
Use 30 mM thiosulphate and up to a maximum of 5 mM NH_4Cl

Thioalkalivibrio nitratireducens ALEN 2 = DSM 14787
Use 30 - 40 mM thiosulphate and 5-10 mM KNO_3

Thioalkalivibrio sp. ALED = DSM 14788
Use 30 mM thiosulphate and up to a maximum of 5 mM NH_4Cl

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926. ALKALIPHILIC THERMOCOCCUS MEDIUM

NaCl	27.70	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_20	5.50	g
KCl	0.65	g
NaBr	0.10	g
$NaHCO_3$	0.32	g
K_2HPO_4	1.00	g
$CaCl_2$ x 2 H_2O	0.05	mg
Trace element solution (see medium 141)	20.00	ml
KI	15.00	mg
H_3BO_3	0.03	g
Distilled water	2000.00	ml

Prepare the medium anaerobically under nitrogen. Do not adjust the pH.

Prepare separate anaerobic stock solutions of, Casamino acids(10%), Yeast extract (10%), and glycine (2M = 150 g/l). A 0.5M polysulphide solution is prepared by dissolving 12.0 g Na_2S x 9 H_2O in oxygen free water, followed by adding 1.6 g sulphur - the solution will be dark yellow.

To the sterile, anaerobic, mineral medium add:
0.12 ml polysulphide/10 ml medium, yeast extract to a final concentration of 0.2% and glycine to a final concentration of 0.1 M for DSM 10322.

0.08 ml polysulphide/10 ml medium, casamino acids to a final concentration of 0.2% for DSM 11906. The may be precipitation of material and the medium will turn pale yellow due to the addition of the polysulphide. The colour will disappear as the strain grows.

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927. HALORHABDUS UTAHENSIS MEDIUM

NaCl	270.0	g
NaBr	0.1	g
$MgSO_4$ x 7 H_2O	20.0	g
KCl	5.0	g
NH_4Cl	2.0	g
Tris-HCl	12.0	g
Yeast extract (Difco)	1.0	g
Glucose	2.0	g
Distilled water	1000.0	ml

Prepare and sterilise separately:

KH_2PO_4	50.0	g
Distilled water	1000.0	ml
$CaCl_2$ x 6 H_2O	100.0	g
Distilled water	1000.0	ml
$FeCl_2$ x 4 H_2O	20.0	g
$MnCl_2$ x 4 H_2O	20.0	g
Distilled water	1000.0	ml

Prepare the medium, and adjust the pH to 7.6 with 5M NaOH.
Autoclave and when the medium has cooled add 2.5 ml of the phosphate solution, 0.5 ml of the calcium chloride solution, and 0.25 ml of the Fe/Mn solution. This strain will not grow in normal media for halobacteria, such as DSM medium 372!

For **DSM 27208** add 2ml/l trace elements SL-7

Trace element solution SL-7:

HCl (25%)	6.5	ml
$FeCl_2$ x 7 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	62.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	17.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	1000.0	ml

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928. ROSEINATRONOBACTER MEDIUM

Na_2CO_3	22.00	g
$NaHCO_3$	8.00	g
NaCl	5.00	g
KNO_3	0.50	g
K_2HPO_4	0.50	g
Yeast extract	1.00	g
Peptone	1.00	g
Sodium acetate	1.60	g
$MgSO_4$	0.06	g
Trace elements solution (see below)	2.00	ml
Distilled water	1000.00	ml

Final pH should be about 10.0

If medium is solidified with agar prepare the medium given above in 500 ml of distilled water and 20 g of agar in another container with 500 ml distilled water. Autoclave and cool to about 50°C before mixing.

Trace elements solution (Pfennig & Lippert,1966)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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929. RHODOVULUM IODOSUM AND R. ROBIGINOSUM MEDIUM

NaCl	26.40	g
$MgCl_2$ x 6 H_20	5.70	g
$MgSO_4$ x 7 H_2O	6.80	g
$CaCl_2$ x 2 H_2O	1.50	g
KCl	0.66	g
KBr	0.09	g
Distilled water	1000.00	ml

Prepare the medium anaerobically under an atmosphere of N_2:CO_2 (8:2 vol:vol) and then add:

$NaHCO_3$ solution		
(84 g/l autoclaved under a CO_2 atmopshere)	30.00	ml/l
KH_2PO_4	0.40	g/l
NH_4Cl	0.25	g/l
$Na_2S_2O_3$	0.50	mM
Trace elements	1.00	ml/l
Selenite/tungstate solution	1.00	ml/l
Vitamin solution	1.00	ml/l
Vitamin B12 solution	1.00	ml/l
Vitamin B1 solution	1.00	ml/l

The pH is adjusted to 6.8

Trace elements:

$FeSO_4$ x 7 H_2O	2.10	g
Na_2EDTA	5.20	g
H_3BO_3	30.00	mg
$MnCl_2$ x 4 H_20	100.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$ZnSO_4$ x 7 H_2O	144.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Adjust the pH to 6.0

Selenite/tungsate solution:

Na_2SeO_3 x 5 H_2O	6.00	mg
Na_2WO_4 x 2 H_2O	8.00	mg
NaOH	200.00	mg
Distilled water	1000.00	ml

Vitamin solutions: See next page!

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Vitamin solution:		
Para-aminobenzoic acid	4.00	mg
Biotin	1.00	mg
Nicotinate	10.00	mg
Pantothenate	5.00	mg
Pyridoxin	15.00	mg
Distilled water	100.00	ml
Vitamin B12 solution:		
Cyanocobalamine	5.00	mg
Distilled water	100.00	ml
Vitamin B1 solution:		
Thiamine	10.00	mg
Distilled water	100.00	ml

To the medium add 5 mM acetate and 10 mM $FeSO_4$ (from a 1 M stock solution kept under nitrogen). When the iron is added to the medium a white floculant precipitate may appear. In the initial stages of growth the precipitate turns orange/brown as the ferrous ions are oxidised to ferrihydrite. Cultivation may take 2-3 weeks at a distance of 30 cm from a 25 W light bulb.

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930. NATRONINCOLA HISTIDINOVORANS MEDIUM

KH_2PO_4	0.200	g
$MgCl_2$ x 6 H_2O	0.100	g
NH_4Cl	1.000	g
KCl	0.200	g
NaCl	80.000	g
Na_2CO_3	6.830	g
$NaHCO_3$	3.830	g
Na_2S x 9 H_2O	0.200	g
Yeast extract	0.200	g
Histidine	5.000	g
Trace elements (see DSM medium 141)	1.000	ml
Vitamin solution (see DSM medium 141)	2.000	ml
Resazurin	0.001	g
Distilled water	1000.000	ml

Final pH 8.9

Prepare the medium without adding the vitamins, histidine, yeast extract, NH_4Cl, Na_2CO_3, and $NaHCO_3$ using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH_4Cl, Na_2CO_3, and $NaHCO_3$ after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave, and to the cooled medium add the vitamins (filter sterilised under N_2), histidine, yeast extract, and Na_2S x 9 H_2O from anaerobic, sterile stock solutions.

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931. ALCALIPHILIC AMPHIBACILLUS STRAINS MEDIUM

KH_2PO_4	0.200	g
$MgCl_2$	0.100	g
NH_4Cl	0.500	g
KCl	0.200	g
Na_2CO_3	63.600	g
$NaHCO_3$	50.400	g
Na_2S x 9 H_2O	0.700	g
Yeast extract	0.200	g
Sucrose	5.000	g
Trace elements (see DSM medium 141)	1.000	ml
Vitamin solution (see DSM medium 141)	10.000	ml
Resazurin	0.001	g
Distilled water	1000.000	ml

Final pH 9.5-10.0

Prepare the medium without adding the vitamins, sucrose, yeast extract, NH_4Cl, Na_2CO_3, and $NaHCO_3$ using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH_4Cl, Na_2CO_3, and $NaHCO_3$ after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave, and to the cooled medium add the vitamins (filter sterilised under N_2), sucrose, yeast extract, and Na_2S x 9 H_2O from anaerobic, sterile stock solutions. DSM 13869 and DSM 13870 were deposited as anaerobic cultures.

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932. HALONATRONUM SACCHAROPHILUM MEDIUM

KH_2PO_4	0.200	g
$MgCl_2$	0.100	g
NH_4Cl	0.500	g
KCl	0.200	g
NaCl	50.000	g
Na_2CO_3	68.000	g
$NaHCO_3$	38.000	g
Na_2S x 9 H_2O	0.700	g
Yeast extract	0.200	g
Sucrose	5.000	g
Trace elements (see DSM medium 141)	1.000	ml
Vitamin solution (see DSM medium 141)	10.000	ml
Resazurin	0.001	g
Distilled water	1000.000	ml

Final pH 9.5-10.0

Prepare the medium without adding the vitamins, sucrose, yeast extract, NH_4Cl, Na_2CO_3, and $NaHCO_3$ using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH_4Cl, Na_2CO_3, and $NaHCO_3$ after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave, and to the cooled medium add the vitamins (filter sterilised under N_2), sucrose, yeast extract, and Na_2S x 9 H_2O from anaerobic, sterile stock solutions.

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933. HALOANAEROBIUM CONGOLENSE MEDIUM

NH_4Cl	1.000	g
K_2HPO_4	0.300	g
KH_2PO_4	0.300	g
$MgCl_2$ x 6 H_2O	10.000	g
$CaCl_2$ x 2 H_2O	0.100	g
KCl	1.000	g
Na-acetate	0.500	g
Cysteine	0.500	g
Trypticase	1.000	g
Yeast extract	1.000	g
NaCl	100.000	g
Trace elements SL-6 (see medium 141)	1.000	ml
Resazurin	0.001	g
Distilled water	1000.000	ml

Prepare the medium and boil, cool under N_2:CO_2 (80:20 v/v). Dispense medium into Hungate tubes or serum bottles under N_2:CO_2 (80:20 v/v), autoclave. To the sterile medium add from sterile stock solutions 2% Na_2S x 9 H_2O (0.2 ml/10 ml), 10% $NaHCO_3$ (0.2 ml/10 ml), 1M glucose (0.2 ml/10 ml) and 1M sodium thiosulphate (0.2 ml/10 ml). Final pH 7.0

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934. THERMOCOCCUS WAIOTAPUENSE MEDIUM

NaCl	1.500	g
Na_2SO_4	4.000	g
KCl	0.700	g
$NaHCO_3$	0.200	g
KBr	0.100	g
H_3BO_3	0.030	g
$MgCl_2$	5.800	g
$CaCl_2$	1.500	g
$SrCl_2$	0.025	g
Tryptone	5.000	g
Yeast extract	1.000	g
Na_2S x 9 H_2O	0.750	g
Resazurin	0.002	g
Sulphur	5 - 10.000	g
Distilled water	1000.000	ml

pH 7.0

Prepare the medium anaerobically under N_2. Steam the medium for 3 hours on three successive days.

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935: DEFERRIBACTER MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.08	g
$MgCl_2$ x 6 H_2O	4.50	g
$CaCl_2$ x 2 H_2O	0.38	g
NaCl	30.00	g
KNO_3	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	2.00	g
Na_2CO_3	1.50	g
Na-pyruvate	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, pyruvate and vitamins), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30-45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate and vitamins from anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The pyruvate and vitamins solutions are sterilized by filtration. The pH of the complete medium should be 6.5 - 6.7.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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936. SALINIBACTER RUBER MEDIUM

NaCl	195.000	g
$MgCl_2$ x 6 H_2O	34.600	g
$MgSO_4$ x 7 H_2O	49.500	g
$CaCl_2$ x 2 H_2O	1.250	g
KCl	5.000	g
$NaHCO_3$	0.250	g
NaBr	0.625	g
Yeast extract	1.000	g
Distilled water	1000.000	ml

Final pH 7.2.

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937. PYES MEDIUM

Peptone from casein	3.0	g
Yeast extract	3.0	g
Sodium succinate	2.3	g
Distilled water	1000.0	ml

Final pH 7.2. Liquid media may be solidified by adding 15 g/l agar.

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938. ST21 AGAR FOR CELLULOSE DEGRADERS

Solution A:		
K_2HPO_4	1.0	g
Yeast extract (Difco)	20.0	mg
Agar	14.0	g
Tap water	600.0	ml
Solution B:		
KNO_3	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
$CaCl_2$ x 2 H_2O	1.0	g
$MnSO_4$ x 7 H_2O	0.1	g
Tap water	300.0	ml
Solution C:		
$FeCl_3$	0.2	g
Tap or distilled water	100.0	ml

Autoclave solutions separately and combine after cooling to 50°C. A slight precipitate does not restrict agar quality. Adjust pH to 7.2 with KOH using pH indicating paper strips before pouring plates. Place 3-4 strips of filter paper (ca 1 cm^2) on the gelated plates and inoculate the filter strips.

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939. METHYLOTROPHIC ARTHROBACTER AND HYPHOMICROBIUM MEDIUM

Na_2HPO_4 x 2 H_2O	7.9	g
KH_2PO_4	1.5	g
NH_4Cl	0.8	g
$MgSO_4$ x 7 H_2O	0.1	g
Trace elements	10.0	ml
Distilled water	1000.0	ml

Final pH 7.2-7.5

After autoclaving add 10 ml methanol to 1 litre of the sterile Mineral Salts Solution.

Trace elements solution:

EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
Distilled water	1000.00	ml

Dissolve the EDTA in about 400 ml of water, then add 9 g NaOH. Dissolve each of the salts individually in about 40-50 ml of water and add them to the EDTA-NaOH solution. Adjust the final pH of the solution to pH 6.0 with 1M NaOH (about 24 ml). Make up to 1 litre with distilled water, store in the dark and do not autoclave the stock solution before it is added to the medium.

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940. HORIKOSHI ALKALINE

D-glucose	10.0	g
Peptone	5.0	g
Yeast extract	5.0	g
KH_2PO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Na_2CO_3	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH 9.0

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941. MEDIUM FOR ARHODOMONAS AND MARINOBACTER

NaCl (for *Arhodomonas*)	150.00	g
NaCl (for *Marinobacter*)	6.00	g
NH_4Cl	1.00	g
KCl	0.10	g
KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.04	g
Na-acetate	1.00	g
Bactopeptone	0.10	g
Trace element solution (SL7)	1.00	ml
Distilled water	1000.00	ml

pH 7.2

Vitamin solution		
(see Medium 141 x 10 conc.)	1.00	ml

Trace element solution SL-7:

Distilled water	1000.0	ml
HCl (25%)	6.5	ml
$FeCl_2$ x 7 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	62.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	17.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg

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942. AGAR WITH E. COLI AS FEED

Ca_2Cl x 2 H_2O	1.0	g
Agar	15.0	g

Adjust pH to 7.2, autoclave and pour plates. Grow *Escherichia coli* (e.g. DSM 498) in liquid culture, collect cells by centrifugation and resuspend them in enough water to receive a thick but ready-to-pipet suspension. Autoclave. Place three separate drops on one $CaCl_2$-agar plate and streak every drop once to receive *E. coli* lines, about 3 cm long, across the *E. coli* drops. Inoculate *E. coli* lines/drops with myxobacteria.

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943: SEDIMENTIBACTER SAALENSIS MEDIUM

$(NH_4)HCO_3$	0.45	g
$CaCl_2$ x 2 H_2O	0.12	g
$MgSO_4$ x 6 H_2O	0.13	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	3.00	g
$NaHCO_3$	3.80	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sodium Potassium phosphate buffer (1 M, pH 7.3)	10.00	ml
Glycine	0.75	g
L-Arginine x HCl	2.11	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O (3% w/v)	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, phosphate buffer, glycine, arginine, vitamins, and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate and adjust pH to 7.3, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add phosphate buffer, glycine, arginine, vitamins (sterilized by filtration), and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Prior to inoculation add 10 - 20 mg sodium dithionite per liter medium from a 5% (w/v) stock solution, freshly prepared under 100% N_2 gas and filter-sterilized.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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944. PSEUDOMONAS CHLOROTIDISMUTANS MEDIUM

Solution A:

KH_2PO_4	0.41	g
Na_2HPO_4	0.53	g
$NaClO_3$	1.06	g
Na-Acetate x 3 H_2O	2.72	g
Resazurin	0.50	mg
Trace element solution SL-10	1.00	ml
Selenite-tungstate-molybdate solution	10.00	ml
Distilled water	900.00	ml

Solution B:

$CaCl_2$	0.11	g
$MgCl_2$	0.10	g
Distilled water	50.00	ml

Solution C:

NH_4HCO_3	0.44	g
$NaHCO_3$	3.73	g
Na_2S x 9 H_2O	0.50	g
Distilled water	50.00	ml

All solutions are prepared anaerobically under N_2 and autoclaved. After cooling, the solutions are combined under N_2 gas. Add 1 ml/l vitamin solution. pH should be 8.5 without further adjustment.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Selenite-tungstate-molybdate solution:

NaOH	500.00	mg
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	3.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
Distilled water	1000.00	ml

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Vitamin solution:

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

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945: MARINITOGA PIEZOPHILA MEDIUM

Final pH: 6.0
Final volume: 1000 ml

NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	30.00	g
Na-acetate	0.83	g
MES (SIGMA)	1.95	g
Yeast extract	5.00	g
Trypticase peptone (BD BBL)	5.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, cysteine and sulfide. Sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic and adjust pH to 6.0 with NaOH. Distribute the medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur. For sterilization heat the cultivation vessels containing medium plus sulfur in boiling water for 2 - 3 hours on each of 3 successive days! Prior to inoculation add cysteine and sulfide and from sterile anoxic stock solutions prepared under 100% N_2 gas.

2. After inoculation pressurize tubes to 2 bar overpressure with sterile 100% N_2 gas.

For DSM 14283: 5.00 g/l maltose can be used as substrate instead of sulfur. Maltose is added to autoclaved medium from an anoxic stock solution, sterilized by filtration.

For DSM 17373: Adjust pH of complete medium to 5.5. After inoculation pressurize tubes to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

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946: NAUTILIA MEDIUM

Synthetic seawater (2 x conc.)	500.00	ml
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Na_2CO_3	1.50	g
Na-formate	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.60	g
Distilled water	500.00	ml

Dissolve ingredients (except sulfur, carbonate, formate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. For sterilization heat cultivation vessels for 1 - 2 hours to 90 - 100°C in a water bath on each of three successive days. Prior to inoculation add carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere and formate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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946: NAUTILIA MEDIUM

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Synthetic seawater (2 x conc.) (from medium 795)

NaCl	55.40	g
SrCl2 x 6 H_2O	0.03	g
$MgSO_4$ x 7 H_2O	14.00	g
KI	0.10	mg
$MgCl_2$ x 6 H_2O	11.00	g
Na_3-citrate	20.00	mg
KCl	1.30	g
$CaCl_2$ x 2 H_2O	1.50	g
NaBr	0.20	g
H_3BO_3	0.06	g
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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947: CALORAMATOR VITERBIENSIS MEDIUM

$(NH_4)_2SO_4$	0.50	g
NH_4Cl	0.50	g
KH_2PO_4	2.00	g
$MgCl_2$ x 6 H_2O	0.04	g
$CaCl_2$ x 2 H_2O	0.04	g
Yeast extract	0.30	g
Glycerol	3.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.13	g
Na_2S x 9 H_2O	0.13	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate and equilibrate pH to 6.3 with 80% N_2 and 20% CO_2 gas mixture. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 6.2 - 6.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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948. OXOID NUTRIENT BROTH

Nutrient broth (Oxoid)*	13.0	g
Distilled water	1000.0	ml

** (composition of Oxoid nutrient broth):*

"Lab-Lemco" powder (Oxoid)	1.0	g
Yeast extract	2.0	g
Peptone	5.0	g
NaCl	5.0	g
Distilled water	1000.0	ml

pH 7.2

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949. SEA WATER YEAST PEPTONE MEDIUM

Yeast extract	3.0	g
Peptone	5.0	g
Filtered, aged sea water	750.0	ml
Distilled water	250.0	ml

Adjust pH to 7.3. Prepare in similar manner to sea water agar.

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950. ONR7a MEDIUM

Solution 1:

NaCl	22.79	g
Na_2SO_4	3.98	g
KCl	0.72	g
NaBr	83.00	mg
$NaHCO_3$	31.00	mg
H_3BO_3	27.00	mg
NaF	2.60	mg
NH_4Cl	0.27	g
Na_2HPO_4 x 7 H_2O	89.00	mg
TAPSO	1.30	g
H_2O dest	500.00	ml

adjust pH to 7.6 with NaOH

Solution 2:

$MgCl_2$ x 6 H_2O	11.18	g
$CaCl_2$ x 2 H_2O	1.46	g
$SrCl_2$ x 6 H_2O	24.00	mg
H_2O dest	450.00	ml

Solution 3:

$FeCl_2$ x 4 H_2O	2.00	mg
H_2O dest	50.00	ml

Autoclave the solutions separately. All solutions should be mixed after autoclaving when they have cooled to at least 50°C. For solid media add to the first solution 15 g/l of Bacto agar or 12 g/l of agarose (Sigma).

DSM 13489 should be grown with 0.1-1% tetradecane or hexadecane (0.2% prefered).
DSM 14913 may be grown with 1% sodium acetate, 0.1-1% tetradecane, or 1% Tween 80.
Tetradecane or hexadecane are filter sterilized.

DSM 26010, DSM 26066 may be grown with 0.5 % sodium acetate.

DSM 26671, DSM 26678, DSM 26710 may be grown with 1.0 % sodium pyruvate.

DSM 29600 may be grown with 1.0 % sodium pyruvate and pH=8.0.

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951. METHYLOPHAGA SULFIDOVORANS MEDIUM

NaCl	15.00	g
$(NH_4)_2SO_4$	0.50	g
$CaCl_2$ x 6 H_2O	0.33	g
KCl	0.20	g
$MgSO_4$ x 7 H_2O	1.00	g
KH_2PO_4	0.02	g
Na_2CO_3	2.00	g
$FeSO_4$ x 7 H_2O	1.00	mg
Trace elements solution SL10 (see below)	1.00	ml
Vitamin solution (see below)	1.00	ml
Methanol	10.00	ml
Agar	15.00	g
Distilled water	998.00	ml

Prepare the medium without methanol and the vitamin solution. Adjust pH to 7.5 (±0.3) with 2 N HCl. Liquid media is prepared in either Hungate tubes (5ml medium) or 100ml serum bottles (10ml medium) sealed with rubber stoppers.
Sterilize by autoclaving. After autoclaving add the methanol (filter sterilised) and the vitamin solution (filter sterilised) from sterile stock solutions.

Trace element solution SL10

HCl (concentrated)	5.000	ml
$FeCl_2$ x 4 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.200	g
H_3BO_3	0.006	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled water	1000.000	ml

Vitamin solution:

Biotin	20.00	mg
Nicotinic acid	200.00	mg
Thiamine	100.00	mg
p-aminobenzoic acid	100.00	mg
Pantothenate	50.00	mg
Pyridoxine x HCl	500.00	mg
Riboflavine	10.00	mg
Vitamin B_{12}	10.00	mg
Distilled water	1000.00	ml

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952. 1/10 NUTRIENT AGAR NO.2

Peptone	1.0	g
Meat extract	1.0	g
NaCl	0.5	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0-7.2.

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953. 1/100 NUTRIENT AGAR NO.2

Peptone	0.1	g
Meat extract	0.1	g
NaCl	50.0	mg
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0-7.2.

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954. MEDIUM FOR H. DOMBROWSKII

Casamino acids	5.00	g
Yeast extract (Oxoid)	5.00	g
TRIS	12.10	g
KCl	2.00	g
$MgCl_2$ x 6 H_2O	20.00	g
$CaCl_2$ x 2 H_2O	0.20	g
NaCl	200.00	g
Agar	20.00	g
Distilled water	1000.00	ml

Adjust pH to 7.4. Add the agar after dissolving all ingredients in the water and adjustment of pH.

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955. AG MEDIUM

Glucose	1.0	g
Glycerol	1.5	g
Peptone	5.0	g
Yeast extract	5.0	g
Malt extract	2.0	g
$CaCO_3$	7.0	g
Distilled water	1000.0	ml

pH 6.8. Agar may be added to solidify media at a final concentration of 1.5%.

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956. MEDIUM FOR DSM 14457 AND 14458

KH_2PO_4	2.000	g
$(NH_4)_2SO_4$	2.000	g
$MgSO_4$ x 7 H_2O	0.125	g
NaCl	0.500	g
$FeSO_4$ x 7 H_2O	0.002	g
Distilled water	1000.000	ml

pH 7.2. To the sterilised medium add sterile methanol (to a final concentration of 0.5%) or methylamine (to a final concentration of 0.3%).

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957. MICROVIRGULA MEDIUM

KH_2PO_4	0.82	g
K_2HPO_4	0.70	g
$MgSO_4$ x 7 H_2O	0.50	g
$(NH_4)_2SO_4$	1.00	g
Yeast extract	0.25	g
Na-succinate	1.50	g
KNO_3	1.50	g
Distilled water	1000.00	ml

Adjust pH 7.0, anaerobic with N_2.

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958. VY/4-SWS AGAR

NaCl	20.00	g
Yeast cell paste (bakers yeast, washed in deion. water) wet weight	2.50	g
Bacto agar	15.00	g
Cyanocobalamine (vitamin B_{12})	0.50	mg
Sea water salts solution (see below)	1000.00	ml

Adjust pH to 7.5 with 1M NaOH, autoclave at 121°C for 20 min. Add filter sterilized cyanocobalamine solution after autoclaving and cooling.

Sea water salts solution:

Ferric citrate	0.01	g
$MgSO_4$ x 7 H_2O	8.00	g
$CaCl_2$ x 2 H_2O	1.00	g
KCl	0.50	g
$NaHCO_3$	0.16	g
H_3BO_3	0.02	g
KBr	0.08	g
$SrCl_2$ x 6 H_2O	0.03	g
di-Na-ß-glycerophosphate	0.01	g
Trace elements solution SL-4	1.00	ml
Distilled water	1000.00	ml

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6 (see below)	100.00	ml
Distilled water	900.00	ml

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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959: PETROTOGA OLEARIA MEDIUM

NaCl	15.00	g
$MgCl_2$ x 6 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	1.00	g
KCl	0.20	g
PIPES	3.40	g
K_2HPO_4	0.35	g
KH_2PO_4	0.35	g
Na-acetate x 3 H_2O	2.72	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Maltose	3.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except maltose and sulfide, adjust pH to 7.0 - 7.2 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add maltose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. The pH of the complete medium should be 7.5.

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960: PELOTOMACULUM MEDIUM

Final pH: 7.0
Final volume: 1002 ml

KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-pyruvate	2.20	g
Yeast extract (OXOID)	0.10	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, pyruvate, yeast extract, vitamins, and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, yeast extract, vitamins, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of vitamins and pyruvate should be sterilized by filtration. The pH of the complete medium should be 7.0.

2. Note: Use 10% (v/v) as inoculum.

For DSM 13752, DSM 21041: Replace pyruvate with 0.92 ml/l ethanol.

For DSM 14795: Replace pyruvate with 0.86 g/l Na-crotonate.

For DSM 14947: Replace pyruvate with 0.72 g/l Na-benzoate.

For DSM 15200, DSM 15578, DSM 16145: Replace pyruvate with 2.00 g/l Na-propionate.

For DSM 16121: Replace pyruvate with 10 ml/l of a sterile anoxic Na-crotonate solution (1 M).

For DSM 16494, DSM 22288: Replace pyruvate with 2.72 g/l Na-formate and 0.10 g/l Na -acetate. Supplement medium with 0.08 g/l coenzyme M (2-mercaptoethanesulfonic acid).

For DSM 17711, DSM 23604: Replace pyruvate with 0.10 g/l Na-acetate and pressurize serum vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

For DSM 17771: Supplement medium with 1.42 g/l Na_2SO_4 and replace pyruvate with 0.41 g/l 4-hydroxybenzoic acid added from a sterile, neutralized and anoxic stock solution.

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Increase concentration of yeast extract to 0.20 g/l.

For DSM 18709, DSM 26646: Replace pyruvate with 0.86 g/l Na-crotonate and increase concentration of yeast extract to 0.50 g/l.

For DSM 24196: Increase amount of yeast extract to 0.20 g/l and replace pyruvate with 0.18 g/l D-glucose added from an anoxic stock solution sterilized by filtration. Use 5% (v/v) as inoculum.

For DSM 24202: Omit yeast extract and replace pyruvate with 0.18 g/l D-glucose added from an anoxic stock solution sterilized by filtration. Use 10% (v/v) as inoculum.

For DSM 102911: Increase amount of yeast extract to 0.50 g/l and replace pyruvate with 0.50 g/l Na-acetate added from an anoxic sterile stock solution. Use 5% (v/v) as inoculum.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg

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Distilled water	1000.00	ml

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961: THERMOVIBRIO MEDIUM

Sodium resazurin (0.1% w/v)	0.50	ml
KNO_3	1.00	g
Yeast extract (OXOID)	1.00	g
Na_2S x 9 H_2O	0.50	g
Synthetic seawater	1000.00	ml

1. Dissolve resazurin in seawater, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle) and autoclave. Add nitrate, yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 6.5, if necessary.

2. After inoculation pressurize bottles to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

3. Note: Alternatively, aged filtered seawater can be used instead of synthetic seawater.

Synthetic seawater

NaCl	27.70	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_2O	5.50	g
KCl	0.65	g
KH_2PO_4	0.50	g
$(NH_4)_2SO_4$	0.25	g
H_3BO_3	0.03	g
NaBr	0.10	g
$CaCl_2$ x 2 H_2O	0.75	g
KI	0.05	mg
SrCl2 x 6 H_2O	15.00	mg
Distilled water	1000.00	ml

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962: THERMINCOLA FERRIACETICA MEDIUM

Na_2-9,10-anthraquinone-2,6-disulfonate (TCI A0308)	8.00	g
NH_4Cl	0.33	g
KH_2PO_4	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
KCl	0.33	g
Yeast extract (OXOID)	0.05	g
Modified Wolin's mineral solution	10.00	ml
$NaHCO_3$	2.50	g
Na-acetate	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate and vitamins), boil medium to dissolve AQDS, then cool to room temperature under 80% N_2 and 20% CO_2 gas atmosphere. Add solid bicarbonate and adjust pH to 6.8 - 7.0. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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963: THERMOANAEROMONAS MEDIUM

K_2HPO_4	0.78	g
KH_2PO_4	0.75	g
Na_3-EDTA	0.04	g
$FeSO_4$ x 7 H_2O	0.01	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.03	g
NaCl	0.20	g
NH_4Cl	0.50	g
D-Glucose	0.50	g
Yeast extract (OXOID)	0.50	g
Trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	5.00	g
$Na_2S_2O_3$ x 5 H_2O	1.24	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, thiosulfate, vitamins and cysteine), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate and adjust pH to 6.8 - 7.0. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

For DSM 26576: Adjust pH of medium to 7.5.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g

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Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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964: CAMINICELLA MEDIUM

Sea Salt (SIGMA)	30.00	g
PIPES buffer (SIGMA)	6.05	g
D-Glucose	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	12.00	g
Peptone (BD Bacto)	1.00	g
Yeast extract (BD Bacto)	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, peptone, yeast extract, and sulfide), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 7.2 - 7.4, then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Add peptone, yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The pH of the complete medium should be 7.5.

For DSM 106824: Adjust pH of complete medium to 6.5 - 6.8.

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965: THERMOANAEROBACTER KREATINOPHILUS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.50	g
NaCl	2.00	g
KCl	0.20	g
$CaCl_2$ x 2 H_2O	0.05	g
Yeast extract (OXOID)	1.00	g
Tryptone (BD Bacto)	2.00	g
Modified Wolin's mineral solution	10.00	ml
Starch (soluble)	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine and thiosulfate), bring medium to the boil then cool to room temperature under 100% N_2 gas atmosphere. Add cysteine and adjust pH to 7.5, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation, add thiosulfate from a sterile anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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966. R3 MEDIUM

Yeast extract	1.00	g
Proteose peptone (Difco No.3)	1.00	g
Casamino acids (Difco)	1.00	g
Glucose	1.00	g
K_2HPO_4	0.60	g
$MgSO_4$ x 7 H_2O	0.10	g
Na pyruvate	0.05	g
Distilled water	1000.00	ml

Final pH should be 7.8

For **DSM 17972** prepare medium without glucose and adjust to pH 8.0 with 1M NaOH. Autoclave at 121°C for 15 minutes.

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967. TEREDINIBACTER TURNERAE MEDIUM

KH_2PO_4	15.3	mg
Na_2CO_3	10.0	mg
Na_2MoO_4 x 2 H_2O	2.5	mg
Na_2EDTA	0.5	mg
Ferric ammonium citrate	3.0	mg
HEPES	5.2	g
Trace elements solution	1.0	ml
Sea water	750.0	ml
Distilled water	250.0	ml

Teredinibacter turnerae will grow on either Sigmacell 100 (cellulose) or Whatman filter paper "1". When grown in the absence of a nitrogen source the strains will fix nitrogen under microaerophilic conditions. Microaerophilic conditions may be achieved by adding 2 g/l agar (semi-solid) and dispensing the medium into 10 ml portions in 16 mm (outside diameter) test tubes - incubate without shaking. The strains may also be grown with gentle shaking if NH_4Cl, 0.27 g/l is added to the medium.

Trace elements (A5 of Rippka et al., 1979):

H_3BO_3	2.860	g
$MnCl_2$ x 4 H_2O	1.810	g
$ZnSO_4$ x 7 H_2O	0.220	g
Na_2MoO_4 x 2 H_2O	0.390	g
$CuSO_4$ x 5 H_2O	0.079	g
Distilled water	1000.000	ml

DSM 104091 may be grown in the presence of 0.5% glucose instead of cellulose.

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968: ANAEROBACILLUS ARSENICISELENATIS MEDIUM

$(NH_4)_2SO_4$	0.10	g
$MgSO_4$	25.00	mg
NaCl	90.00	g
K_2HPO_4	0.15	g
KH_2PO_4	0.08	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$NaNO_3$	1.25	g
Na-DL-lactate	1.70	g
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	10.60	g
$NaHCO_3$	4.20	g
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, hydrogencarbonate and reducing agents), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add solid carbonate and bicarbonate, adjust pH to 9.8, dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation, add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 9.8, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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968: ANAEROBACILLUS ARSENICISELENATIS MEDIUM

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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971. HALOMONAS MAGADIENSIS MEDIUM

Glucose	10.0	g
Peptone (Difco)	5.0	g
Yeast extract (Difco)	5.0	g
KH_2PO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
NaCl	40.0	g
Na_2CO_3	10.0	g
Agar	20.0	g
Distilled water	1000.0	ml

NaCl and Na_2CO_3 were autoclaved separately and added to the organic components at 60°C before pouring the agar media. Final pH should be about 10.0.

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972. MYCT MEDIUM

KH_2PO_4	13.60	g
$(NH_4)_2SO_4$	4.00	g
$MgCl_2$	0.20	g
Sodium citrate	0.25	g
$FeSO_4$ x 7 H_2O	25.00	mg
$MnSO_4$ x 4 H_2O	25.00	mg
Yeast extract	3.00	g
Casein hydrolysate	3.00	g
Tween 80	1.00	g
Cyclomaltoheptaose (ß-cyclodextrin)	7.00	g
Distilled water	1000.00	ml

pH 6.5.

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973. MARINITHERMUS HYDROTHERMALIS MEDIUM

Yeast extract	1.00	g
Tryptone	1.00	g
NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
$NiCl_2$ x 6 H_2O	0.50	mg
Na_2Se_3 x 5 H_2O	0.50	mg
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	10.00	mg
Trace element solution (see Medium 141)	10.00	ml
Distilled water	990.00	ml

Final pH 7.0.

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974. 1/2 YTSS MEDIUM

Yeast extract	2.00	g
Tryptone	1.25	g
Sea salts (Sigma)	20.00	g
Distilled water	1000.00	ml

Final pH 7.0
Solid media is prepared by adding 15 g/l agar.

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975. OCEANITHERMUS PROFUNDUS MEDIUM

NH_4Cl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
KCl	0.33	g
KNO_3	1.00	g
NaCl	30.00	g
HEPES (10mM)	2.38	g
Yeast extract	0.20	g
Tryptone	1.00	g
Sucrose	2.00	g
Vitamin solution (see medium 141)	1.00	ml
Trace elements (see medium 141)	1.00	ml
Distilled water	1000.00	ml

Prepare the medium anaerobically, under nitrogen, omitting the $CaCl_2$, $MgCl_2$, KNO_3, tryptone, yeast extract, vitamins and sucrose. The pH should be 7.0-7.5. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of nitrogen and autoclave. To the sterile, cooled medium add, from sterile stock solutions the $CaCl_2$, $MgCl_2$, KNO_3, tryptone, yeast extract, vitamins and sucrose. The $CaCl_2$, $MgCl_2$, KNO_3, tryptone, yeast extract, and sucrose stock solutions should be autoclaved, while the vitamin solution is sterile filtered. This strain grows anaerobically in the presence of nitrate, but microaerophilic growth has been reported provided the oxygen concentration does not exceed 6 % (v/v). Under anaerobic conditions a redox agent, such as sulphide is not required.

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976. METHYLOPHAGA ALCALICA MEDIUM

KH_2PO_4	1.00	g
KNO_3	1.00	g
$MgSO_4$ x 7 H_2O	0.22	g
NaCl	30.00	g
Na_2CO_3	5.00	g
Trace elements	1.00	ml
Distilled water	1000.00	ml

Final pH 9.5

Trace elements solution:

Ferric citrate	30.00	mg
$CaCl_2$ x 2 H_2O	30.00	mg
$MgCl_2$ x 4 H_2O	5.00	mg
$ZnSO_4$ x 7 H_2O	5.00	mg
$CuSO_4$ x 5 H_2O	0.50	g
Distilled water	1000.00	ml

Prepare the medium without the Na_2CO_3, which can be sterilised separately by autoclaving. 10 ml/l sterile methanol is added to the cooled medium. When preparing liquid media cool the mineral salts solution and Na_2CO_3 to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salts solution and autoclave. Cool the Na_2CO_3 stock solution and agar to 50-55°C before mixing.

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977. VULCANITHERMUS MEDIUM

NH_4Cl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
KCl	0.33	g
KNO_3	0.33	g
NaCl	25.00	g
PIPES (12mM)	3.60	g
Yeast extract	0.50	g
Tryptone	1.00	g
Sucrose	1.00	g
Vitamin (see medium 141)	1.00	ml
Trace elements (see medium 141)	1.00	ml
Distilled water	1000.00	ml

Prepare the medium anaerobically, under nitrogen, omitting the $CaCl_2$, $MgCl_2$, KNO_3, tryptone, yeast extract, vitamins and sucrose. The pH should be 6.8. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of nitrogen and autoclave. To the sterile, cooled medium add, from sterile stock solutions the $CaCl_2$, $MgCl_2$, KNO_3, tryptone, yeast extract, vitamins and sucrose. The $CaCl_2$, $MgCl_2$, KNO_3, tryptone, yeast extract, and sucrose stock solutions should be autoclaved, while the vitamin solution is sterile filtered. This strain grows anaerobically in the presence of nitrate, but aerobic growth has been reported provided the oxygen concentration does not exceed 16% (v/v). Under anaerobic conditions a redox agent, such as sulphide is not required.

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979. PETROBACTER SUCCINIMANDENS MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	1.00	g
NH_4Cl	1.00	g
Yeast extract	1.00	g
Trace elements (see medium 141)	10.00	ml
Vitamin solution (see medium 141)	5.00	ml
Distilled water	1000.00	ml

Add to the basic medium for:

Geococcus nitratireducens

Na acetate	0.16	g
Glucose	3.60	g

Petrobacter succinimandens

Succinic acid	2.36	g

When grown aerobically prepare the medium without the vitamins, adjust the pH to 7.0 with 10M KOH, and sterilise for 45 min at 110°C. On cooling add the vitamins from a filter sterilised stock solution. Both *Geococcus nitratireducens* (DSM 15495) and *Petrobacter succinimandens* (DSM 15512) are supplied from aerobically grown cultures.

Anaerobic growth may be obtained by gassing the medium with N_2:CO_2 (80:20), to which 10mM KNO_3 has been added, and dispensing the medium under N_2:CO_2 (80:20). Sterilise by autoclaving at 110°C for 45 min, and to the cold medium add the filter sterilised vitamins. A reducing agent is not required.

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981: GEOTHERMOBACTER MEDIUM

NaCl	19.00	g
$MgCl_2$ x 6 H_2O	9.00	g
$MgSO_4$ x 7 H_2O	0.15	g
$CaCl_2$ x 2 H_2O	0.30	g
KCl	0.50	g
KH_2PO_4	0.42	g
$(NH_4)_2SO_4$	0.10	g
NaBr	0.05	g
SrCl2 x 6 H_2O	0.02	g
KNO_3	1.01	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.00	g
Yeast extract	0.10	g
DL-Malic acid	1.34	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.05	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, yeast extract, malic acid, vitamins and cysteine) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add yeast extract, malic acid, vitamins (sterilized by filtration), and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be 6.2.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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982. MY10-12 MEDIUM

Malt extract	20	g
Yeast extract	5	g
NaCl	100	g
Glucose	120	g
Agar	20	g
Dist. water	1000	ml

Sterilize by steaming for 30 min. Overheating will cause softening. Final a_w is 0.88.

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985: DEFERRIBACTER AUTOTROPHICUS MEDIUM

NaCl	18.00	g
$MgCl_2 \times 6\ H_2O$	4.00	g
KCl	0.34	g
NH_4Cl	0.25	g
$CaCl_2$	0.11	g
K_2HPO_4	0.18	g
$Fe(NH_4)_2(SO_4)_2 \times 7\ H_2O$	20.00	mg
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
KNO_3	1.00	g
Na-acetate	1.60	g
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, nitrate, acetate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration), nitrate, acetate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The pH of the complete medium should be 6.5 -7.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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986: PARAMALEDIVIBACTER MEDIUM

Sea Salt (SIGMA)	30.00	g
NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
Peptone	0.50	g
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
D-Glucose	4.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose, vitamins, cysteine and sulfide), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, glucose, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. The pH of the complete medium should be 7.0.

For DSM 103478: Adjust pH of complete medium to pH 7.5 - 8.0.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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987. ISP2 MEDIUM

Yeast extract (Difco)	4.0	g
Malt extract (Difco)	10.0	g
Dextrose (Difco)	4.0	g
Agar	20.0	g
Distilled water	1000.0	ml

pH 7.2

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988. YEAST-PEPTONE-SUCCINATE MEDIUM

Yeast extract	3.0	g
Peptone	3.0	g
Sodium succinate	2.3	g
Distilled water	1000.0	ml

Final pH 7.2. The medium may be solidified by adding 15 g/l agar.

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989. ACETIC ACID BACTERIUM MEDIUM

Bacto peptone	5.0	g
Yeast extract	5.0	g
Glucose	5.0	g
$MgSO_4$ x 7 H_2O	1.0	g
Distilled water	1000.0	ml
Agar (if needed)	15.0	g

pH 6.6-7.0

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990. YPS MEDIUM

Sea salts (Sigma)	35.00	g
PIPES	3.46	g
Yeast extract	1.00	g
Peptone	4.00	g
Elemental sulphur	5.00	g
NH_4Cl	0.50	g
KH_2PO_4	0.35	g
$CaCl_2$	0.20	g
$FeCl_3$	6.70	mg
Na_2WO_4	2.90	mg
Resazurin	0.10	mg
Na_2S x 9 H_2O	0.25	g

Prepare the medium without the yeast extract, peptone and sodium sulphide. Boil the medium and cool under nitrogen. Adjust the pH to 6.8. Dispense into hungate tubes or serum bottles under a nitrogen atmosphere. Sterilise the medium at 100°C for 3 hours on 3 consecutive days. Add the peptone and yeast extract from sterile (10% w/v) stock solutions. The medium is reduced by adding a sterile, neutralised 5% w/v sodium sulphide solution to an end concentration of 0.025%. Final pH should be 6.8

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991: CALDISPHAERA MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Trisodium citrate x 2 H_2O	2.94	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Yeast extract (OXOID)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, yeast extract, vitamins and sulfide), adjust pH to 3.5 with 10 N H_2SO_4 and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 100 ml bottles) containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1 - 2 hours on each of 3 successive days. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 4.0 - 4.5, if necessary.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg

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991: CALDISPHAERA MEDIUM

Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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992. BACILLUS FILIFORMIS MEDIUM

yeast extract	10.00	g
Na-citrate	3.00	g
KCl	2.00	g
$MgSO_4$ x 7 H_2O	1.00	g
Na_2CO_3	3.00	g
NaCl	100.00	g
$MnCl_2$ solution see below	1.00	ml
$FeSO_4$ solution see below	1.00	ml
Distilled water	1000.00	ml

$MnCl_2$ solution:

$MnCl_2$ x 4 H_2O	0.36	g
Distilled water	1000.00	ml

$FeSO_4$ solution:

$FeSO_4$ x 7 H_2O	50.00	g
Distilled water	1000.00	ml

Sodium carbonate and sodium chloride should be separately autoclaved. pH 9.0.

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993. ISP 5 MEDIUM

L-asparagine (anhydrous basis)	1.0	g
Glycerol	10.0	g
K_2HPO_4 (anhydrous basis)	1.0	g
Distilled water	1000.0	ml
Trace salts solution (see medium 84)	1.0	ml
Agar	20.0	g

The pH of trace salts solution is about 7.0-7.4. Do not adjust if it is within this range.
Liquefy agar by steaming at 100°C for 15-20 minutes.
Sterilize in flasks for pouring into Petri dishes. Seven standard 90 mm dishes containing 25 ml per dish.

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994. MINERAL MEDIUM

Sorbit or D-Glucose	3.6	g
NH_4Cl	0.6	g
KH_2PO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.4	g
NaCl	0.4	g
$CaCl_2$ x 2 H_2O	0.5	mg
SL4 (10 x conc., see medium 14)	1.0	ml
Vitamin solution (10 x conc., see medium 461)	5.0	ml
Distilled water	1000.0	ml

pH 6.8.

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995. NPB MEDIUM

Tryptone peptone (Difco)	10.0	g
Yeast extract (Difco)	2.0	g
$MgSO_4$ x 7 H_2O	1.0	g
K_2HPO_4	1.0	g
KH_2PO_4	0.5	g
D-glucose	5.0	g
Distilled water	1000.0	ml

For solid medium 15 g of agar is mixed, and autoclaved. pH 7.0.
Inoculate the cells to 5 ml of NPB medium, and incubate at 30°C with shaking (160 strokes per min.). Cell turbidity at 660 nm after 4 days of cultivation will be 0.4-0.5. On NPB agar plate, colony formation is observed after 3-4 days of incubation at 30°C.

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997. HNW MEDIUM

$NaNO_3$	1.000	g
$NaHCO_3$	1.000	g
Na_2WO_4 x 2 H_2O	0.100	mg
Na_2S	0.500	g
Trace vitamin solution (see medium 141)	10.000	ml
DMJ synthetic seawater (see below)	1000.000	ml

To prepare the medium, all compounds of DMJ seawater were dissolved in 1 liter of distilled deionized water, and the pH was adjusted to around 7.0 with NaOH at room temperature prior to autoclaving. After autoclaving, filter-sterilized $NaNO_3$ solution (100 g/l), $NaHCO_3$ solution (100 g/l), Na_2S solution (100 g/l); pH 7.5) and trace vitamin solution were added. Then the tubes were tightly sealed with butyl rubber stoppers under a gas phase of 80% H_2 + 20% CO_2 (300 kPa).

Synthetic seawater:

NaCl	30.000	g
K_2HPO_4	0.140	g
$CaCl_2$ x 2 H_2O	0.140	g
NH_4Cl	0.250	g
$MgSO_4$ x 7 H_2O	3.400	g
$MgCl_2$ x 6 H_2O	4.180	g
KCl	0.330	g
$NiCl_2$ x 6 H_2O	0.500	mg
Na_2SeO_3 x 5 H_2O	0.500	mg
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.010	g
Trace mineral solution (see below)	10.000	ml

Trace mineral solution:

$C_6H_9NO_6$	1.500	g
$MgSO_4$ x 7 H_2O	3.000	g
$MnSO_4$ x 2 H_2O	0.500	g
NaCl	1.000	g
$FeSO_4$ x 7 H_2O	0.100	g
$CoSO_4$ x 7 H_2O	0.180	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnSO_4$ x 7 H_2O	0.180	g
$CuSO_4$ x 5 H_2O	0.010	g
$KAl(SO_4)_2$ x 12 H_2O	0.020	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.010	g
$NiCl_2$ x 6 H_2O	0.025	g
Na_2SeO_3 x 5 H_2O	0.300	mg

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998. MEDIUM FOR ROSEOSPIRA

KH_2PO_4	0.30	g
NH_4Cl	0.50	g
$CaCl_2$ x 2 H_2O	0.05	g
$MgCl_2$ x 6 H_2O	1.00	g
$MgSO_4$ x 7 H_2O	0.50	g
NaCl	20.00	g
Trace element solution SL12 (see below)	1.00	ml
Yeast extract	0.50	g
Distilled water	1000.00	ml

Media were autoclaved and cooled under N_2/CO_2 (90/10, v/v). Vitamin V7 solution (1 ml/l) and $NaHCO_3$, (0.15% w/v) were then aseptically added to the medium. Final pH was adjusted to 6.8-7.0 and the medium was dispensed into sterile 50 ml screw-capped bottles. Organic substrates (5 mM Na-acetate and/or 5 mM di-Na-succinate) were added as substrates before use. In addition, for strains DSM 15114 and AT2115, Na_2S x 9 H_2O (0.02% w/v) were also added to the medium prior to utilisation. Pure cultures, grown in 50-ml screw-capped bottles were stored at +4°C in the dark for preservation.

Vitamins V7 solution (Pfennig et Trüper, 1992):

Distilled water	1000.00	ml
Biotine	2.00	mg
p-aminobenzoate	10.00	mg
Thiamine	10.00	mg
Pantothenate	5.00	mg
Pyridoxamine	50.00	mg
Vitamine B_{12}	20.00	mg
Nicotinate	20.00	mg

Sterilized by filtration.

Trace element solution SL12:

Distilled water	1000.00	ml
H_3BO_3	300.00	mg
$FeSO_4$ x 7 H_2O	1.10	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 2 H_2O	50.00	mg
$ZnCl_2$	42.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	18.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg

Sterilized by filtration.

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999. HALORHODOSPIRA MEDIUM

KH_2PO_4	1.00	g
$CaCl_2$ x 2 H_2O	0.05	g
NH_4Cl	0.50	g
NaCl	100.00	g
$MgCl_2$ x 6 H_2O	2.00	g
$MgSO_4$ x 7 H_2O	1.00	g
Distilled water	1000.00	ml

After autoclaving 20 min at 121°C, the following sterile solutions are added:

$NaHCO_3$ (20%)	10.00	ml
Na_2S x 9 H_20 (10%)	7.50	ml
Vitamin solution V7 (see medium 998)	1.00	ml
Trace element solution SL12 (see medium 998)	1.00	ml

The pH is adjusted to pH 7.2-7.4 For better growth of pure cultures, the growth medium should be supplemented with acetate (2 mM final concentration), succinate (1 mM final concentration) and yeast extract (0.5 g/l). Pure cultures should be incubated at 30°C and light intensity of 500 lux from a tungsten lamp (16 h light, 8 h dark). Pure cultures in liquid medium can be stored in 60 ml screw cap bottles at 4°C in the dark.

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1001. BASAL MEDIUM

$NaHCO_3$	2.500	g
NH_4Cl	0.250	g
NaH_2PO_4 x H_2O	0.600	g
KCl	0.100	g
Vitamin Mix (see below)	10.000	ml
Mineral Mix (see below)	10.000	ml
Distilled water	980.000	ml

This medium should not be exposed to direct sunlight! Add all basal medium ingredients. Bring the final volume of the medium to 1.0 liter. Dispense to appropriate culture containers. Bubble the medium with 80:20 N_2:CO_2 (final pH sould be 6.8 to 7.0) - approximately 10 ml of media (anaerobic culture tube) should be gassed for 5 min in the aqueous phase (bubbled) and the headspace gassed for one minute prior to sealing the container. Sterilize per usual procedure. Add electron donor (Acetate-final conc. of 10 mM-recipe below) and electron acceptor (Fe(III)NTA-final conc. of 10 mM-recipe below) from sterile, anaerobic stock solutions using a sterile syringe and needle flushed with anaerobic gas. Store out of direct light.

1.0 M Acetate Stock Solution:
Add 13.6 g sodium acetate to ca. 80 ml distilled water. Bring to final volume of 100 ml. Bubble with anaerobic gas (nitrogen) for 45 min. Seal and sterilize.

500 mM Fe(III)NTA Stock solution:
Add 8.2 g $NaHCO_3$ to ca. 70 ml distilled water. Add 12.8 g of Na_3Nitrilotriacetic acid (NTA) to ca. 70 ml distilled water. Add 13.5 g $FeCl_3$ x 6 H_2O to ca 70 ml distilled water. Adjust pH to 6.5 using 10N NaOH. Bring solution to final volume of 100 ml. Ingredients will go into solution after stirring for ca. 15 min. Bubble with anaerobic gas (N_2) for 45 min and then filter sterilize (0.2 µm filter) into a sterile, anaerobic, serum bottle.

Vitamin mixture:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine HCl	10.000	mg
Riboflavin	5.000	mg
Thiamine	5.000	mg
Nicotinic acid	5.000	mg
Pantothenic acid	5.000	mg
Vitamin B_{12}	0.100	mg
p-aminobenzoic acid	5.000	mg
Thioctic acid	5.000	mg
Distilled water	1000.000	ml

Continued on next page

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Mineral mixture:

NTA	1.500	g
$MgSO_4$	3.000	g
$MnSO_4$ x H_2O	0.500	g
NaCl	1.000	g
$FeSO_4$ x 7 H_2O	0.100	g
$CaCl_2$ x 2 H_2O	0.100	g
$CoCl_2$ x 6 H_2O	0.100	g
$ZnCl_2$	0.130	g
$CuSO_4$ x 5 H_2O	0.010	g
$AlK(SO_4)_2$ x 12 H_2O	0.010	g
H_3BO_3	0.010	g
Na_2MoO_4	0.025	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2WO_4 x 2 H_2O	0.025	g
Distilled water	1000.000	ml

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1002. ECTOTHIORHODOSYNUS MEDIUM

KH_2PO_4	0.5	g
NH_4Cl	0.5	g
NaCl	20.0	g
$MgCl_2$ x 6 H_2O	0.2	g
$CaCl_2$	0.1	g
Na_2CO_3	5.0	g
$NaHCO_3$	5.0	g
Sodium acetate	1.0	g
Yeast extract	0.1	g
$Na_2S_2O_3$ x $5H_2O$	0.5	g
Na_2S x 9 H_2O	0.1	g
Trace elements SL6 (see medium 27)	1.0	ml
Vitamin B_{12}	20.0	µg
Distilled water	1000.0	ml

Prepare the NH_4Cl, $CaCl_2$, carbonate, bicarbonate and sulfide as a single sterile stock solution and add to the sterile medium after autoclaving. The pH should be adjust to 9-9.5.

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1003. HYDROGEN-OXYDIZING MEDIUM

Place 1000 ml of anaerobic water (CO_2 water) in a flask. Add the following compounds:

$MgSO_4$ x 7 H_2O	7.00	g
NaS_2O_3	2.00	g
$CaCl_2$ x 2 H_2O	0.40	g
KCl	0.48	g
$MgCl_2$	0.78	g
MES	1.95	g

Add solutions A, B, and D (see recipes below):

Solution A	2.00	ml
Solution B	1.50	ml
Solution D (Trace minerals)	10.00	ml

pH the media to 6.0 and gas the flask containing the media with CO_2 for 20 min at least. Gas first culture tube in rack and place 5 ml of media in previous tube. Place stopper on loaded culture tube, crimp tube cap onto stopper. Autoclave the medium for 20 min., 121°C. Add 1 ml of O_2 to each tube before inoculation. After the inoculation the tubes are pressurized with H_2 (138 KP).

Solution A (100x solution):

NH_4Cl	100.00	g
$MgCl_2$ x H_2O	100.00	g
$CaCl_2$ x 2 H_2O	40.00	g
Distilled water	1000.00	ml

Adjust the pH to 4 with HCl.

Solution B (500x solution):

K_2HPO_4 x 3 H_2O	200.00	g
Distilled water	1000.00	ml

Trace mineral solution (100x): *see next page*

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Trace mineral solution (100x):

Na-EDTA x 2 H_2O	500.00	mg
$CoCl_2$ x 6 H_2O	150.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
$FeSO_4$ x 7 H_2O	100.00	mg
$ZnCl_2$	100.00	mg
$AlCl_3$ x 6 H_2O	40.00	mg
Na-tungstate x 2 H_2O	30.00	mg
CuCl	20.00	mg
Ni_2SO_4 x 6 H_2O	20.00	mg
Se-acid	10.00	mg
HBO_3	10.00	mg
Na_2MoO_4 x 2 H_2O	10.00	mg
Distilled water	1000.00	ml

Adjust the pH of the solution to 3.0 with HCl.

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1004: ANAEROLINEA MEDIUM

KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
Yeast extract	2.30	g
D-Glucose	2.20	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, yeast extract, vitamins, glucose and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate and adjust pH to 7.0. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration), glucose, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 7.0, if necessary.

For DSM 16554, DSM 16555, DSM 17877: Replace glucose with 7.20 g/l sucrose and reduce amount of yeast extract to 0.10 g/l.

For DSM 16556: Omit glucose and reduce amount of yeast extract to 0.10 g/l.

For DSM 22659: Increase amount of glucose to 5.00 g/l.

For DSM 23815: Replace glucose with 2.00 g/l cellobiose added after autoclaving from a sterile anoxic stock solution sterilized by filtration.

For DSM 103421: Reduce amount of yeast extract to 0.1 g/l

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg

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Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1005: THERMODESULFOBIUM MEDIUM

K_2HPO_4	0.78	g
KH_2PO_4	0.75	g
Na_2-EDTA	0.04	g
$FeSO_4$ x 7 H_2O	0.01	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.03	g
Trace element solution	10.00	ml
NaCl	0.20	g
NH_4Cl	0.50	g
Na_2SO_4	2.80	g
Na-acetate	0.15	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except vitamins and cysteine), adjust pH to 5.5 with 10 N H_2SO_4 and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml of medium in 50 ml bottles) and autoclave. Prior to inoculation add vitamins (sterilized by filtration) and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 5.5 - 6.0, if necessary.

2. After inoculation pressurize vials to 0.5 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1006: ALLISONELLA MEDIUM

DL-Histidine	7.80	g
Yeast extract	4.00	g
Trypticase peptone (BD BBL)	1.00	g
K_2HPO_4	0.29	g
KH_2PO_4	0.29	g
$(NH_4)_2SO_4$	0.48	g
NaCl	0.48	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	64.00	mg
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
Volatile fatty acid mixture	3.10	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, fatty acid mixture and cysteine), bring medium to the boil, then cool to room temperature under 100% CO_2 gas atmosphere. Add solid carbonate, fatty acid mixture and cysteine and adjust pH to 6.0. Dispense medium under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Adjust pH of complete medium to 6.0, if necessary.

Volatile fatty acid mixture (from medium 330)

Acetic acid	548.50	ml
Propionic acid	193.50	ml
Butyric acid	129.00	ml
n-Valeric acid	32.25	ml
iso-Butyric acid	32.25	ml
DL-2-Methylbutyric acid	32.25	ml
iso-Valeric acid	32.25	ml

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1007. MINERAL MEDIUM

KNO_3	250.0	mg
KH_2PO_4	100.0	mg
$MgSO_4$ x 7 H_2O	50.0	mg
$CaCl_2$ x 2 H_2O	10.0	mg
Trace elements	1.0	ml
Distilled water	1000.0	ml

Trace elements:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
$MnCl_2$ x 4 H_2O	0.03	g
Distilled water	1000.00	ml

Final pH 5.5-6.0.

DSM 15673 may be grown on either 0.5-1.0% methanol or under a gas phase of 20% methane in air.
DSM 16984 has been grown on 20% methane in air.
DSM 24478, 24479, 24480, 24481, 24492, 24493 and 24527 have been grown on 30-50% methane in air.
Strains should be grown with shaking when grown on methane.

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1008: SEDIMINISPIROCHAETA MEDIUM

Trypticase peptone (BD BBL)	2.00	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-thioglycolate	1.00	g
Cellobiose	2.00	g
Charcoal-filtered, natural seawater	800.00	ml
Distilled water	200.00	ml

1. Dissolve ingredients except thioglycolate and cellobiose, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add thioglycolate and adjust pH of medium to 7.5 with 10 N KOH. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 15 min. Add cellobiose from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration.

2. Note: Bottled seawater from Biomaris GmbH can be used instead of filtered seawater.

For DSM 16308, DSM 19205, DSM 19230: Replace cellobiose with 5.0 g/l D-glucose.

For DSM 100344: Replace cellobiose with 5.0 g/l D-xylose.

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1009. TCG MEDIUM

Tryptone	3.0	g
Casitone	5.0	g
Glucose	4.0	g
Seawater (see below)	1000.0	ml

Artifical seawater (ASW, eg., Instant Ocean) = 32 g seasalt disolved in 1000 ml distilled water.

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1010: DESULFOPILA CORRODENS MEDIUM

NaCl	26.40	g
$MgCl_2$ x 6 H_2O	5.70	g
$MgSO_4$ x 7 H_2O	6.80	g
KCl	0.66	g
$CaCl_2$ x 2 H_2O	1.47	g
KBr	0.09	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
Na_2CO_3	1.00	g
Na-DL-lactate	2.30	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except dihydrogenphosphate, ammonium chloride, carbonate, lactate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add dihydrogenphosphate, ammonium chloride, lactate, vitamins and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The vitamin solutions should be sterilized by filtration. Adjust pH of the complete medium to 7.2 - 7.5, if necessary.

For DSM 15769: Replace lactate with 0.45 g/l benzenepropanoic acid (3-phenylpropionic acid), which is added to the medium after autoclaving from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1011: SULFURIMONAS MJ MEDIUM

NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
NH_4Cl	0.25	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.01	g
Modified Wolin's mineral solution	10.00	ml
$NaHCO_3$	1.50	g
$Na_2S_2O_3$ x 5 H_2O	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, thiosulfate and vitamins), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add bicarbonate, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions. Solutions of vitamins and thiosulfate are sterilized by filtration and stored under N_2, whereas the solution of bicarbonate is prepared under 80% N_2 and 20% CO_2 gas mixture and autoclaved. Adjust pH of complete medium to 6.7.

2. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.

For DSM 23290: Supplement medium with 2.00 g/l $NaNO_3$. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture. Do not add sterile air!

For DSM 24660: Omit thiosulfate and supplement medium with 4.00 g/l yeast extract and 4.00 g/l Trypton peptone. After autoclaving the medium is reduced with 0.30 g/l Na_2S x 9 H_2O added from a sterile anoxic stock solution (3% w/v) prepared under 100% N_2 gas and the pH adjusted to 6.5. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture. Do not add sterile air!

For DSM 28671: Omit pressurizing vials with 80% N_2 and 20% CO_2 gas mixture.

For DSM 101780, DSM 101688: Supplement medium with 2.00 g/l KNO_3. Do not add overpressure of 80% N_2 and 20% CO_2 and do not add sterile air!

Modified Wolin's mineral solution (from medium 141)

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Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1012: PEREDIBACTER (DN) MEDIUM

Nutrient broth (BD Difco)	0.80	g
Casamino acids (BD Bacto)	0.50	g
Yeast extract (OXOID)	0.10	g
$CaCl_2$ x 2 H_2O	0.30	g
$MgCl_2$ x 6 H_2O	0.60	g
Distilled water	1000.00	ml

1. Dissolve ingredients except calcium and magnesium chloride and adjust pH of the medium to 7.2 with NaOH. After autoclaving add calcium and magnesium from sterile stock solutions.

2. Before inoculation prepare a suspension of prey cells in 30-40 ml medium using a 250 ml flask. Usually the resuspension of two overnight cultures of the prey strain on agar slants of DSMZ medium 54 will yield a final concentration of 10^8 to 10^9 cells per ml culture broth.

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1013: HALOBACTERIOVORAX MEDIUM

Artificial sea water	700.00	ml
Modified Wolin's mineral solution	10.00	ml
Distilled water	300.00	ml

Mix ingredients and adjust pH of the final solution to 7.5 - 8.0. Sterilize by autoclaving at 121°C for 20 min. Grow prey bacterium (DSM 15416) on agar plates using medium 514. For 50 ml prey medium suspension use one agar plate incubated for 24 hours and wash off grown cells in 3 - 5 ml medium. The prey medium suspension should contain approx. 10^7 to 10^9 cells. Inoculate the complete medium immediately after preparation and incubate the suspension with shaking until a decrease in turbidity is visible.

Artificial sea water (from medium 343)

NaCl	27.70	g
$MgSO_4 \times 7\ H_2O$	7.00	g
$MgCl_2 \times 6\ H_2O$	5.50	g
KCl	0.65	g
NaBr	0.10	g
H_3BO_3	30.00	mg
SrCl2 x 6 H_2O	15.00	mg
Citric acid	10.00	mg
KI	0.05	mg
$CaCl_2 \times 2\ H_2O$	2.25	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg

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Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Main sol. 514 (from medium 514)

Bacto peptone	5.00	g
Yeast extract (Bacto)	1.00	g
Fe(III) citrate	0.10	g
NaCl	19.45	g
$MgCl_2$ (anhydrous)	5.90	g
Na_2SO_4	3.24	g
$CaCl_2$	1.80	g
KCl	0.55	g
$NaHCO_3$	0.16	g
KBr	0.08	g
SrCl2	34.00	mg
H_3BO_3	22.00	mg
Na-silicate	4.00	mg
NaF	2.40	mg
$(NH_4)NO_3$	1.60	mg
Na_2HPO_4	8.00	mg
Distilled water	1000.00	ml

Final pH should be 7.6 ± 0.2 at 25°C. If using the complete medium from Difco add 37.40 g to 1 litre water.

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1014: THIOALKALIVIBRIO HALOPHILUS MEDIUM

NaCl	175.00	g
K_2HPO_4	1.50	g
NH_4Cl	0.50	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
$MgCl_2$ x 6 H_2O	0.20	g
$MgSO_4$ x 7 H_2O	0.25	g
$Na_2S_2O_3$	5.00	g
$NaHCO_3$	4.00	g
Distilled water	1000.00	ml

Dissolve sodium chloride, hydrogenphosphate, ammonium chloride and trace elements and fill solution in screw capped Erlenmeyer flasks to 10% of volume. Autoclave at 110°C for 30 min. Add magnesium chloride, magnesium sulfate, thiosulfate and bicarbonat from sterile stock solutions and adjust pH of the medium to 8.0 - 8.5.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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1015. YPGA

Yeast extract	7.0	g
Bactopeptone	7.0	g
Glucose	7.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.3.

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1016. IDIOMARINA MEDIUM

Glucose	10.0	g
Proteose peptone	5.0	g
Yeast extract	3.0	g
Malt extract	3.0	g
NaCl	30.0-60.0	g
Agar (if required)	18.0	g
Distilled water	1000.0	ml

DSM 16139 and DSM 16140 have been grown in medium containing 45 g/l NaCl.

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1017. YPG MEDIUM

Yeast extract	10.0	g/l
Peptone (Difco)	10.0	g/l
Glucose	70.0	g/l
Distilled water	1000.0	ml

Adjust the pH to 6.0 with dilute HCl. Solid media may be prepared by adding 15g/l agar.

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1018. HALOFERAX SULFURIFONTIS MEDIUM

Yeast extract	5.0	g/l
NaCl	150.0	g/l
$MgCl_2$	20.0	g/l
K_2SO_4	0.5	g/l
$CaCl_2$	0.1	g/l
Distilled water	1000.0	ml

pH 7.0

The medium may be solidified by adding 20 g/l agar.

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1019. ROUF'S MEDIUM

Yeast extract	5.00	g
Peptone	5.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 6 H_2O	0.05	g
$Fe(NH_3)$citrate	0.015	g
$MnSO_4$ x 4 H_2O	0.05	g
$FeCl_3$ x 4 H_2O	0.01	g
Vitamin solution (see medium 141)	10.00	ml
Trace elements solution (see below)	1.00	ml
Distilled water	1000.00	ml
pH 7.1 (adjusted with NaOH if needed)		

The medium may be solifidied with 17 g/l agar.

Trace elements solution:

Nitrilotriacetic acid	12.80	g
$CoCl_2$ x 6 H_2O	0.17	g
$CaCl_2$ x 2 H_2O	0.10	g
$FeSO_4$ x 7 H_20	0.10	g
$MnCl_2$ x 4 H_2O	0.10	g
NaCl	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
Distilled water	1000.00	ml

First dissolve the nitrilotriacetic acid and adjust the pH to 6.5 with KOH, then add the minerals. The final pH is 7.0, adjusted with KOH.

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1020: SULFURICURVUM (MBM) MEDIUM

$NaNO_3$	0.20	g
KH_2PO_4	0.20	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-4	2.00	ml
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients, except sodium thiosulfate, then sparge solution with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 7.0, then distribute medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 100 ml bottles) and autoclave. Prior to inoculation, add thiosulfate from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

2. After inoculation, pressurize culture vessels to 0.5 bar overpressure with sterile 100% H_2 gas.

Trace element solution SL-4 (from medium 14)

Na_2-EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.

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1021. SODALIS GLOSSINIDIUS MEDIUM

Solution A:

Yeast extract	6.20	g
Lactalbumin hydrolysate or		
Casein hydrolysate	8.10	g
D-Glucose	5.00	g
KCl	0.25	g
$MgCl_2$ x 6 H_2O	0.12	g
$CaCl_2$ x 2 H_2O	0.25	g
NaCl	8.70	g
$NaHCO_3$	0.15	g
Agar	17.00	g
Distilled water	900.00	ml

Adjust pH to 8.0.

Solution B:

NaH_2PO_4 x 2 H_2O	0.28	g
Distilled water	100.00	ml

Sterilize solution A and B separately at 121°C for 20 min. Cool to 50°C, then combine. To 4 parts of the above, add 1 part sterile foetal or new borne calf serum. Liquid medium should be filter sterilized instead of autoclaved.

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1022: CRYPTOANAEROBACTER MEDIUM

4-Hydroxybenzoic acid	0.45	g
K_2HPO_4	0.40	g
NH_4Cl	0.40	g
Yeast extract (BD Bacto)	5.00	g
Casamino acids (BD Bacto)	1.00	g
Trace element solution SL-10	2.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
***C. sporogenes* supernatant**	350.00	ml
$MgCl_2 \times 6\ H_2O$	0.08	g
$CaCl_2 \times 2\ H_2O$	0.06	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	650.00	ml

1. Dissolve ingredients (except bicarbonate, C. sporogenes supernatant, magnesium chloride, calcium chloride, and vitamins), adjust pH to 7.0 - 7.5 and boil medium for 1 min, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Dissolve solid bicarbonate, adjust pH to 7.8, dispense the solution under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add the appropriate amount of sterile and anoxic supernatant of C. sporogenes and complete the medium by adding magnesium chloride, calcium chloride and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N_2 gas. The final pH of the medium should be 7.5 - 8.0.

2. It may be necessary to add 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 gas and filter-sterilized), if the medium is not completely reduced after inoculation.

3. Note: For transfers use 10% (v/v) inoculum.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg

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Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

C. sporogenes supernatant

Cultivate *Clostridium* sp. DSM 754 for 5 to 8 days at 37°C in the medium 1022, but omit 4-hydroxybenzoic acid, replace the *C. sporogenes* supernatant with distilled water and add after autoclaving 0.30 g/l Na_2S x 9 H_2O from a sterile anoxic stock solution prepared under 100% N_2 gas. Adjust pH of the complete medium to 7.0. Disrupt cells of the grown culture by autoclaving at 121°C for 20 min . Centrifuge autoclaved culture at 18000 x g for 20 min. Discard cell pellet and store the supernatant in screw capped bottles at -20°C . Before use sterilize the supernatant by autoclaving under 100% N_2 gas atmosphere in vials suitable for anaerobic cultivation.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1023. MANNING MEDIUM

$(NH_4)_2SO_4$	6.00	g
KCl	0.20	g
K_2HPO_4	0.20	g
$MgSO_4$ x 7 H_2O	1.00	g
$Ca(NO_3)_2$	0.02	g
Distilled water	1000.00	ml

Solution A:

$FeSO_4$ x 7 H_2O	33.40	g/l

Solution B:

Yeast extract	0.20	g/l

Solution A is separately autoclaved and added to the medium. The medium is supplemented with Solution B (yeast extract). Medium pH adjusted by 0.1 N H_2SO_4 to pH 2.5 - 2.7.

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1024. NITRATIRUPTOR AND NITRATIFRACTOR MEDIUM

$NaNO_3$	1.000	g
$NaHCO_3$	1.000	g
$Na_2S_2O_3$ x 5 H_20	1.000	g
Sulfur,powdered	3.000	g
Trace solution (see medium 141)	10.000	ml
Vitamin solution (see medium 141)	10.000	ml
DMJ synthetic seawater (see medium 997)	1000.000	ml

Prepare the medium under an atmosphere of H_2/CO_2 (80:20) without adding the vitamins and $NaHCO_3$ in serum bottles and seal the serum tubes with butyl rubber stoppers. Steam medium for 3 hours on each of 3 successive days. To the sterile medium add, from filter sterilised stock solutions, the $NaHCO_3$ and vitamin solution.
Increase the 80% H_2 + 20% CO_2 gas phase pressure to 300 kPa. The final pH is 7.0.

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1025. MARICAULIS MEDIUM

Sigma Sea salts	30.0	g
NH_4Cl	0.5	g
Distilled water	1000.0	ml

Autoclave and cool the medium. Add from sterile stock solutions, 20 ml 50xPYE and 5 ml 0.2 mg/ml riboflavin (filter sterilised), and 2 ml 50% glucose per liter of medium.

50xPYE:

Peptone	100.0	g
Yeast extract	50.0	g
Distilled water	1000.0	ml

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1026. XED-AGAR

Xylan	7.0	g
Yeast extract	3.0	g
Agar	18.0	g
Distilled water	1000.0	ml

pH 7.0

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1027. YEAST STARCH AGAR (A)

yeast extract	2.0	g
soluble starch	10.0	g
agar	15.0	g
distilled water	1.0	l

adjust pH to 7.3

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1028: THERMINCOLA MEDIUM (CO)

NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.33	g
KH_2PO_4	0.50	g
Wolfe's mineral elixir	1.00	ml
Na-acetate	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	0.50	g
Na_2CO_3	0.50	g
Na_2S x 9 H_2O	1.00	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	2.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except carbonates, sulfide, yeast extract and vitamins, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add carbonates and sulfide to the medium, dispense under 100% carbon monoxide gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 10 ml medium in 50 ml bottles) and autoclave. Prior to inoculation add yeast extract and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N_2 gas. If the medium is not completely reduced after autoclaving (remains pink) add 0.30 g/l sodium sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 8.0 with a sterile anoxic solution of 1 N HCl.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

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First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1029. BBL ACTINOMYCES BROTH

BBL Actinomyces broth (see below)	57.0	g
Distilled water	1000.0	ml

final pH 6.9

BBL Actinomyces broth (BBL 210920)

Potassium Phosphate	15.0	g
Ammomium Sulfate	1.0	g
Magnesium Sulfate	0.2	g
Calcium Chloride	0.01	g
Infusion Broth	25.0	g
Dextrose	5.0	g
L-Cysteine x HCL	1.0	g
Pancreatic Digest of Casein	4.0	g
Yeast Extract	5.0	g
Soluble Starch	1.0	g

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1031. YMA-MEDIUM

K_2HPO_4	0.2	g
$MgSO_4$	0.2	g
Mannitol	10.0	g
Yeast extract	0.3	g
NaCl	0.05	g
Agar	20.0	g
Distilled water	1000.0	ml

adjust to pH 7.0

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1033. THERMUS MEDIUM

Nitrilotriacetic acid ($C_6H_9NO_6$)	0.10	g
$CaSO_4$ x 2 H_2O	0.06	g
$MgSO_4$ x 7 H_2O	0.10	g
NaCl	0.008	g
KNO_3	0.10	g
$NaNO_3$	0.69	g
Na_2HPO_4	0.1	g
Traceelements solution	10.0	ml
0.17 mM $FeCl_3$ x 6 H_2O	10.0	ml
Tryptone (Bacto Trypton 211705)	1.0	g
Yeast extract (Oxoid LP0021, BD 212750)	1.0	g
Agar	15.00	g
Distilled water	1000.0	ml

Dissolve the nitrilotriacetic acid ($C_6H_9NO_6$), adjust the pH to 8.0 with 1 M NaOH.
Dissolve the remaining ingredients in the nitrilotriacetic acid solution and adjust to pH 8.2 with 1 M NaOH.
Autoclave at 121° C for 15 minutes.

Traceelements Solution

$MnSO_4$ x H_2O	0.2200	g
$ZnSO_4$ x 7 H_2O	0.0500	g
H_3BO_3	0.0500	g
$CuSO_4$ x 5 H_2O	0.0025	g
Na_2MoO_4 x 2 H_2O	0.0025	g
$CoCl_2$ x 6 H_2O	0.0046	g
Distilled water	1000.00	ml

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1034. ALKALIPHILIC HALOMONAS MEDIUM

Yeast extract	10.0	g
Sodiumcitrate	3.0	g
$MgSO_4$ x 7 H_2O	1.0	g
Na_2CO_3	3.0	g
NaCl	100.0	g
Solution A	1.0	ml
Solution B	1.0	ml
Distilled water	1000.0	ml

Solution A:

$MnCl_2$ x 4 H_2O	0.36	g
Distilled water	1000.0	ml

Solution B:

$FeSO_4$ x 7 H_2O	50.0	g
Distilled water	1000.0	ml

Na_2CO_3 and NaCl were autoclaved separately.

adjust to pH 9.0

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1035. COLLIMONAS MEDIUM

KH_2PO_4	1.0	g
NaCl	5.0	g
Tryptone Soy Broth	3.0	g
Distilled water	1000.0	ml

adjust pH to 6.5 before autoclaving.

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1036: ACETIVIBRIO ALKALICELLULOSI MEDIUM

NH_4Cl	0.50	g
KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
KCl	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.00	g
$NaHCO_3$	7.60	g
NaCl	10.00	g
Yeast extract	0.20	g
Cellobiose	3.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except cellobiose and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose after autoclaving from an anoxic stock solution prepared under 100% N_2 gas sterilized by filtration and sulfide from a sterile anoxic stock solution autoclaved under 100% N_2 gas. Adjust pH of the complete medium to pH 8.8-9.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg

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Distilled water	1000.00	ml

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1037: THIOMONAS DELICATA MEDIUM

NH_4Cl	0.30	g
KH_2PO_4	1.50	g
Na_2HPO_4	4.50	g
$MgSO_4$ x 7 H_2O	0.10	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Yeast extract	1.00	g
Na-aspartate	1.00	g
Agar, for solid medium (optional)	15.00	g
Distilled water	1000.00	ml

Dissove ingredients, adjust medium to pH 6.0, dispense into suitable culture vessels and autoclave.

For DSM 18570: Prepare medium without aspartate.

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1038: ACIDICALDUS MEDIUM

$(NH_4)_2SO_4$	0.45	g
KCl	0.05	g
$MgSO_4$ x 7 H_2O	0.50	g
KH_2PO_4	0.05	g
$Ca(NO_3)_2$ x 4 H_2O	0.02	g
D-Glucose	1.00	g
Yeast extract (OXOID)	0.20	g
Distilled water	1000.00	ml

Dissolve ingredients except glucose and yeast extract, adjust pH to 2.5, dispense in suitable cultivation vessels and autoclave. Add glucose and yeast extract after autoclaving from sterile stock solutions.

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1039. ANACKER-ORDAL AGAR

Tryptone peptone (DIFCO)	0.5	g
Yeast extract (DIFCO)	0.5	g
Na-acetate	0.2	g
Meat extract (OXOID)	0.2	g
Agar	11.0	g
Tap water	1000.0	ml

Adjust to pH 7.2 prior to autoclaving with KOH.
It is important to use indicator paper for pH adjustment and not a pH electrode!

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1040: MARIDESULFOVIBRIO MEDIUM

Sea Salt (SIGMA)	35.00	g
Wolfe's mineral elixir	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na-DL-lactate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, lactate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add lactate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2, if necessary.

For DSM 15518: Replace lactate with 3.0 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution and adjust pH of complete medium to 6.0.

For DSM 17682, DSM 111985: Reduce amount of Na-lactate to 0.6 g/l and replace Wolin's vitamin solution of medium 141 with the vitamin solution of medium 521.

For DSM 24848, DSM 29592: Replace lactate with 3.0 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 105309: Replace lactate with 3.0 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution and adjust pH of complete medium to 7.5 -8.0.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g

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$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1041: DESULFUROMUSA FERRIREDUCENS MEDIUM

Sea Salt (SIGMA)	35.00	g
Wolfe's mineral elixir	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
Na-acetate	1.60	g
Na_2-fumarate	3.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, fumarate, vitamins, and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, fumarate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of vitamins and fumarate should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2, if necessary.

For DSM 101009: Omit sulfide and adjust pH of complete medium to 6.8.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1042: VULCANIBACILLUS MEDIUM

Sea Salt (SIGMA)	30.00	g
$NaNO_3$	1.00	g
Wolfe's mineral elixir	2.00	ml
$VOSO_4$ x 5 H_2O (0.01% w/v)	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$FeSO_4$ x 7 H_2O	0.10	g
$NaHCO_3$	1.00	g
Yeast extract	0.50	g
Na-pyruvate	1.00	g
Wolin's vitamin solution (10x)	2.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins), adjust pH to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense the medium under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. Add ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Prepare the stock solution of ferrous sulfate by dissolving 1% (w/v) $FeSO_4$ x 7 H_2O in 0.1 N H_2SO_4. The stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.8-7.0.

2. Note: It may be necessary to add 10-20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under 100% N_2 and filter-sterilized), if the medium is not completely reduced after inoculation.

For DSM 23802: Replace pyruvate with 2.50 g/l D-glucose added to the autoclaved medium from an anoxic sterile stock solution.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g

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Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1043: IGNISPHAERA MEDIUM

$(NH_4)_2SO_4$	1.30	g
$CaCl_2$ x 2 H_2O	74.00	mg
$MgSO_4$ x 7 H_2O	0.28	g
KH_2PO_4	0.28	g
Yeast extract (OXOID)	0.10	g
Trypticase peptone (BD BBL)	2.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$FeCl_3$ x 6 H_2O (0.1% w/v in 0.2 N HCl)	0.50	ml
L-Cysteine HCl x H_2O	0.30	g
Starch (soluble)	2.00	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferric chloride, cysteine, starch and sulfide), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add ferric chloride and solid cysteine, then adjust pH to 6.3. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Prior to inoculation add starch and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 6.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust

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final to pH 7.0 with KOH.

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1044. GLUCONACETOBACTER RHAETICUS MEDIUM

Glucose	50.0	g
Yeast extract	5.0	g
Agar	15.0	g
Distilled water	1000.0	ml

adjust pH 6.5

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1045. THERMUS SP. MEDIUM

Peptone	8.0	g
Yeast extract	4.0	g
NaCl	2.0	g
Distilled water	1000.0	ml

adjust pH 7.0

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1046. ANOXYBACILLUS AMYLOLYTICUS MEDIUM

Yeast extract	6.0	g
NaCl	6.0	g
Tap water	1000.0	ml

adjust pH 5.6

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1047. DIFCO RAKA-RAY NO.3 MEDIUM

Yeast extract	5.0	g
Tryptone	20.0	g
Liver Concentrate	1.0	g
Maltose	10.0	g
Fructose	5.0	g
Glucose	5.0	g
Betaine Hydrochloride	2.0	g
Diammonium Citrate	2.0	g
Potassium Aspartate	2.5	g
Magnesium Sulfate	0.98	g
Manganese Sulfate	0.42	g
Dipotassium Phosphate	2.0	g
N-Acetyl Glucosamine	0.5	g
Potassium Glutamate	2.5	g
Agar	16.0	g
Distilled water	1000.0	ml

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1048. GAUZE'S SYNTHETIC MEDIUM NO.1

Soluble starch	20.0	g
KNO_3	1.0	g
NaCl	0.5	g
$MgSO_4$ x 7 H_2O	0.5	g
K_2HPO_4	0.5	g
$FeSO_4$ x 7 H_2O	10.0	mg
Agar	15.0	g
Distilled water	1.0	l

Adjust pH 7.4

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1049. SELENATE REDUCER MEDIUM

Solution A:		
DL-Na-lactate	1.12	g
KCl	1.30	g
KH_2PO_4	0.20	g
NaCl	23.00	g
NH_4Cl	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	3.00	g
Resazurin	0.50	mg
Agar	15.00	g
Distilled water	940.00	ml
Solution B:		
Na_2SeO_4	1.89	g
Distilled water	10.00	ml
Solution C:		
$NaHCO_3$	1.00	g
Distilled water	10.00	ml
Solution D:		
Na_2S x 9 H_2O	0.10	g
Distilled water	10.00	ml
Solution E:		
Na_2SeO_3	0.003	g
Na_2WO_4	0.008	g
Distilled water	100.00	ml
Solution F:		
Vitamin solution (see medium 461)		
Solution G:		
Trace element solution SL-10 (see medium 320)	1.00	ml
Distilled water	9.00	ml
Solution H:		
1,4-Naphthoquinone	2.00	mg
Hemin	0.50	mg
Distilled water	100.00	ml

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After cooling of medium A, add per 100 ml:
1.0 ml solution B,
2.5 ml solution C,
1.0 ml solution D,
0.1 ml solution E,
0.5 ml solution F,
1.0 ml solution G and
1.0 ml solution H.
Adjust pH to 7.0.

It is recommended to cultivate strain DSM 17993 on agar plates which may be prepared under aerobic conditions. Plates are then preincubated under anaerobic conditions prior to inoculation. They will stay pink coloured.

For liquid medium, prepare solution A, distribute to anaerobic cultivation vessels, heat to the boil and cool to room temperature while gassing with N_2 : CO_2 (80:20). Close vessels and autoclave. Prepare solutions B, C, D, E and H under anaerobic conditions and autoclave. Solutions F and H are filter sterilized and flushed with N_2. Selenate (solution B) might be replaced by nitrate (solution: 1.00 g KNO3 in 10 ml).

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1050. DEXTRAN MEDIUM

Dextran	10.0	g
Polypepton	5.0	g
NH_4NO_3	2.0	g
KH_2PO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.5	g
$CaCl_2$ x 2 H_2O	0.25	g
Agar	15.0	g
Distilled water	1000.0	ml

adjust to pH 6.8

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1051: TEPIDANAEROBACTER MEDIUM

KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Yeast extract	2.30	g
Wolin's vitamin solution	2.00	ml
D-Glucose	2.20	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, yeast extract, vitamins, glucose, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration), glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.0-7.2, if necessary.

For DSM 21860: Omit glucose, increase amount of ammonium chloride to 2.50 g/l, decrease amount of yeast extract to 0.20 g/l and supplement medium with 1.00 ml/l selenite-tungstate solution (see medium 385). As substrate add 1.10 g/l betaine to the autoclaved medium from an anoxic stock solution sterilized by filtration.

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	0.20	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.17	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1052. BOSEA MEDIUM

Yeast extract	10.00	g
ACES	10.00	g
Activated charcoal	2.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 6.9 prior to addition of agar.

Same as solutions A and B of medium 585, CYE-ACES agar.

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1053: SULFURIMONAS PARALVINELLA MEDIUM

NaCl	20.00	g
K_2HPO_4	0.09	g
KH_2PO_4	0.07	g
$CaCl_2$	0.80	g
NH_4Cl	0.25	g
$NaNO_3$	1.00	g
$MgSO_4$ x 7 H_2O	4.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.33	g
$Fe_2(SO_4)_3$ x H_2O	0.01	g
Modified Wolin's mineral solution	10.00	ml
$Na_2S_2O_3$ x 5 H_2O	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
$NaHCO_3$	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, bicarbonate and vitamins), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.8 with NaOH and dispense under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (up to a volume of 20%) that contain already the appropriate amount of sulfur. Autoclave at a temperature of **110°C** for 20 min. Add vitamins and bicarbonate to the autoclaved medium from sterile anoxic stock solutions. The solution of vitamins is sterilized by filtration and stored under 100% N_2 gas, whereas the solution of bicarbonate is prepared under 80% N_2 and 20% CO_2 gas mixture and autoclaved. Adjust the pH of the complete medium to 6.5.

2. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g

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$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1054. A1-MEDIUM

Yeast extract	4.0	g
Bacto peptone	2.0	g
Starch	10.0	g
Seawater (Biomaris 089 0.75 bottle) (natural or artificial)	1.0	l
Agar	20.0	g

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1055: DESULFOVERMICULUS MEDIUM

NaCl	100.00	g
$MgSO_4$ x 7 H_2O	10.00	g
KCl	6.00	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	1.00	g
KH_2PO_4	0.10	g
Yeast extract	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-L-lactate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, lactate, vitamins, and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add lactate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

For DSM 18834: Replace Na-L-lactate with 1.0 g/l Na_2-DL-malate.

For DSM 24312: Omit Na-L-lactate and add 3.4 g/l Na-formate, 0.16 g/l Na-acetate and 5.0 g/l $Na_2S_2O_3$ from sterile anoxic stock solutions prepared under 100% N_2 gas.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1056: DESULFOSALSIMONAS MEDIUM

NaCl	60.00	g
$MgSO_4$ x 7 H_2O	10.00	g
KCl	6.00	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	1.00	g
KH_2PO_4	0.10	g
Yeast extract	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-propionate	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, propionate, vitamins and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add propionate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

2. Prior to use add sterile 100% H_2 gas to 0.5 bar overpressure.

3. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) can be used to completely reduce the medium and may stimulate growth at the beginning.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1057: ETHANOLIGENES MEDIUM

Tryptone (BD BBL)	4.00	g
Beef extract (BD BBL)	2.00	g
Yeast extract (OXOID)	1.00	g
NaCl	4.00	g
K_2HPO_4	1.50	g
$FeSO_4$ x 7 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
D-Glucose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, glucose and vitamins), sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH to 6.0, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add glucose and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.0, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1058: THIOHALOPHILUS MEDIUM

NaCl	120.00	g
K_2HPO_4	1.50	g
NH_4Cl	0.50	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
$CaCl_2$ x 2 H_2O	0.05	g
$MgSO_4$ x 7 H_2O	0.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
$NaHCO_3$	5.00	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N_2 and 20% CO_2 gas mixture for at least 30 - 45 min to remove dissolved oxygen and to saturate the solution with CO_2. Dispense solution in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 50% volume under air atmosphere. Close vials with butyl rubber septa to prevent free exchange of oxygen with the external atmosphere and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, bicarbonate and vitamins from sterile stock solutions and adjust pH of the medium to 7.5 - 7.8 using a sterile stock solution of sodium carbonate (5% w/v).

2. Note: Use at least 10% (v/v) as inoculum.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg

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1058: THIOHALOPHILUS MEDIUM

Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1059: GEOALKALIBACTER MEDIUM

NH_4Cl	0.50	g
KCl	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
KH_2PO_4	0.20	g
NaCl	1.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	3.00	g
$NaHCO_3$	10.00	g
Sulfur (powdered)	10.00	g
Yeast extract	0.10	g
Na-acetate	2.50	g
Distilled water	1000.00	ml

Dissolve ingredients (ecxept carbonate, hydrogencarbonate, sulfur, yeast extract and acetate), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add solid carbonate and bicarbonate, adjust pH to 9.0 - 9.2, and dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by autoclaving at **110°C** for 20 min. Add yeast extract and acetate from sterile anoxic stock solutions prepared under 100% N_2 gas.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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1061. MODIFIED TH AGAR

Tryptone	5.00	g
Yeast extract	3.00	g
NaCl	5.00	g
$MnSO_4$ x H_2O	0.01	g
Trace element solution SL-6 (see medium 27)	1.00	ml
Agar	15.00	ml
Distilled water	1000.00	ml

adjust to pH 7.5

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1062: DESULFITOBACTERIUM (PCE II) MEDIUM

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
$FeSO_4$ x 7 H_2O	22.00	mg
Seven vitamins solution	1.00	ml
Yeast extract	2.00	g
Na-pyruvate	4.50	g
Na_2-fumarate	6.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, ferrous iron sulfate, vitamins and organic substrates), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferrous iron sulfate (dissolved in 0.1 N H_2SO_4), vitamins, pyruvate, fumarate and yeast extract from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Stock solutions of ferrous iron sulfate, vitamins, pyruvate and fumarate should be sterilized by filtration. Adjust pH of the complete medium to 7.5, if necessary.

2. Note: Addition of 10 - 20 mg sodium dithionite per litre (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1063. MICROCELLA ALKALIPHILA MEDIUM

Trypticase soy broth	30.00	g
Yeast extract	3.00	g
Distilled water	944.00	ml
Mineral salt solution (see medium 335)	50.00	ml
Vitamine solution (see medium 212)	5.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml

After sterilization add sterile 1M Na-sesquicarbonate solution to achieve the pH of 9.5 (see medium 31).

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1064. BREVIBACILLUS LEVICKII MEDIUM

Yeast extract	2.00	g
$(NH_4)_2SO_4$	1.25	g
KH_2PO_4	1.50	g
$MnSO_4$ x H_2O	2.50	mg
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	12.50	mg
Agar	18.00	g
Distilled water	1000.00	ml

Adjust pH to 5.5; autoclave at 121° for 15 minutes.

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1065. M1-NOCARDIOPSIS ARABIA MEDIUM

Starch	10.0	g
Yeast extract	4.0	g
Peptone	2.0	g
NaCl	20.0	g
Agar	18.0	g
Sea water	1000.0	ml

Adjust pH to 7.2

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1066. MARINOBACTER LUTAOENSIS MEDIUM

Bacto peptone	4.00	g
Yeast extract	2.00	g
NaCl	25.00	g
$MgCl_2 \times 6H_2O$	2.00	g
Distilled water	1000.00	ml

Adjust pH to 7.0

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1068: DESULFOPILA MSL86 MEDIUM

KH_2PO_4	0.50	g
NH_4Cl	1.00	g
Na_2SO_4	1.00	g
NaCl	10.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	2.00	g
Trace element solution SL-10	1.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-DL-lactate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except lactate, vitamins and reducing agents), adjust pH to 7.5 and sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add lactate, vitamins, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg

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1068: DESULFOPILA MSL86 MEDIUM

Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1069. MODIFIED BIEBL AND PFENNIG'S MEDIUM

KH_2PO_4	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
$MgSO_4$ x 7 H_2O	2.00	g
NH_4Cl	0.34	g
NaCl	20.00	g
Yeast extract	0.40	g
Ferric citrate (0.1% w/v)	5.00	ml
Trace element solution (SL-7)	1.00	ml
Distilled water	1000.00	ml

Bubble the medium with nitrogen gas and fill 10 ml in 15 ml tubes with a rubber septum under a stream of nitrogen gas.

Trace element solution SL-7:

Distilled water	1000.0	ml
HCl (25%)	1.0	ml
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	60.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$CuCl_2$ x 2 H_2O	20.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	40.0	mg

For DSM 18064

Final pH 6.8

Add the following components after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B_{12} (2 mg/l filter sterilised)	1.0	ml/l

For DSM 18713 and 18859 and 19784

Add the following components after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B_{12} (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O	1.0	mM
$Na_2S_2O_3$ x 5 H_2O	6.0	mM
$NaHCO_3$ (10%, autoclaved in sealed, half full vessels under CO_2)	10.0	ml/l

DSM 18713 and 18859 Final pH 7.2

DSM 19784 Final pH 6.9

Continued on next page

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For DSM 19947

Final pH 6.9

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B12 (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O	1.0	mM
$Na_2S_2O_3$ x 5 H_2O	4.0	mM
$NaHCO_3$ (10%, autoclaved in sealed, half full vessels under CO_2)	10.0	ml/l

For DSM 18985

Reduce the $MgSO_4$ x 7 H_2O to 1.5 g/l

Final pH 6.8

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B_{12} (2 mg/l filter sterilised)	1.0	ml/l
Either Na_2S x 9 H_2O	1.0	mM
or $Na_2S_2O_3$ x 5 H_2O	2.0	mM
$NaHCO_3$ (10%, autoclaved in sealed, half full vessels under CO_2)	10.0	ml/l

For DSM 21808 and 21809

Final pH 7.5

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B_{12} (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O 1M	3.0	ml/l
$NaHCO_3$ (10%, autoclaved in sealed, half full vessels under CO_2)	10.0	ml/l

For DSM 24766

Reduce the $MgSO_4$ x 7 H_2O to 1.00 g/l

Final pH 7.0

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B_{12} (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O	1.0	mM
$NaHCO_3$ (10%, autoclaved in sealed, half full vessels under CO_2)	10.0	ml/l

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1070. YM MEDIUM

Yeast extract	0.5	g
Mannitol	10.0	g
K_2HPO_4	0.5	g
NaCl	0.2	g
$CaCl_2$ x 2 H_2O	0.2	g
$MgSO_4$ x 7 H_2O	0.1	g
Distilled water	1000.0	ml

adjust to pH 7.0

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1071. PY-BROTH

Polypepton	2.000	g
Bacto yeast extract (Difco)	0.500	g
NaCl	30.000	g
$MgCl_2$ x 6 H_2O	5.000	g
$CaCl_2$ x 2 H_2O	0.005	g
Na_2MoO_4 x 7 H_2O	0.005	g
$CuCl_2$ x 2 H_2O	0.004	g
$FeCl_3$ x 6 H_2O	0.006	g
Agar	15.000	g
Distilled water	1000.000	ml

pH 8.0

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1074: DESULFOGLAEBA MEDIUM

Sea Salt (SIGMA)	25.00	g
Na_2SO_4	3.50	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Yeast extract	0.05	g
Na-pyruvate	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
n-Decane	1.00	ml
Na_2S x 9 H_2O	0.10	g
L-Cysteine HCl x H_2O	0.10	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, yeast extract, pyruvate, vitamins, n-decane and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes or serum bottles) and autoclave. Add yeast extract, pyruvate, vitamins, n-decane and reducing agents from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of vitamins and pyruvate should be sterilized by filtration. Adjust pH of complete medium to 7.2 - 7.4.

2. Note: Use 20% (v/v) as inoculum and incubate vials in an inverted position.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

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First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1075. PPES-II AGAR MEDIUM

Peptone	2.0	g
Proteose peptone No. 3 (Difco)	1.0	g
Soytone	1.0	g
Yeast extract	1.0	g
Fe(III)-EDTA	0.1	g
Agar	15.0	g
Artificial sea water (see below)	1.0	l

Adjust pH to 7.8.

Artificial sea water:

NaCl	30.0	g
KCl	0.7	g
$MgCl_2$ x 6 H_2O	10.8	g
$MgSO_4$ x 7 H_2O	5.4	g
$CaCl_2$ x 2 H_2O	1.0	g
Distilled water		

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1076. SP4 MEDIUM

Tryptone	5.00	g
Peptone	2.65	g
PPLO broth	1.75	g
Distilled water	375.00	ml

Adjust pH to 7.4; autoclave at 121°C for 15 minutes.

Add aseptically:

Fetal bovine serum (heat-inactivated)	90.0	ml
CMRL medium 1066 (10x concentrated; GIBCO)	25.0	ml
Yeast extract solution (20%, autoclaved)	17.5	ml
Yeastolate (20%, autoclaved)	5.0	ml
Phenol red solution (1%, filter-sterilized)	1.0	ml
L-Glutamine (200 mM; GIBCO)	1.7	ml

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1078. FRIIS MEDIUM

Bacto brain heart infusion	0.82	g
PPLO broth (GIBCO)	0.87	g
Hank's balanced salt solution (BSS, see below)	50.00	ml
Distilled water	78.00	ml

Adjust to pH 7.4.

After autoclaving add:

Porcine serum (heat-inactivated)	30.000	ml
25% Yeast extract (autoclaved)	3.320	ml
1% Phenol red solution (filter-sterilized)	0.133	ml
Distilled water (autoclaved)	0.343	ml

Hank's BSS:
25 ml solution 1 (4 g NaCl, 0.2 g KCl, 0.05 g $MgSO_4$ x 7 H_2O, 0.05 g $MgCl_2$ x 6 H_2O dissolved in 25 ml distilled water; then 0.0925 g $CaCl_2$ added and dissolved) and 25 ml of solution 2 (0.075 g Na_2HPO_4 x 2 H_2O, 0.03 g KH_2PO_4 dissolved in 25 ml distilled water) are autoclaved separately and then combined with 450 ml sterile H_2O.

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1081. HORIKOSHI-1 MEDIUM

Glucose	10.0	g
Polypepton	5.0	g
Yeast extract	5.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Agar	15.0	g
Distilled water	900.0	ml

After autoclaving, aseptically add 100 ml of a 10% Na_2CO_3 solution to the medium. Check final pH to be about 10.0.

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1082: AMINIPHILUS MEDIUM

KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
NH_4Cl	1.00	g
NaCl	10.00	g
KCl	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	0.50	g
Modified Wolin's mineral solution	10.00	ml
Trypticase peptone (BD BBL)	10.00	g
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine), then sparge medium for 30 - 45 min with 100% N_2 gas atmosphere to make it anoxic. Add cysteine, adjust pH to 7.1 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. The pH of the complete medium should be 7.0 - 7.2.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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1083: ACIDULIPROFUNDUM MEDIUM

NaCl	30.00	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
$CaCl_2$ x 2 H_2O	0.38	g
KCl	0.33	g
NaBr	0.05	g
$(NH_4)_2SO_4$	0.10	g
KH_2PO_4	0.28	g
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_3-citrate	2.94	g
Yeast extract	1.00	g
Tryptone (BD Bacto)	1.00	g
Sulfur (powdered)	10.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of powdered sulfur and autoclave for 60 min at **105°C**. Prior to inoculation add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH of complete medium to 4.5.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

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First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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1084: METHERMICOCCUS (METHANOGEN) MEDIUM

KCl	0.34	g
NH_4Cl	0.25	g
K_2HPO_4	0.20	g
NaCl	24.00	g
$MgCl_2$ x 6 H_2O	10.20	g
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Sludge fluid	5.00	ml
Methanol (50% v/v)	16.00	ml
2-Mercaptoethanesulfonic acid (coenzyme M)	2.50	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, sludge fluid, methanol, coenzyme M, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sludge fluid, methanol (50% v/v solution), coenzyme M (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 6.0 - 6.5, if necessary.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

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1085: HOWARDELLA MEDIUM

Casitone (BD Bacto)	20.00	g
Yeast extract (BD Bacto)	5.00	g
Na_2HPO_4	5.00	g
$MgCl_2$ x 6 H_2O	1.10	g
Sodium resazurin (0.1% w/v)	0.50	ml
Urea	1.00	g
Na-thioglycolate	0.75	g
Distilled water	1000.00	ml

Dissolve ingredients (except urea and thioglycolate), boil medium for 1 min, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Add thioglycolate, dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add urea from a 10% (w/v) stock solution sterilized by filtration and stored under 100% N_2 gas. Adjust pH of complete medium to 7.4.

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1086: DESULFATIRHABDIUM MEDIUM

Na_2HPO_4 x 2 H_2O	0.53	g
KH_2PO_4	0.41	g
NH_4Cl	0.30	g
$CaCl_2$ x 2 H_2O	0.11	g
$MgCl_2$ x 6 H_2O	0.10	g
NaCl	0.30	g
Na_2SO_4	2.80	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.02	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-benzoate	0.43	g
Na-crotonate solution (1 M)	20.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	980.00	ml

1. Dissolve ingredients (except carbonate, benzoate, crotonate, vitamins and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add benzoate, crotonate, vitamins and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The crotonate and vitamin solutions should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2.

2. After inoculation pressurize the vessels with sterile 80% N_2 and 20% CO_2 gas mixture to 0.7 bar overpressure.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Na-crotonate solution (1 M) (from medium 870)

Crotonic acid (ALDRICH 113018)	86.00	g
NaOH (10 N)	100.00	ml
Distilled water	900.00	ml

Dissolve crotonic acid in 800 ml distilled water, add around 100 ml of 10 N NaOH and adjust pH to around 7. Then add water to reach a volume of 1000 ml. Sterilize by filtration under 100% N_2 gas atmosphere.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1087. MANNITOL-YEAST EXTRACT-PEPTONE (MYP)

D-Mannitol	25.0	g
Yeast extract	5.0	g
Peptone	3.0	g
Agar	15.0	g
Distilled water	1000.0	ml

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1090: PYROBACULUM CALIDIFONTIS MEDIUM

Tryptone (BD Bacto)	10.00	g
Yeast extract	1.00	g
$Na_2S_2O_3$ x 5 H_2O	3.00	g
Distilled water	1000.00	ml

Dissolve ingredients and adjust to pH 7.0 with NaOH before autoclaving.

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1091. HALOQUADRATUM WALSBYI MEDIUM

NaCl	195.00	g
$MgSO_4$ x 7 H_2O	50.00	g
$MgCl_2$ x 6 H_2O	35.00	g
KCl	5.00	g
$NaHCO_3$	0.25	g
$NaNO_3$	1.00	g
$CaCl_2$ x 2 H_2O	0.50	g
KH_2PO_4	0.05	g
NH_4Cl	0.03	g
Tris buffer 1M	20.00	ml
Distilled water	1000.00	ml

Add after autoclaving:

Yeast extract	0.05	g
Sodium pyruvate	1.00	g

Final pH 7.4

Yeast extract and sodium pyruvate are added from sterile (autoclaved) stock solutions to the respective concentrations.

For **DSM 29227** use 4.0 g/l sodium pyruvate

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1092: THERMOSULFIDIBACTER MEDIUM

Sea Salt (SIGMA)	30.00	g
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	3.00	g
$NaHCO_3$	2.50	g
Yeast extract	2.00	g
Wolin's vitamin solution (10x)	2.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, bicarbonate, yeast extract, vitamins and sulfide) and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes) containing already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Autoclave at **110°C** for 20 min. Add yeast extract, vitamins and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2.

2. After inoculation pressurize vessels with sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1094: METHANOSPHAERULA (PEAT) MEDIUM

Solution A	943.00	ml
Solution B	13.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Solutions B, C, D and G are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions E and F are prepared under 100% N_2 gas atmosphere and autoclaved. To complete the medium add appropriate amounts of solutions B to G to the sterile solution A in the sequence indicated and let medium equilibrate overnight. Adjust pH of complete medium to 5.7, if necessary.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

3. Note: The complete medium is not stable and can be stored only for a short time.

For DSM 25616: Supplement medium with 0.02 g/l yeast extract added to the sterile medium from an anoxic stock solution sterilized by filtration. Adjust pH of complete medium to 6.2. Use 5-10 vol% as inoculum and incubate while shaking at 100 - 200 rpm.

For DSM 25820: Supplement medium with 0.02 g/l yeast extract added to the sterile medium from an anoxic stock solution sterilized by filtration. Use 5-10 vol% as inoculum and incubate while shaking at 100 - 200 rpm.

Solution A

KCl (0.1% w/v)	1.50	ml
KH_2PO_4 (0.1% w/v)	13.60	ml
NH_4Cl (0.1% w/v)	26.80	ml
Trace element solution	1.00	ml
Distilled water	900.00	ml

Solution B

Tris-HCl buffer (1.0 M solution, pH 8.0)	7.20	ml
Na_3-NTA x H_2O (0.5 M solution)	4.80	ml
$TiCl_3$ (15% w/v solution in HCl; Riedel-de Haen)	0.55	ml

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Solution C

2-(N-Morpholino)ethanesulfonic acid (MES)	1.95	g
NaOH	0.20	g
Distilled water	20.00	ml

Adjust to pH 7.5 with NaOH.

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

2-Mercaptoethanesulfonic acid (Coenzyme M)	0.08	g
Distilled water	10.00	ml

Solution F

Na-acetate	0.41	g
Distilled water	10.00	ml

Solution G

Na_2S x 9 H_2O	9.60	mg
Distilled water	10.00	ml

Trace element solution

Na_2-EDTA	37.23	g
$FeCl_2$ x 4 H_2O	1.34	g
$AlK(SO_4)_2$ x 12 H_2O	3.45	g
$CaCl_2$ x 2 H_2O	2.34	g
$MgSO_4$ x 7 H_2O	1.56	g
$CoCl_2$ x 6 H_2O	24.00	mg
$ZnCl_2$	75.00	mg
H_3BO_3	19.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2Mo_4 x 2 H_2O	24.00	mg
$MnSO_4$ x 4 H_2O	26.00	mg
$CuSO_4$ x 5 H_2O	9.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1095: NATRANAEROBIUS MEDIUM

NaCl	100.00	g
KH_2PO_4	0.20	g
$MgCl_2$	0.10	g
NH_4Cl	0.50	g
Yeast extract (OXOID)	10.00	g
Tryptone (BD Bacto)	10.00	g
Trace element solution SL-10	1.00	ml
Na_2CO_3	68.00	g
$NaHCO_3$	38.00	g
L-Cysteine HCl x H_2O	0.70	g
Wolin's vitamin solution (10x)	1.00	ml
Sucrose	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonates, cysteine, sucrose and vitamins), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add carbonate, hydrogencarbonate and cysteine and adjust pH to 8.5. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add sucrose and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration.

For DSM 18760: Adjust pH of medium to 9.5.

For DSM 18770: Adjust pH of medium to 9.7 and replace sucrose with 5.0 g/l Casamino acids added from a sterile anoxic stock solution after autoclaving.

For DSM 23380: Adjust pH of medium to 9.5 and replace sucrose with 5.0 g/l D-glucose added from a sterile anoxic stock solution after autoclaving.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1096. UREAPLASMA MEDIUM

Solution A:

PPLO-Broth	14.70	g
L-Cystein HCl	0.10	g
Fish-Sperm DNA	0.20	g
Phenol red	0.02	g
Urea	0.40	g
Water	700.00	ml

Solution B:

2% Yeastolate (BD)	100	ml
Horse serum	200	ml
Isovitalex	5	ml

Adjust solution A for final pH 6.0 with HCl. Autoclave solution A at 121°C for 15 min. Cool to room temperature and aseptically add solution B filter sterilised.

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1097: SULFUROSPIRILLUM MV MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.70	g
NH_4Cl	0.20	g
KH_2PO_4	0.20	g
$CaCl_2$ x 2 H_2O	0.20	g
Trace element solution SL-10	2.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na_2-fumarate	1.60	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, fumarate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fumarate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of vitamins and fumarate should be sterilized by filtration. Adjust pH of complete medium to 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g

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Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1098: ARCHAEOGLOBUS INFECTUS MEDIUM

Sea Salt (SIGMA)	35.00	g
NH_4Cl	0.10	g
KH_2PO_4	0.05	g
Na-acetate	1.60	g
Yeast extract	0.10	g
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.14	g
Seven vitamins solution	1.00	ml
$Na_2S_2O_3$ x 5 H_2O	2.50	g
DL-Dithiothreitol	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, coenzyme M, vitamins, thiosulfate, and dithiothreitol), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes) to 30% of their volume and autoclave. Add coenzyme M, vitamins, thiosulfate, and dithiothreitol from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The vitamin and dithiothreitol solutions should be sterilized by filtration. Adjust the pH of the complete medium to 6.5.

2. After inoculation pressurize culture vessels to 1.5 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g

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Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1099: DESULFOLUNA MEDIUM

NaCl	20.00	g
KH_2PO_4	0.50	g
NH_4Cl	1.00	g
Na_2SO_4	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	2.00	g
Yeast extract	0.50	g
Trace element solution SL-10	1.00	ml
Na-L-lactate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cysteine form a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH of the complete medium to 6.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1100: DESULFACINUM MEDIUM

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	7.00	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
$MgCl_2$ x 6 H_2O	3.10	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.50	g
Na-L-lactate	2.00	g
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, lactate and sulfide) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add lactate and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.2, if necessary.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.

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1101: DESULFONATRONOSPIRA MEDIUM

NaCl	15.00	g
K_2HPO_4	0.50	g
$NaHCO_3$	15.00	g
Na_2CO_3	95.00	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.05	g
Na-L-lactate	2.20	g
Na_2SO_3	1.30	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.24	g
Distilled water	1000.00	ml

Dissolve sodium chloride and hydrogenphospate, then sparge solution with 100% N_2 gas for 30 -45 min to make it anoxic. Add and dissolve carbonates, adjust pH to 10, dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ammonium chloride, magnesium chloride, trace elements, yeast extract, lactate, sulfite, vitamins, and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas. Stock solutions of sulfite (freshly prepared) and vitamins should be sterilized by filtration.

For DSM 19491: Replace sulfite with 5.00 g/l sodium thiosulfate and lactate with 2.20 g/l sodium pyruvate.

For DSM 100427: Replace sulfite with 2.80 g/l sodium sulfate and lactate with 0.16 g/l Na -acetate and 3.40 g/l Na-formate.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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MEDIUM FOR ENHYDROMYXA SALSUGINOSA

Ferric iron citrate	0.02	g
$CaCl_2$ x 2 H_2O	0.20	g
$MgSO_4$ x 7 H2O	1.60	g
KCl	0.10	g
NaCl	10.00	g
Na-Caseinate	0.50	g
HEPES	2.00	g
$NaHCO_3$	0.16	g
H_3BO_3	20.00	mg
KBr	80.00	mg
$SrCl_2$ x 6 H_2O	30.00	mg
Agar	20.00	g
Trace element solution SL-4 (see medium 14)	2.00	ml
Distilled water	998.00	ml
Vitamin B_{12}	0.50	mg

Adjust to pH 7.2, check and adjust pH after autoclaving. Add vitamin B_{12} from a filter sterilized stock solution after autoclaving.

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1104: DETHIOBACTER MEDIUM

NaCl	6.00	g
K_2HPO_4	0.50	g
$NaHCO_3$	8.00	g
Na_2CO_3	22.00	g
Sulfur (powdered)	1.50	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.05	g
Ethanol absolute	0.60	ml
Na-acetate	0.40	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.24	g
Distilled water	1000.00	ml

1. Dissolve sodium chloride and hydrogenphosphate, then sparge solution with 100% N_2 gas for 30 -45 min to make it anoxic. Add and dissolve carbonates, then dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1- 2 hours on each of 3 successive days. After sterilization add ammonium chloride, magnesium chloride, trace elements, yeast extract, ethanol, acetate, thiosulfate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 9.5.

2. After inoculation add sterile 100% H_2 gas to 0.5 bar overpressure.

For DSM 19089: Replace ethanol with 1.10 g/l sodium butyrate.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g

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Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1105. NITRILIRUPTOR ALKALIPHILUS MEDIUM

Basic mineral medium, pH 10, 0.6M Na +:

Na_2CO_3	22.0	g
$NaHCO_3$	8.0	g
NaCl	6.0	g
K_2HPO_4	0.5	g
H_2O dest.	1000.0	ml

Dissolve in this order in 1L preferably MilliQ water. Sterilize in closed bottles at 120°C 20 min. After cooling, usually there will be some precipitate on the bottom. That´s why it is handy to have another empty 1L bottle to decant clear top solution into it.

After sterilization add from concentrated steril stock solutions:

1) 1 ml Trace metals solution
2) 1 ml 20% $MgSO_4$ x $7H_2O$ solution
3) 10 µl 2M $Na_2S_2O_3$ x $5H_2O$ solution pH 10.0
4) 1 ml 0.01 % Vitamin B_{12} solution
5) 10 ml of 1M Isobutyroamide (8.7 g in 100 ml H_2O dest) solution

solution 1, 2 and 3 you can autoclave
solution 4 and 5 you have to filter-sterilize

Trace metals solution (Pfennig & Lippert, 1966):

EDTA (Trilon B)	5.00	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$	0.10	g
$MnCl_2$	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$	0.01	g
$NiCl_2$ x 2 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O or NH_4MoO_4	0.02	g
H_2O dest	1000.00	ml

pH final 3-4 by HCl

Sodium thiosulfate solution (available):

50% solution of $Na_2S_2O_3$ (Fluka), pH 9-10 >> filter sterilize or 110°C 20 min

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1106: SPOROSALIBACTERIUM MEDIUM

NaCl	40.00	g
NH_4Cl	1.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.20	g
Yeast extract	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$MgCl_2$ x 6 H_2O	3.00	g
Na_2CO_3	1.00	g
D-Glucose	3.60	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except magnesium chloride, carbonate, glucose and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 -45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add magnesium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The glucose solution should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.

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1107. DIALISTER MEDIUM

Trypticase (BBL)	17.0	g
Yeast extract	3.0	g
NaCl	3.0	g
KNO_3	3.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.
Incubate in oxygen-free, 5 - 10% CO_2 containing atmosphere.

To 10 ml of the above medium add aseptically:

Na-L-lactate (50% (w/v))	0.10	ml
Haemin (0.5 mg/ml)	0.10	ml
$NaHCO_3$ (10% (w/v))	0.10	ml
Glucose (40% (w/v))	0.10	ml
Cystein-HCl x 2 H_2O (5% (w/v))	0.05	ml
DTT (100 mM)	0.05	ml
Vitamin K_1 (see below)	0.05	ml
Na-formate (6% (w/v))	0.50	ml
Na-fumarate (6% (w/v))	0.50	ml
Horseblood (sterile)	0.50	ml

Vitamin K_1 solution:
Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Filter-sterilize all solutions and incubate them in oxygen-free atmosphere.

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1108. CYS MEDIUM

NZ Case (Wako pure chemicals, Japan)=Peptone from Casein	3.000	g
Yeast Extract	2.000	g
Soluble Starch	1.000	g
NaCl	3.000	g
$MgCl_2$	0.125	g
$CaCl_2$	0.025	g
$FeSO_4$ x 7 H_2O	0.010	g
Gelrite	8.000	g
H_2O	900.000	ml

Add 100 µl each of Trace Metal solutions according to the following order:

Na_2MoO_4 x 2 H_2O	12.00	g
$VOSO_4$ x H_2O	1.00	g
$MnCl_2$ x 4 H_2O	5.00	g
$ZnSO_4$ x 7 H_2O	0.60	g
$CuSO_4$ x 5 H_2O	0.15	g
$CoCl_2$ x 6 H_2O	8.00	g
$NiCl_2$ x 6 H_2O	0.20	g

Adjust to pH 7.5 at room temperature and add water to the final volume of 1 liter.
Sterilize with and autoclave at 121°C for 15 min.

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1109. BTT MEDIUM

Glucose	10.0	g
Yeast Extract	1.0	g
Meat Extract	1.0	g
Casitone	2.0	g
Agar	20.0	g
H_2O dest.	1000.0	ml

Adjust to pH 5.5 – 6.0 (can optionally be buffered with 25 mM MES).

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1110: NAUTILIA PROFUNDICOLA MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
$CaCl_2$ x 2 H_2O	0.15	g
KCl	0.50	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	5.00	g
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, carbonate, vitamins and sulfide), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (to 20% of volume) that contain already the appropriate amount of sulfur. For sterilization heat cultivation vessels in a water bath to 90 - 100°C for 1 - 2 hours on each of three successive days. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust the pH of the complete medium to 7.0.

2. After inoculation, pressurize culture vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1112: CALDITERRIVIBRIO MEDIUM

NH_4Cl	0.54	g
KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
Na-acetate	0.82	g
$NaNO_3$	0.85	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg

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Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1113. LEPTOSPIRA-MEDIUM

Leptospira Medium Base EMJH (Difco)	2.3	g
agarose	1.5	g
water	900.0	ml
Leptospira Enrichment EMJH	100.0	ml

Dissolve the agarose in water by boiling. Add the Leptospira base and dissolve completely. Autoclave at 121°C for 15 min. Allow the medium to cool to 50-55°C. Warm the Leptospira Enrichment to approximately 25°C and add to the warm medium. Mix uniformly and dispense aseptically into sterile tubes as requested. Final pH is 7.5 at 25°C.

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1114: THIOFABA MEDIUM

Solution A	972.00	ml
Solution B	10.00	ml
Solution C	10.00	ml
Solution D	10.00	ml
Solution E	1.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into serum vials (to 20% of volume) and autoclave. Solution B is autoclaved separately under 100% N_2 gas and solution C under 80% N_2 and 20% CO_2 gas atmosphere. Solutions D and E are prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. The pH of the complete medium should be 6.5.

2. After inoculation cultivation vials are pressurized with an amount of sterile air that is equivalent to 30 vol% of the cultivation vial.

Solution A

$MgCl_2 \times 6\ H_2O$	0.75	g
$CaCl_2 \times 2\ H_2O$	0.15	g
NH_4Cl	0.54	g
Modified Wolin's mineral solution	2.00	ml
Distilled water	970.00	ml

Solution B

KH_2PO_4	1.19	g
K_2HPO_4	0.21	g
Distilled water	10.00	ml

Solution C

Na_2CO_3	0.50	g
Distilled water	10.00	ml

Solution D

$Na_2S_2O_3 \times 5\ H_2O$	1.25	g
Distilled water	10.00	ml

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Solution E

Wolin's vitamin solution (10x)	1.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1115: CONGREGIBACTER (SYPHC) MEDIUM

Sea Salt (SIGMA)	35.00	g
Wolfe's mineral elixir	1.00	ml
NH_4Cl	0.10	g
Yeast extract	1.00	g
Na-pyruvate	1.10	g
HEPES (SIGMA)	2.50	g
L-Histidine	0.04	g
L-Cysteine HCl x H_2O	0.04	g
Seven vitamins solution	0.50	ml
KH_2PO_4	0.05	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except vitamins and hydrogenphosphate) and adjust pH to 7.7. After autoclaving add vitamins and hydrogenphosphate from sterile stock solutions sterilized by filtration. Adjust pH of complete medium to 7.3 - 7.5, if necessary.

2. Note: Growth of most strains is stimulated by using microaerophilic conditions with only 10% (v/v) oxygen in the head space gas atmosphere. For microaerophilic growth medium is prepared without HEPES, sparged with 80% N_2 and 20% CO_2 gas mixture to make it anoxic and then dispensed under air atmosphere into serum vials to 50% volume. After autoclaving the pH is adjusted to 7.5 using a sterile anoxic stock solution of Na_2CO_3 (5% w/v) prepared under 80% N_2 and 20% CO_2 gas mixture.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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1115: CONGREGIBACTER (SYPHC) MEDIUM

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1116. STEROIDOBACTER MEDIUM

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KH_2PO_4	0.20	g
KCl	0.50	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.15	g
Na_2SO_4	0.07	g
$NaNO_3$	0.42	g
Distilled water	1000.00	ml

For growth with testosterone, dissolve testosterone in acetone (20 mg/ml), dispense portions of the solution, e.g. 0.1 ml portions for 10 ml medium, to anaerobic culture tubes. Let the solvent evaporate to dryness. Dispense 10 ml portions of medium and stream with N_2/CO_2 (80:20). Close the vessels and autoclave. Treat the vessels in an ultrasonic bath to detach and suspend the testosterone.
For growth with heptanoate, omit testosterone. Sterilize the gassed medium in anaerobic vessels by autoclaving. After cooling, add 325 mg/l heptanoic acid from a 20fold, pH adjusted, filter sterilized anaerobic stock solution.

After autoclaving, add (per 10 ml) 0.3 ml $NaHCO_3$-solution (84 g/l, autoclaved under CO_2-atmosphere), 1 µl trace element solution SL 10 (see below), 1 µl selenite tungstate solution (see below), and 1 µl vitamin solution (see below). Adjust pH to 7.2. Incubate the cultures in the dark for up to 4 weeks. Briefly shake the cultures once per day.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

Selenite and tungstate solution:

NaOH	0.5	g
Na_2SeO_3 x 5 H_2O	3	mg
Na_2WO_4 x 2 H_2O	4	mg
Distilled water	1000	ml

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Vitamin solution:

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

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1117. PYS MEDIUM

Peptone	8.0	g
Yeast extract	4.0	g
NaCl	2.0	g
Tap water	1000.0	ml

Adjust to pH 7.2

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1118. MD1-MEDIUM

Casitone	3.0	g
$CaCl_2$ x 2 H_2O	0.7	g
$MgSO_4$ x 7 H_2O	2.0	g
Trace element solution SL 4	1.0	ml
Distilled water	1000.0	ml

After autoclaving and cooling, add 0.5 mg /l vitamin B_{12} from a filter sterilized stock solution. pH should be 7.0 -7.2.

Trace element solution SL-4:

EDTA	0.500	g
$FeSO_4$ x 7 H_2O	0.200	g
$ZnSO_4$ x 7 H_2O	0.010	g
$MnCl_2$ x 4 H_2O	0.003	g
H_3BO_3	0.030	g
$CoCl_2$ x 6 H_2O	0.020	g
$CuCl_2$ x 2 H_2O	0.001	g
$NiCl_2$ x 6 H_2O	0.002	g
Na_2MoO_4 x 2 H_2O	0.003	g
Distilled water	1000.000	ml

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1119. GNYS MEDIUM

Sodium gluconate	3.00	g
Yeast extract (Difco)	1.00	g
$(NH_4)_2SO_4$	1.00	g
KH_2PO_4	1.00	g
NaCl	0.20	g
$MgCl_2 \times 6H_2O$	0.20	g
$CaCl_2 \times 2H_2O$	0.05	g
Trace element solution SL8 (see below)	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 4.0 - 4.5.
For solid medium, add 1.8% agar (final) and adjust pH to 5.0 to avoid hydrolysis of agar.

Trace element solution SL8

Na_2·EDTA	5.2	g
$FeCl_2 \times 4H_2O$	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2 \times 4H_2O$	100.0	mg
H_3BO_3	62.0	mg
$CoCl_2 \times 6H_2O$	190.0	mg
$CuCl_2 \times 2H_2O$	17.0	mg
$NiCl_2 \times 6H_2O$	24.0	mg
$Na_2MoO_4 \times 2H_2O$	36.0	mg
Distilled water	1000.0	ml

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1120. PYSE MEDIUM

Peptone	8.00	g
Yeast extract	3.00	g
NaCl	15.00	g
KCl	0.35	g
$MgSO_4 \times 7H_2O$	2.65	g
$CaSO_4 \times 2H_2O$	0.65	g
$MgCl_2 \times 6H_2O$	5.40	g
Distilled water	1000.00	ml

Adjust to pH 7.5.

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1121. MMJS MEDIUM (modified)

NaCl	20.00	g
K_2HPO_4	0.09	g
KH_2PO_4	0.07	g
$CaCl_2$ x 2 H_2O	0.80	g
NH_4Cl	1.25	g
$MgSO_4$ x 7 H_2O	4.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.33	g
Fe –citrate	0.01	g
FeSO4 x 7 H2O	0.01	g
$Na_2S_2O_3$ x 5 H_2O	3.00	g
Sulfur, powdered	3.00	g
Trace mineral solution (see below)	10.00	ml
Distilled water	1000.00	ml

The pH of the medium is adjusted with NaOH to 6.8. Steam medium for 3 hours on each of 3 successive days. The separately autoclaved, concentrated solutions including each of follows are added to the medium. Then a mix gas (80%N_2, 20%CO_2) is purged for 5 min. Finally, the mix gas (79%N_2, 20%CO_2, 1%O_2) is compressed into gas phase (>80% volume of the tube or bottle) at 2 atm.

Vitamin solution (see below)	final concentration	1.0	ml/l
$NaHCO_3$	final concentration	0.2	%

Trace mineral solution:

Nitrilotriacetic acid	1.500	g
$MnSO_4$ x x H_2O	0.500	g
$CoSO_4$ x 7 H_2O	0.500	g
$ZnSO_4$ x 7 H_2O	0.180	g
$CuSO_4$ x 5 H_2O	0.010	g
$KAI(SO_4)_2$ x 12 H_2O	0.020	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.001	g
$SrCl_2$ x 6 H_2O	0.010	g
NaBr	0.010	g
KI	0.010	g
$NiCL_2$ x 6 H_2O	0.100	g
Na_2SeO_3 x 5 H_2O	0.100	g
$Fe_2(SO_4)_3$ x xH_2O	1.000	g
H_2WO_4	0.100	g

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Distilled water	1000.000 ml

Vitamin solution:

Biotin	0.0020 g
Folic acid	0.0020 g
Pyridoxine-HCl	0.0100 g
Thiamine-HCl-$2H_2O$	0.0050 g
Riboflavin	0.0050 g
Nicotinic acid	0.0050 g
D-Ca-pantothenate	0.0050 g
Vitamin B_{12}	0.0001 g
p-Aminobenzoic acid	0.0050 g
Lipoic acid	0.0050 g
Distilled water	1000.0000ml

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1122. MEDIUM K

$(NH_4)_2SO_4$	2.000	g
KH_2PO_4	2.000	g
NaCl	0.500	g
$MgSO_4$ x 7 H_2O	0.125	g
$FeSO_4$ x 7 H_2O	0.002	g
Agar Difco	20.000	g
Distilled water	1000.000	ml

Adjust to pH 7.2.
After autoclaving add methanol (sterilized separately) to a concentration of 1% (v/v).

For DSM 23053
+ 0.02 % Yeast extract (Difco)
Add 0.5% Methanol after autoclaving.

For DSM 25212, for DSM 25216 and for DSM 25045
Add 2.0% Methanol after autoclaving.

For DSM 23053
Add 0.02% Yeast extract.

For DSM 103579
Add 0.5% Methanol after autoclaving.

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1123. YED MEDIUM, salted

Glucose	7.0	g
Yeast extract	4.0	g
NaCl	10.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0

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1124. HD (1:10 diluted)

Casein peptone	0.50	g
Glucose	0.10	g
Yeast extract	0.25	g
If required, Agar-agar	15.00	g
Distilled water	1000.00	ml

Adjust to pH 5.0

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1125. HALOPHILIC MEDIUM

$MgCl_2$ x 6 H_2O	20.00	g
K_2SO_4	5.00	g
$CaCl_2$ x 2 H_2O	0.10	g
Yeast extract	0.10	g
NH_4Cl	0.50	g
KH_2PO_4	0.05	g
Sucrose	0.50	g
NaCl	180.00	g
HEPES	6.00	g
Distilled water	1000.00	ml

Adjust to pH 6.5

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1126. TELMATOSPIRILLUM MEDIUM

K_2HPO_4	0.25	g
KH_2PO_4	1.00	g
$(NH_4)_2SO_4$	1.00	g
$CaCl_2$	0.10	g
$MgSO_4$	0.40	g
Na-EDTA	0.01	g
$FeCl_3$ x 6 H_2O	1.00	mg
KI	0.20	mg
$CoCl_2$ x 6 H_2O	0.20	mg
$MnCl_2$ x 4 H_2O	0.80	mg
$ZnSO_4$	0.80	mg
H_3BO_2	0.10	mg
Na_2MoO_4 x 2 H_2O	0.10	mg
$CuCl_2$	0.10	mg
$NiCl_2$ x 6 H_2O	0.20	mg
Na-citrate	2.00	g
Distilled water	1000.00	ml

Prepare the medium anaerobically under nitrogen. Adjust to pH 5.5 – 6.5 with 1M H_3PO_4. Dispense into serum bottles or Hungate tubes (under a nitrogen atmosphere) that can be sealed with rubber stoppers.

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1127. TPT 18 MEDIUM

Glucose	500	mg
Yeast extract	100	mg
Casitone (pancreatic digest of casein)	100	mg
$MgSO_4$ x 7 H_2O	50	mg
$CaCl_2$ x 2 H_2O	20	mg
Agar (for solid medium)	14	g
Distilled water	1000	ml

Adjust to pH 6.0.

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1129. PHOTOTROPHIC MEDIUM

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	1.00	g
NaCl	20.00	g
NH_4Cl	0.60	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	0.40	g
Ferric citrate solution (0.1%)	5.00	ml
Trace element solution SL7 (see below)	1.00	ml
Distilled water	1000.00	ml

Bubble the medium with nitrogen gas and fill 10 ml in 15 ml tubes with a rubber septum under a stream of nitrogen gas.

Trace element solution SL7:

HCl (25%, v/v)	1.0	ml
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	60.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$CuCl_2$ x 2 H_2O	20.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	40.0	mg
Distilled water	1000.0	ml

<u>For DSM 17935</u>

Final pH 6.5

Add the following component after autoclaving from sterile stock solutions:

Sorbitol	3.0	g/l
Sodium pyruvate	3.0	g/l

<u>For DSM 17936</u>

Final pH 6.8

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Na_2S x 9 H_2O	1.0	mM

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For DSM 18632

Increase the NH_4Cl to 0.68 g/l

Final pH 7.2

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B12 (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O	1.0	mM

For DSM 18805

Final pH 7.2

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B_{12} (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O	1.0	mM

For DSM 18858

Final pH 6.8

Add the following component after autoclaving from sterile stock solutions:

Sodium pyruvate	3.0	g/l
Vitamin B12 (2 mg/l filter sterilised)	1.0	ml/l
Na_2S x 9 H_2O 1M	3.0	ml/
$NaHCO_3$ (10%, autoclaved in sealed, half full vessels under CO_2)	10.0	ml/l

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1130. RE-101/102 MEDIUM

NaCl	175.0	g
$MgCl_2$ x $6H_2O$	20.0	g
K_2SO_4	5.0	g
$CaCl_2$ x $2H_2O$	0.1	g
Yeast extract	5.0	g
Distilled water	1000.0	ml

Adjust to pH 6.8-7.2

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1131. HYDROGENIVIRGA OKINAWENSIS MEDIUM

Modified MJ synthetic sea water (see below)	1000.0	ml
Trace vitamins (see next page)	10.0	ml
$NaNO_3$	1.0	g
$NaHCO_3$	1.0	g
Sulfur (powder)	3.0	g

Mix ingredients except $NaHCO_3$ and vitamin solution, adjust pH to 7.0 with NaOH, and autoclave under a N_2 atmosphere. Filter-sterilize 8% $NaHCO_3$ solution and add to the medium. Distribute the medium into culture vessels (e.g., 20 ml in 120 ml serum bottles) containing sulfur under a stream of a H_2-CO_2 (4:1, v/v) gas mixture, and seal with butyl rubber stoppers. Steam medium for 3 hr on each of 3 successive days. Prior to inoculation, add trace vitamins (sterile filtrated and stocked under a N_2 atmosphere) to the medium. Pressurize the inoculated bottles to 200 kPa H_2-CO_2 (4:1, v/v).

Modified MJ synthetic sea water:

NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.50	g
NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
$FeSO_4$ x 7 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	1.00	mg
Na_2SeO_3 x 5 H_2O	1.00	mg
Trace element solution (see below)	10.00	ml
Distilled water	1000.00	ml

Trace element solution:

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x X H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.10	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$	0.01	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

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Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1132. LC 2

$MgSO_4$ x 7 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.20	g
KNO_3	0.05	g
SL-10 (see below)	1.00	ml
H_2O	1000.00	ml

Dispense 5 ml aliquots into Hungate tubes. After autoclaving add the following components per liter:

Phosphate buffer (pH 6.8, see below)	2.0	ml
2 M HEPES buffer (pH 7.0)	1.0	ml
Fe-NH_4EDTA (4mg/ml)	1.0	ml
Methanol	0.3	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.0	ml
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	6.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	990.0	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Phosphate buffer:

Na_2HPO_4	3.6	g
KH_2PO_4	1.4	g
H_2O	100.0	ml

Adjust to pH 6.8.

For DSM 22980 after autoclaving add 1 ml of vitamine solution 141 (see Medium141) to 1 litre of the sterile medium.

Incubate in sealed containers. The strain held in the DSMZ has been cultivated on methanol, but the original publication indicates that the strain will also grow on methane.

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1133. ENRICHED CYTOPHAGA AGAR MEDIUM

Tryptone	2.0	g
Beef extract	0.5	g
Yeast extract	0.5	g
Sodium acetate	0.2	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2-7.4 and autoclave at 121°C/15 min.

For soft agar for the maintenance of active cultures reduce agar content to 4.0 g/l. Dispense in 3 to 4 ml amounts in 7 ml screw-capped bottles (bijou bottles).

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1135. HD-MEDIUM, 1:10 diluted, modified

Solution A:

Casein peptone	0.50	g
Glucose	0.10	g
Yeast extract	0.25	g
MES (2-[N-Morpholino]ethane sulfonic acid)	1.95	g
Double distilled water for liquid medium	1000.00	ml
Double distilled water for solid medium	500.00	ml

Solution B:

1.5% washed agar (with double distilled water)		
Double distilled water	500.00	ml

Adjust to pH 5.5 (with NaOh/HCl)
Sterilize separately. For solid medium combine solution A with solution B at 50°C.

Liquid and solid medium possible. Incubation at 20°C, 4 days.

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1137. RUBRITALEA MEDIUM

Peptone	1.5	g
Yeast extract	1.5	g
Glucose	1.5	g
Tris-HCl (1M, pH 7.5)	5.0	ml
Hutners basal salts (see below)	20.0	ml
Artifical sea water (see below)	975.0	ml

Adjust to pH 7.5

Hutners basal salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44" (see below)	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.0	mg
$ZnSO_4$ x 7 H_2O	1095.0	mg
$FeSO_4$ x 7 H_2O	500.0	mg
$MnSO_4$ x H_2O	154.0	mg
$CuSO_4$ x 5 H_2O	39.2	mg
$Co(NO_3)_2$ x 6 H_2O	24.8	mg
$Na_2B_4O_7$ x 10 H_2O	17.7	mg
Distilled water	1000.0	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

Artificial sea water, 3 times concentrated:

NaCl	70.43	g
Na_2SO_4	11.75	g
$NaHCO_3$	2.88	g
KCl	1.99	g
KBr	0.29	g
$MgCl_2$ x 6 H_2O	31.86	g
$CaCl_2$ x 2 H_2O	4.35	g
H_3BO_3	0.08	g
Distilled water	1000.00	ml

Add $NaHCO_3$ from a filter-sterilized stock solution when the medium has cooled.

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1138. HALOPIGER MEDIUM

NaCl	195.00	g
$MgCl_2 \times 6H_2O$	32.50	g
$MgSO_4 \times 7H_2O$	50.80	g
$CaCl_2 \times 2H_2O$	0.80	g
KCl	5.00	g
$NaHCO_3$	0.16	g
NaBr	0.60	g
Yeast extract	5.00	g
Distilled water	1000.00	ml

Adjust to pH 8.0

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1139. PYG MEDIUM (B)

Polypepton (Nihon Pharmaceutical Co.)	5.0	g
Tryptone (Difco)	5.0	g
Yeast extract (Difco)	10.0	g
Glucose	10.0	g
Salts solution (see below)	40.0	ml
Distilled water	960.0	ml

Adjust pH to 7.2

Salts solution:

$CaCl_2$	0.2	g
$MgSO_4$ x 7 H_2O	0.4	g
K_2HPO_4	1.0	g
KH_2PO_4	1.0	g
$NaHCO_3$	10.0	g
NaCl	2.0	g
Distilled water	1000.0	ml

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1140. PYG MEDIUM (E)

Bacto peptone (Difco)	5.0	g
Beef extract (Difco)	3.0	g
Yeast extract (Difco)	0.2	g
Glucose	5.0	g
NaCl	0.5	g
$MgSO_4$ x 7 H_2O	1.5	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0

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1141. THERMOGYMNOMONAS MEDIUM

$(NH_4)_2\ SO_4$	0.20	g
KH_2PO_4	3.00	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
Yeast extract (Difco)	1.00	g
Glucose	10.00	g
Distilled water	1000.00	ml

Adjust pH to 3.0 with 10 N H_2SO_4. Separately autoclave yeast extract and glucose as 10% solutions, and add aseptically to the medium.

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1142. RHODOBLASTUS MEDIUM

Yeast extract	0.10	g
Na_2-succinate	1.00	g
KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-6 (see below)	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 5.7

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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1143. TY MEDIUM

Tryptone	5.0	g
Yeast extract	3.0	g
$CaCl_2$ x 2 H_2O	0.9	g
Distilled water	1000.0	ml

Adjust to pH 6.8

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1144. SINGULISPHAERA MEDIUM

N-acetylglucosamine	1.0	g
KH_2PO_4	0.1	g
Hutners basal salts (see below)	20.0	ml
Na-ampicillin (may be omitted for pure cultures)	0.2	g
Peptone	0.1	g
Yeast extract	0.1	g
Agar-agar (Difco)	18.0	g
Distilled water	1000.0	ml

Adjust pH to 5.8.

Hutners basal salts:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$(NH_4)MoO_7O_{24}$ x 4 H_2O	9.25	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.0	mg
$ZnSO_4$ x 7 H_2O	1095.0	mg
$FeSO_4$ x 7 H_2O	500.0	mg
$MnSO_4$ x H_2O	154.0	mg
$CuSO_4$ x 5 H_2O	39.2	mg
$Co(NO_3)_2$ x 6 H_2O	24.8	mg
$Na_2B_4O_7$ x 10 H_2O	17.7	mg
Distilled water	1000.0	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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1145. MS-MEDIUM (MODIFIED)

Anaerobic double distilled water	800.00	ml
MS Buffer (see below)	200.00	ml
Elemental sulphur	5.00	g
NaS_2O_3 x 5 H_2O	2.00	g
$MgSO_4$ x 7 H_2O	7.00	g
KCl	0.48	g
$CaCl_2$ x 2 H_2O	0.40	g
$MgCl_2$ x 6 H_2O	0.80	g
Solution A (see below)	20.00	ml
Solution B (see below)	1.50	ml
Solution D (see below)	10.00	ml

The medium is prepared anaerobically under nitrogen gas and dispensed into Hungate tubes or serum bottle sealed with rubber septa. The medium to gas phase ratio should be about 1:10 (v:v). Sterilise the medium by heating at 100° C for 3 hours on three consecutive days.

Final pH of the medium for DSM 19534 is pH 6.0-6.8

Final pH of the medium for DSM 19557 is pH 5.9-6.5

MS Buffer:
100 mM NaOH saturated with CO_2

Each of the solutions below were prepared with anaerobic double distilled water and prepared under constant gassing with nitrogen.

Solution A:

NH_4Cl	100.00	g
$MgCl_2$ x 6 H_2O	100.00	g
$CaCl_2$ x 2 H_2O	40.00	g
Distilled water	1000.00	ml

Adjust to pH 4 with HCl

Solution B:

K_2HPO_4 x 3H_2O	200.00	g
Distilled water	1000.00	ml

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Solution D – Trace mineral solution (Ferguson and Mah, 1983):

Na_2-EDTA x 2 H_2O	500.00	mg
Na_2WO_4 x 2 H_2O	30.00	mg
$CoCl_2$ x 6 H_2O	150.00	mg
$CuCl_2$ x 2 H_2O	20.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
$NiSO_4$ x 6 H_2O	20.00	mg
$FeSO_4$ x 7 H_2O	100.00	mg
H_2SeO_3	10.00	mg
$ZnCl_2$	100.00	mg
H_3BO_3	10.00	mg
$AlCl_3$ x 6 H_2O	40.00	mg
$NaMoO_4$ x 2 H_2O	10.00	mg
Distilled water	1000.00	ml

Adjust pH to 3.0 with HCl

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1146. VENENIVIBRIO STAGNISPUMANTIS MEDIUM

KCl	0.50	g
$MgCl_2$ x 6 H_2O	1.36	g
$MgSO_4$ x 7 H_2O	7.00	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	0.20	g
KH_2PO_4	0.25	g
Trace minerals (see below)	10.00	ml
MES	1.95	g
Distilled water	1000.00	ml

Adjust the pH to 5.5 and gas the media with N_2/CO_2 (80/20) for 15 min at least.
Fill 20 ml medium into 100 ml serum bottles, gas the flask containing the media with H_2/CO_2 (80/20) and seal with a rubber stopper. Autoclave the medium for 20 min at 121°C.
After the inoculation the tubes are pressurized with $H_2/CO_2/O_2$ (79%/15%/6%,170 kPa).
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Trace mineral solution:

Na-EDTA x 2 H_2O	500.00	mg
$CoCl_2$ x 6 H_2O	150.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
$FeSO_4$ x 7 H_2O	100.00	mg
$ZnCl_2$	100.00	mg
$AlCl_3$ x 6 H_2O	40.00	mg
Na-tungstate x 2 H_2O	30.00	mg
CuCl	20.00	mg
Ni_2SO_4 x 6 H_20	20.00	mg
$NaSeO_3$	10.00	mg
H_3BO	10.00	mg
Na_2MoO_4 x 2 H_2O	10.00	mg
Distilled water	1000.00	ml

Adjust the pH of the solution to 3.0 with HCl.

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1147. RV5 MEDIUM

EDTA	10.00	mg
$MgSO_4$ x 7 H_2O	200.00	mg
$CaCl_2$ x 2 H_2O	75.00	mg
NaCl	15.00	g
NH_4Cl	0.52	g
DL-malic acid	4.00	g
K_2HPO_4	1.00	g
BICINE buffer (Sigma)	10	mM
Trace elements (see below)	1.00	ml
Distilled water	1000.00	ml

Filter-sterilize and add after autoclaving:

Thiamine	1.00	mg
Vitamin B_{12}	20	µg
Biotin	30	µg

The pH of the medium was adjusted to 9.5 by addition of NaOH and then sterilized by autoclaving. Before inoculating medium RV5, 0.1% (w/v) $NaHCO_3$ was added to tubes, bottles, or molten media from a 5% (w/v) filter-sterilized stock solution. For routine culture, medium RV5 supplemented with 0.05% (w/v) Difco yeast extract was used.

Trace elements:

EDTA	5.20	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 4 H_2O	0.10	g
$FeCl_2$ x 4 H_2O	1.50	g
H_3BO_3	6.00	mg
$CuCl_2$ x 2 H_2O	17.00	mg
Na_2MoO_4 x 2 H_2O	188.00	mg
$NiCl_2$ x 6 H_2O	25.00	mg
$ZnCl_2$	70.00	mg
$VoSO_4$ x 2 H_2O	30.00	mg
Na_2WO_4 x 2 H_2O	2.00	mg
$NaHSeO_3$	2.00	mg
Deionized water	1000.00	ml

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1148: TINDALLIA MEDIUM

NaCl	30.00	g
KCl	0.20	g
K_2HPO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
NH_4Cl	1.00	g
Yeast extract	0.50	g
Peptone	5.00	g
Trace element solution (mg per 200 ml)	1.00	ml
Resazurin	1.00	mg
Distilled water	1000.00	ml

1. Prepare the medium omitting Na_2CO_3, $NaHCO_3$, Na_2S x 9 H_2O, and Vitamin solution.
2. Boil the medium and cool under nitrogen, add Na_2CO_3 and $NaHCO_3$.

$NaHCO_3$	24.00	g
Na_2CO_3	2.76	g

3. Adjust pH to 9.5-10.0 with 10M KOH.
4. Dispense into tubes or bottle fitted with rubber stoppers.
5. Autoclave at 121 °C for 15 min.
6. Add Na_2S x 9 H_2O (neutralized) and Vitamin solution from sterile stock solutions

Na_2S x 9 H_2O (from neutralized stock solution)	0.40	g
Wolin's vitamin solution	2.00	ml

Trace element solution (mg per 200 ml)

$MnCl_2$ x 4 H_2O	720.00	mg
$Fe(NH_4)_2(SO_4)_2$ x 12 H_2O	400.00	mg
$FeSO_4$ x 7 H_2O	200.00	mg
$CoCl_2$ x 6 H_2O	200.00	mg
$ZnSO_4$ x 7 H_2O	200.00	mg
Na_2MoO_4 x 2 H_2O	20.00	mg
$NiCl_2$	100.00	mg
$CuSO_4$ x 5 H_2O	20.00	mg
$AlK(SO_4)_2$ x 12 H_2O	20.00	mg
H_3BO_3	20.00	mg
HCl (concentrated)	5.00	ml
Distilled water	200.00	ml

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1148: TINDALLIA MEDIUM

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1149. OCEANITHERMUS MEDIUM

K_2HPO_4	0.78	g
KH_2PO_4	0.75	g
Trace element solution (see below)(see medium 318)	10.00	ml
Vitamin solution (see below)	10.00	ml
$MgCl_2$ x 6 H_2O	0.36	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl_2	0.53	g
Na_2SO_4	0.14	g
NaCl	20.00	g
Yeast extract	2.00	g
$NaNO_3$	1.70	g
Distilled water	1000.00	ml

Prepare the medium without vitamins, boil and cool under a nitrogen:carbon dioxide (80:20 v/v) atmosphere. Vitamins (filter sterilized and stored under nitrogen) and 100 ml 0.6 M $NaHCO_3$ solution (sterilized and stored under nitrogen) are added from stock solutions.
The pH should be pH 6.5.

Trace element solution:

Nitrilotriacetic acid (NTA)	12.800	g
$FeCl_3$ x 6 H_2O	1.350	g
$MnCl_2$ x 4 H_2O	0.100	g
$CoCl_2$ x 6 H_2O	0.024	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnCl_2$	0.100	g
$CuCl_2$ x 2 H_2O	0.025	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.024	g
NaCl	1.000	g
$NiCl_2$ x 6 H_2O	0.120	g
Na_2SeO_3 x 5 H_2O	0.026	g
Distilled water	1000.000	ml

Vitamin solution:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine-HCl	10.000	mg

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Thiamine-HCl x $2H_2O$	5.000	mg
Riboflavin	5.000	mg
Nicotinic acid	5.000	mg
D-Ca-pantothenate	5.000	mg
Vitamin B_{12}	0.100	mg
p-Aminobenzoic acid	5.000	mg
Lipoic acid	5.000	mg
Distilled water	1000.000	ml

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1151. SDO MEDIUM

NaCl	100.00	g
$MgSO_4$ x 7 H_2O	3.45	g
$MgCl_2$ x 6 H_2O	3.00	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
$CaSO_4$ x 2 H_2O	0.25	g
KH_2PO_4	0.14	g
$NaHCO_3$	1.00	g
Yeast extract	0.10	g
Trace elements (see below)	1.00	ml
Vitamin solution (see below)	1.00	ml
Distilled water	1000.00	ml

Adjust to pH 6.0 (with HCl). For agar plates, add 20 g/l of agar

Trace elements (SL 6):

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.0	ml

Vitamin solution (DSM Med. 141):

Biotin	2.0	mg
Folic acid	2.0	mg
Pyridoxine-HCl	10.0	mg
Thiamine-HCl x 2 H_2O	5.0	mg
Riboflavin	5.0	mg
Nicotinic acid	5.0	mg
D-Ca-pantothenate	5.0	mg
Vitamin B_{12}	0.1	mg
p-Aminobenzoic acid	5.0	mg
Lipoic acid	5.0	mg
Distilled water	1000.0	ml

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1152. PM MEDIUM

KH_2PO_4	4.00	g
Na_2HPO_4	11.20	g
NaCl	4.00	g
$MgCl_2$ x 6 H_2O	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
NH_4Cl	2.00	g
$CaCl_2$	0.01	g
$FeSO_4$ x 7 H_2O	0.00	g
Trace element solution (see below)	10.00	ml
Na acetate	10.00	g
Distilled water	1000.00	ml

Adjust to pH 7.2. The medium may be solidified by adding 20.0 g/l agar.

Trace element solution (SL 10):

HCl (25%; 7.7 M)	10.0	ml
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	6.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	990.0	ml

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1153. R3 A MEDIUM

Yeast extract	1.0	g
Proteose peptone (Difco No.3)	1.0	g
Casamino acids (Difco)	1.0	g
Glucose	1.0	g
Boiled soluble starch	1.0	g
Sodium pyruvate	0.5	g
K_2HPO_4	0.6	g
$MgSO_4$ x 7 H_2O	0.1	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2

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1156. HALOPHILIC METHANOTROPHIC BACTERIUM MEDIUM

H_2O	1000.000	ml
$MgSO_4$ x 7 H_2O	0.100	g
NH_4Cl	0.500	g
Na_2HPO_4 x 2 H_2O	0.700	g
KH_2PO_4	0.300	g
NaCl	65.000	g
$CaCl_2$ x 6 H_2O	0.010	g
$FeSO_4$ x 7 H_2O	0.005	g
Trace Element Solution (see below)	1.000	ml

pH-value 7.2 (Adjust to 7.6 with 10% NaOH before sterilization)

Trace Element Solution:
1 ml of trace element solution contains:

$ZnSO_4$ x 7 H_2O	0.44	mg
$CuSO_4$ x 5 H_2O	0.20	mg
$MnSO_4$ x 2 H_2O	0.17	mg
Na_2MoO_4 x 2 H_2O	0.06	mg
H_3BO_3	0.10	mg
$CoCl_2$ x 6 H_2O	0.08	mg

Culture bottles: 120-ml Serum bottles with 25 ml medium closed with butyl rubber serum caps and aluminium crimps

Gas supply: 2 vol.% CO_2; 20 vol.% CH_4; 78 vol.% air
(Alternate cultivation method: instead of a methane-containing headspace, add 0.5% (w/v) methanol to the medium)

Culture conditions: 30°C; shaking culture on rotary shaker (200 rpm)

Notes
pH is critical, final pH must be between 6.5-7.5;
some insoluble phosphates may precipitate during medium preparation (should not be more than OD 0.005).

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1157. PYEM MEDIUM

Peptone	2.0	g
Yeast extract	2.0	g
NH_4Cl	0.5	g
Distilled water	1000.0	ml

After autoclaving and cooling add:

Riboflavin (0.2 mg/ml) filter-sterilized	5.0	ml
50% Glucose (autoclaved)	2.0	ml
20% $MgSO_4$ (autoclaved)	1.0	ml
10% $CaCl_2$ (autoclaved)	1.0	ml

Final pH 7.0

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1158. HALOMONAS SYMIRNIENSIS MEDIUM

Yeast extract	5.0	g
$MgSO_4$ x 7 H_2O	3.0	g
KCl	2.0	g
NaCl	100.0	g
Tri-sodium citrate	3.0	g
Distilled water	1000.0	ml

Minimum NaCl concentration must be 5%. Adjust to pH 6.9. The medium may be solidified by adding 15.0 g/l agar.

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1159. GYM STREPTOMYCES MEDIUM 10% NACL

Glucose	4.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
$CaCO_3$	2.0	g
NaCl	100.0	g
Agar	15.0	g
H_2O dest.	1000.0	ml

Adjust pH to 7.2. Delete $CaCO_3$ if liquid medium is used.

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1160. PAYNE, SEGHAL & GIBBONS MEDIUM

Casamino acids (Difco)	7.50	g
Yeast extract	10.00	g
Trisodium citrate	3.00	g
KCl	2.00	g
$MgSO_4$ x 7 H_2O	20.00	g
$FeCl_2$ x 4 H_2O	36.00	mg
$MnCl_2$ x 4 H_2O	0.36	mg
NaCl	250.00	g
Distilled water	1000.00	ml

Adjust pH to 7.4. For solid medium add 20.0 g agar.

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1161. DUBOS SALTS MEDIUM

$NaNO_3$	0.5	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.5	g
KCl	0.5	g
$FeSO_4$ x 7 H_2O	10.0	mg
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2. Autoclave at 121°C/15 min. Dispense into slopes. When the agar has solidified, place a strip of sterile filter paper on to the surface of each slope. Inoculate on to the filter paper.

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1163: KOSMOTOGA OLEARIA MEDIUM

NaCl	30.00	g
PIPES (SIGMA)	6.70	g
KCl	0.33	g
$MgCl_2 \times 2\ H_2O$	0.90	g
$MgSO_4 \times 7\ H_2O$	1.40	g
NH_4Cl	0.25	g
$CaCl_2 \times 2\ H_2O$	0.14	g
KH_2PO_4	0.45	g
Yeast extract	5.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Maltose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, maltose and vitamins. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then add and dissolve cysteine and adjust pH to 6.8 with NaOH. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add maltose and vitamins after autoclaving from anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration.

For DSM 105848: Supplement medium with 1.50 g/l Na-thiosulfate added to the autoclaved medium from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1164. HALOMICROBIUM KATESII MEDIUM

$NaHCO_3$	0.166	g
NaBr	0.350	g
$CaCl_2$ x 2 H_2O	0.916	g
KCl	5.000	g
$MgSO_4$ x 7 H_2O	49.830	g
NaCl	195.000	g
$FeCl_3$ x 6 H_2O (0.5%)	7	drops
Glucose	1.000	g
Proteose peptone	5.000	g
Yeast extract	5.000	g
Distilled water	1000.000	ml

Adjust to pH 7.2

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1165. SPIROCHAETA AMERICANA MEDIUM

NaCl	30.00	g
Na_2CO_3	2.76	g
$NaHCO_3$	24.00	g
KCl	0.20	g
K_2HPO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
NH_4Cl	1.00	g
Na_2S x 9 H_2O	0.40	g
Yeast extract	0.50	g
D-glucose	5.00	g
Vitamin Solution (see below)	2.00	ml
Trace element solution (see below)	1.00	ml
Resazurin	1.00	mg
Distilled water	1000.00	ml

Adjust to pH 9.4

Vitamin Solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Trace element solution:

$MnCl_2$ x 4 H_2O	720.00	mg
$Fe(NH_4)(SO_4)_2$ x 12 H_2O	400.00	mg
$FeSO_4$ x 7 H_2O	200.00	mg
$CoCl_2$ x 6 H_2O	200.00	mg
$ZnSO_4$ x 7 H_2O	200.00	mg
Na_2MoO_4 x 2 H_2O	20.00	mg
$NiCl_2$	100.00	mg
$CuSO_4$ x 5 H_2O	20.00	mg

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$AlK(SO_4)_2$ x 12 H_2O	20.00	mg
H_3BO_3	20.00	mg
HCl	5.00	ml
Distilled water	200.00	ml

Prepare the medium without the Na_2S, yeast extract, glucose, Na_2CO_3, $NaHCO_3$ and vitamins. Heat the medium in a boiling water bath and cool under a stream of nitrogen. Add the Na_2CO_3, and $NaHCO_3$ to the cooled medium and check the pH. Dispense into appropriate vessels (Hungate tubes or serum bottles) and autoclave. To the sterile medium add Na_2S, yeast extract, and glucose from autoclaved stock solutions. The vitamin solution is filter sterilised and added to the sterilised medium.

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1166: DESULFONATRONUM THIOAUTOTROPHICUM MEDIUM

NaCl	6.00	g
K_2HPO_4	1.00	g
Na_2SO_4	2.80	g
$NaHCO_3$	8.00	g
Na_2CO_3	22.00	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.05	g
Na-formate	3.40	g
Na-pyruvate	0.55	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.24	g
Distilled water	1000.00	ml

Dissolve sodium chloride, hydrogenphosphate and sodium sulfate, then sparge solution with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonates, dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ammonium chloride, magnesium chloride, trace elements, yeast extract, formate, pyruvate, thiosulfate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of vitamins and thiosulfate should be sterilized by filtration. Adjust pH of complete medium to 9.5 - 10.

For DSM 22071: Replace formate and pyruvate with 1.60 g/l Na-acetate as substrate and supplement medium with 3.00 g/l sulfur. For sterilization steam sulfur for 3 hours on each of 3 successive days. Add sulfur aseptically to the autoclaved medium while retaining anoxic conditions.

For DSM 22341: Do not add thiosulfate and sulfide. Replace formate and pyruvate with 2.00 g/l D-glucose as substrate, added after autoclaving from an anoxic stock solution sterilized by filtration

For DSM 22410: Prepare medium without formate and adjust pH of final medium to 9.5.

For DSM 22429: Replace formate and pyruvate with 2.00 g/l D-glucose as substrate, added after autoclaving from anoxic stock solutions sterilized by filtration. Adjust pH of final medium to 9.0 - 9.5

For DSM 24176, DSM 24629: Replace formate and pyruvate with 1.00 g/l D-galacturonic acid sodium salt as substrate, added after autoclaving from an anoxic stock solution sterilized by filtration.

For DSM 24179: Replace formate and pyruvate with 2.00 g/l D-xylose added after

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autoclaving from an anoxic stock solution sterilized by filtration.

For DSM 24257, DSM 29847: Replace formate and pyruvate with 1.10 g/l Na-butyrate added after autoclaving from an anoxic stock solution sterilized by filtration.

For DSM 24258: Replace formate and pyruvate with 1.92 g/l Na-propionate added after autoclaving from an anoxic stock solution sterilized by filtration.

For DSM 24400: Replace pyruvate with 0.20 g/l Na-acetate.

For DSM 29990: Replace formate and pyruvate with 20.00 ml/l of a 1 M Na-crotonate solution sterilized by filtration and added after autoclaving (see medium 870).

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1167. NSY-MEDIUM FOR POLYNUCLEOBACTER

Nutrient broth	1.0	g
Peptone from soybean (e.g. Bacto Soytone)	1.0	g
Yeast extract	1.0	g
Tap water	1000.0	ml

The following inorganic solution may substitute for tap water:

$MgSO_4$ x 7 H_2O	75.0	mg
$Ca(NO_3)_2$ x 4 H_2O	43.0	mg
$NaHCO_3$	16.0	mg
KCl	5.0	mg
K_2HPO_4 x 3 H_2O	3.7	mg
Trace element solution SL-4 (see DSM-medium 14)	0.1	ml
Distilled water	1000.0	ml

Adjust to pH 7.2.

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1168. YPS MEDIUM

Yeast extract	4.0	g
Peptone	2.0	g
Sea Salts (Sigma)	25.0	g
Distilled water	1000.0	ml

Agar may be added (15 g/l) to solidify the medium.

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1170. METHYLONATRUM MEDIUM

K_2HPO_4	1.00	g
KNO_3	1.00	g
Na_2CO_3	11.00	g
Methanol	2.00	ml
NaCl	18.00	g
NH_4Cl	0.16	g
$MgSO_4$ x 7 H_2O	0.24	g
NaS_2O_3 x 5 H_2O	24.00	mg
Trace elements (SL6, medium 27)	1.00	ml
Vitamin B_{12}	100.00	mg
Distilled water	1000.00	ml

pH 10.0

Prepare the medium without methanol, vitamin B_{12}, $MgSO_4$ x 7 H_2O and NaS_2O_3 x 5 H_2O in closed vessels with a medium to headspace ratio of 1:5 to 1:10. Add the methanol, $MgSO_4$ x 7 H_2O and NaS_2O_3 x 5 H_2O from autoclaved stock solutions after sterilisation of the medium. Vitamin B_{12} is added from a filter sterilised stock solution to the sterile medium. Incubate the strains with shaking at 150 rpm. Growth is faster in the presence of 20 mM sodium acetate instead of methanol.

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1171. METHYLOHALOMONAS MEDIUM

K_2HPO_4	2.00	g
NH_4Cl	0.50	g
NaCl	120.00	g
$MgSO_4$ x 7 H_2O	0.50	g
NaS_2O_3 x 5 H_2O	0.024	g
Trace elements (SL6, medium 27)	1.00	ml
$NaHCO_3$	1.70	g
Vitamin B_{12}	100.00	µg
Methanol	2.00	ml
Distilled water	1000.00	ml

pH 7.5

Prepare the medium without methanol, vitamin B_{12} $MgSO_4$ x 7 H_2O, $NaHCO_3$ and NaS_2O_3 x 5 H_2O in closed vessels with a medium to headspace ratio of 1:5 to 1:10. Add the methanol, $MgSO_4$ x 7 H_2O, $NaHCO_3$ and NaS_2O_3 x 5 H_2O from autoclaved stock solutions after sterilisation of the medium. Vitamin B_{12} is added from a filter sterilised stock solution to the sterile medium. Incubate the strains with shaking at 150 rpm. Growth is faster in the presence of 20mM sodium acetate instead of methanol.

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1172. YPG MEDIUM

Yeast extract	1.0	g
Peptone	1.0	g
Glucose	1.0	g
Distilled water	1000.0	ml

pH 5.6 - 6.0

Agar may be added (15 g/l) to solidify the medium.

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1173. MODIFIED MEDIUM 514

To medium 514 increase the Bacto peptone concentration to 10 g/l, the Bacto yeast extract concentration to 5 g/l, and add a further 20 g/l NaCl.

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1174. NITRINCOLA MEDIUM

K_2HPO_4	0.25	g
NH_4Cl	0.50	g
Yeast extract	0.10	g
Na acetate	10.00	g
NaCl	17.50	g
$Na_2B_4O_7$	4.00	g
Distilled water	1000.00	ml

Adjust the pH to 9.0 with 1M NaOH before autoclaving.

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1175. ALKALIFLEXUS MEDIUM

NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.05	g
KH_2PO_4	0.20	g
Na_2CO_3	7.40	g
$NaHCO_3$	18.50	g
Na_2S x 9 H_2O	0.50	g
Yeast extract	0.20	g
Cellulobiose	3.00	g
Distilled water	1000.00	ml

pH 10.0

Prepare the medium without Na_2CO_3, $NaHCO_3$, Na_2S x 9 H_2O, cellulobiose or yeast extract. Boil the medium in a water bath and cool under a stream of nitrogen. Add the Na_2CO_3 and $NaHCO_3$ to the cooled medium (the pH should be about 10.0) and dispense the medium into Hungate tubes or serum bottles under a stream of nitrogen. Seal the tubes under nitrogen gas and autoclave. To the cooled medium at the appropriate amounts of Na_2S x 9 H_2O, cellulobiose and yeast extract from sterile, anaerobic stock solutions (10% Na_2S x 9 H_2O, 5% cellulobiose, 10% yeast extract).

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1176. HALOVIBRIO MEDIUM

NaCl	120.00	g
K_2HPO_4	3.00	g
NH_4Cl	0.50	g
Trace elements (see medium 1369)	1.00	ml
HEPES	2.38	g
Distilled water	1000.0	ml

Adjust pH to 7.5. Dispense the medium into sealed bottles and autoclave.

After sterilization add from sterile stock solutions add:

$MgSO_4$ x 7 H_2O (1M)	2.0	ml/l
Sodium acetate (2M)	10.0	ml/l
Yeast extract (10%)	2.0	ml/l

Trace elements solution (Pfennig & Lippert,1966)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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1177. RUBITELEA MEDIUM

Starch	10.0	g
Yeast extract	4.0	g
Peptone	2.0	g
Sea water	1000.0	ml

Agar may be added (15 g/l) to solidify the medium.

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1178. AQUINCOLA MEDIUM

Yeast extract	1.0	g
Peptone	1.0	g
Fructose	0.5	g
Distilled water	1000.0	ml

pH 7.0

Agar may be added (15 g/l) to solidify the medium.

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1180. METHYLOMICROBIUM MEDIUM

NaCl	30.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.02	g
KNO_3	1.00	g
Trace elements (see below)	1.00	ml
Distilled water	1000.00	ml

Dispense the medium into the culture vessels. For small volumes serum bottles or Hungate tubes may be used. The gas phase to liquid ratio should be about 10:1. Use a gas phase containing air, 50% methane and autoclave at 121°C for 15 minutes. When the growth medium is cool the $NaHCO_3$, the Na_2CO_3 and phosphate buffer are added. Liquid cultures should be grown with shaking.

To the sterile medium add from sterile stock solutions:

$NaHCO_3$ (1M)	50.0	ml/l
Na_2CO_3 (1M)	5.0	ml/l

Phosphate buffer:	20.0	ml/l
KH_2PO_4	14.0	g
Na_2HPO_4 x 12 H_2O	30.0	g
Distilled water	1000.0	ml

Final pH of the medium: 8.5-9.0

Trace elements:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
Distilled water	1000.00	ml

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1181. METHYLOCELLA SILVERSTRIS MEDIUM

$MgSO_4$ x 7 H_2O	0.200	g
$CaCl_2$ x 6 H_2O	0.006	g
Fe(III)NH_4-EDTA	4.000	mg
$NaNO_3$	0.200	g
Trace element solution (see below)	0.500	ml
Phosphate buffer (see below)	5.000	ml
KH_2PO_4	0.272	g
Na_2HPO_4 x 12 H_2O	0.717	g
Distilled water	1000.000	ml

Dispense the medium into the culture vessels. For small volumes serum bottles or Hungate tubes may be used. The gas phase to liquid ratio should be about 1:10. Use a gas phase containing air, 20-30% methane and autoclave at 121°C for 15 minutes. When the growth medium is cool the phosphate buffer is added.
Liquid cultures should be grown with shaking.

Autoclave at 121°C for 15 min.

Add to the sterile medium from a filter sterilised phosphate buffer:

NaH_2PO_4 x 2 H_2O	28.71	g
Na_2HPO_4 x 12 H_2O	5.73	g
Distilled water	1000.00	ml

pH 5.8

Final pH of the medium is 5.8.

Trace element solution:

$ZnSO_4$ x 7 H_2O	10.000	mg
$MnCl_2$ x 4 H_2O	3.000	mg
H_3BO_3	30.000	mg
$CuCl_2$ x 6 H_2O	1.000	mg
$CaCl_2$ x 2 H_2O	20.000	mg
Na_2MoO_4 x 2 H_2O	3.000	mg
Distilled water	1000.000	ml

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1182. AQUISALIMONAS MEDIUM

Solution A

Glucose	10.0	g
Peptone	5.0	g
Yeast extract	5.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Distilled water	500.0	ml

Solution B

Na_2CO_3	20.0	g
NaCl	80.0	g
Distilled water	500.0	ml

Autoclave solutions A and B separately and mix when cool. The medium may be solidified by adding 20 g/l agar to solution A. Solutions A and B are then cooled to 50°C, mixed and plates poured immediately.

Final pH of the medium is 9.5.

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1183. ROSEICYCLUS MEDIUM

KCl	0.3	g
NH_4Cl	0.3	g
$MgSO_4$	2.0	g
Na_2SO_4	15.0	g
Malic acid	1.0	g
Na acetate	1.0	g
Yeast extract	1.0	g
Bactopeptone	0.5	g
Trace elements (see below)	2.0	ml
Vitamins (see below)	2.0	ml
Distilled water	1000.0	ml

pH is about 5.9 before autoclaving.

Add to the autoclaved medium the following, from sterile stock solutions

K_2HPO_4 (10%)	3.0	ml
$NaHCO_3$ (10%)	5.0	ml
$CaCl_2$ (10%)	0.5	ml

The pH of the final medium should be 8.0-8.5.

Vitamin solution

Nictotinic acid	400.0	mg
Thiamine	400.0	mg
Biotin	80.0	mg
Vitamin B_{12}	0.5	mg
Distilled water	1000.0	ml

Filter sterilise

Trace elements solution

$FeSO_4$ x 7 H_2O	300.0	mg
$ZnSO_4$ x 7 H_2O	5.0	mg
$MnCl_2$ x 4 H_2O	3.0	mg
H_3BO_3	2.0	mg
$CoCl_2$ x 6 H_2O	1.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	3.0	mg
Na_2MoO_4 x 2 H_2O	3.0	mg
Distilled water	1000.00	ml

Adjust the pH to 3.0 with HCl and filter sterilise, store at 4°C.

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1184. MEDIUM FOR HALOPHILIC ARCHAEA

Yeast extract	5.00	g
KCl	5.00	g
$MgCl_2$ x 6 H_2O	32.50	g
$MgSO_4$ x 7 H_2O	50.80	g
$CaCl_2$ x 2 H_2O	0.80	g
$NaHCO_3$	0.16	g
NaBr	0.60	g
NaCl	195.00	g
Distilled water	1000.00	ml

The medium may be solidified by adding 20.0 g/l agar.

Final pH 6.5-7.0.

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1185. SMB MEDIUM

NaCl	50.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.01	g
NH_4NO_3	1.00	g
KH_2PO_4	2.00	g
Na_2HPO_4	3.00	g
Na_2CO_3	0.10	g
$MnSO_4$ x 2 H_2O (1% solution)	2.00	ml
$FeSO_4$ x 7 H_2O (1% solution)	1.00	ml
Tryptone	5.00	g
Yeast extract	2.00	g
Distilled water	1000.00	ml

pH 6.8 – 7.1

The medium may be solidified by adding 15.0 g/l agar.

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1186. GRANULIBACTER MEDIUM

Glucose	50.0	g
$CaCO_3$	12.5	g
Yeast extract	5.0	g
Distilled water	1000.0	ml

The medium may be solidified by adding 15.0 g/l agar.

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1188: SPIROCHAETA CELLOBIOSIPHILA MEDIUM

Sea Salt (SIGMA)	35.00	g
Wolfe's mineral elixir	1.00	ml
Yeast extract	1.00	g
Trypticase peptone (BD BBL)	0.50	g
Cellobiose	2.00	g
Distilled water	1000.00	ml

Dissolve compounds except cellobiose, adjust pH to 7.5 and dispense medium under air atmosphere into Hungate-type tubes or serum vials to 30% volume and autoclave. Add cellobiose after autoclaving from a stock solution sterilized by filtration.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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1189. AEROBIC SULFOLOBALES MEDIUM WITHOUT SULFUR

Solution A:

Salt base solution (see below)	1000.0	ml
Yeast extract (Difco)	0.5	g

Autoclave salt base solution and yeast extract (as a 10% solution) separately, and combine after cooling.

Salt base solution:

$(NH_4)_2\ SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$MnCl_2$ x 4 H_2O	1.80	mg
$Na_2B_4O_7$ x 10 H_2O	4.50	mg
$ZnSO_4$ x 7 H_2O	0.22	g
$CuCl_2$ x 2 H_2O	0.05	mg
Na_2MoO_4 x 2 H_2O	0.03	mg
$VOSO_4$ x n H_2O	0.03	mg
$CoSO_4$ x 7 H_2O	0.01	mg
Distilled water	1000.00	ml

Adjust to pH 2.5 with 10 N H_2SO_4.

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1190: FERROUS SULFATE/YEAST EXTRACT MEDIUM

Yeast extract	0.20	g
50x Heterotrophic basal salts solution	20.00	ml
Ferrous sulfate solution (27.8%)	20.00	ml
Distilled water	960.00	ml

Dissolve yeast extract in distilled water and add basal salts solution. Adjust to desired pH with H_2SO_4. Dispense in suitable cultivation vessels and autoclave at 121°C for 20 min. When cool, add filter sterilized ferrous sulfate solution.

For DSM 19468: Adjust to pH 2.0.

For DSM 19497: Adjust pH of basal medium to 2.0.

For DSM 19514: Adjust pH of complete medium to 1.8 - 2.0.

For DSM 28176: Reduce amount of ferrous sulfate solution to 5.5 ml, supplement medium after sterilization with 1.0 g/l D-glucose and adjust pH to 2.5.

50x Heterotrophic basal salts solution

Na_2SO_4 x 10 H_2O	7.50	g
$(NH_4)_2SO_4$	22.50	g
KCl	2.50	g
$MgSO_4$ x 7 H_2O	25.00	g
KH_2PO_4	2.50	g
$Ca(NO_3)_2$ x 4 H_2O	0.70	g
Distilled water	1000.00	ml

Ferrous sulfate solution (27.8%)

$FeSO_4$ x 7 H_2O	278.00	g
Distilled water	1000.00	ml

Adjust pH to 1.8 with H_2SO_4; sterilize by filtration.

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1191. EGGC

Na-acetate	0.3	g
Casaminoacids	0.3	g
Glucose	0.6	g
Salt solution	10.0	ml
Agar	15.0	g
H_2O demin. ad	1000.0	ml

Please add after autoclaving:

Vitamin solution	5	ml
Phosphate solution	2	ml

Salt solution:

$(NH_4)_2SO_4$	1.000	g
KCl	0.500	g
$MgSO_4$ x 7 H_2O	0.500	g
$CaCl_2$ x 2 H_2O	0.200	g
$CaCO_3$	0.200	g
Fe_3Cl x 6 H_2O	0.005	g
H_2O demin. ad	1000.000	ml

Phosphate solution:

Na_2HPO_4	2.480	g
NaH_2PO_4	0.308	g
H_2O demin. ad	20.000	ml

Adjust pH to 7.2

Vitamin solution:

Riboflavin	10.000	mg
Thiamine-HCl x 2 H_2O	50.000	mg
Nicotinic acid	50.000	mg
Pyridoxine-HCl	50.000	mg
Ca-pantothenate	50.000	mg
Biotin	0.100	mg
Folic acid	0.200	mg
Vitamin B_{12}	1.000	mg
H_2O demin. ad	100.000	ml

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1192. BM MEDIUM

Difco marine broth (Difco 2216)	37.4	g
Distilled water	1000.0	ml
Biotin	20.0	µg
Vitamin B_{12}	1.0	µg
Methanol	4.0	ml
(Agar, if necessary	15.0	g)

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1193. CULTIVATION MEDIUM FOR CHLAMYDIAE

Cultivation of L929 (ACC 2) or HeLa (ACC 57) cells

IMDM-Medium	45.0	ml
Fetal Bovine Serum	5.0	ml
Aminoacids (100 x)	0.5	ml

Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

Prepare a 25 cm^2 flask and seed cells according to standard protocols (see DSMZ catalogue for ACC 2 and ACC 57). Incubate at 37°C plus 5% CO_2. When a confluent layer has formed, infection can be carried out.

Exchange medium with 6 ml of Infection Medium (as above with the addition of 1 µg/ml cycloheximide (final concentration)) and add 500 − 1000 µl of EB stock solution (thawed quickly to 37°C).

Centrifuge for 1 h onto the cell layer at 1600 rpm at 20°C.

Incubate at 37°C + 5% CO_2. Control cells daily and look for inclusions. Not all Chlamydiae form well-visible inclusions, ultimately, immunofluorescence or in situ-hybridization techniques are necessary to visualize inclusions.

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1194. HALORUBRUM CALIFORNIENSE MEDIUM

NaCl	156.00	g
$MgCl_2$ x 6 H_2O	26.00	g
MgSO4 x 7 H_2O	40.60	g
KCl	4.00	g
NaBr	0.46	g
$NaHCO_3$	0.12	g
$CaCl_2$ x 2 H_2O	0.66	g
Yeast extract	10.00	g
Agar (for solid media)	20.00	g
Distilled water	1000.00	ml

Adjust to pH 7.2 and autoclave.

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1195. KUSHNERIA AURANTIA MEDIUM

NaCl	78.00	g
$MgCl_2$ x 6 H_2O	13.00	g
$MgSO_4$ x 7 H_2O	20.30	g
KCl	2.00	g
NaBr	0.23	g
$NaHCO_3$	0.06	g
$CaCl_2$ x 2 H_2O	0.33	g
Yeast extract	5.00	g
Agar (for solid media)	20.00	g
Distilled water	1000.00	ml

Adjust to pH 7.2 – 7.4 and autoclave.

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1196. ZAVARZINELLA FORMOSA MEDIUM

KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 6 H_2O	0.05	g
NaCl	0.01	g
N-acetylglucosamine	1.00	g
Glucose	0.50	g
Peptone	0.10	g
Yeast extract	0.10	g
Casamino acids	0.10	g
"Metals 44" (see medium 590)	1.00	ml
Agar (for solid media)	15.00	g
Distilled water	1000.00	ml

Final pH 5.8 – 6.0

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1198. Vero B4 Medium

Cultivation of Vero B4 (ACC 33) cells

IMDM-Medium	45.0	ml
Fetal Bovine Serum	5.0	ml
Aminoacids (100 x)	0.5	ml

Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

Prepare a 25 cm^2 flask and seed cells according to standard protocols (see DSMZ catalogue for ACC 33). Incubate at 37°C plus 5% CO_2. When a confluent layer has formed, infection can be carried out.

Infect cells with 1 ml of *Rickettsia* stock solution (thawed quickly to 37°C).

Centrifuge for 1 h onto the cell layer at 1600 rpm at 20°C.

Incubate at 28°C. Up to 90% of Vero cells should be infected after 15 days. Specific PCRs can be performed to verify the presence of rickettsiae. Ultimately, in situ-hybridization can be used to visualize them.

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1199. K7 MEDIUM

Glucose	1.0	g
Yeast extract	1.0	g
Peptone	1.0	g
Distilled water	1000.0	ml

For DSM 19966, adjust to pH 5.5 and add 20 g l^{-1} agar-agar if required. For DSM 25088, adjust pH to 6.5. In that case, 14 g l^{-1} agar is sufficient.

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1200. ANAEROMYXOBACTER-MEDIUM

Solution A:

NaCl	1.0	g
$MgCl_2$ x 6 H_2O	0.5	g
KH_2PO_4	0.2	g
NH_4Cl	0.3	g
KCl	0.3	g
$CaCl_2$ x 2 H_2O	15.0	mg
Na-acetate	0.4	g
Trace element solution SL 10B (see below)	1.0	ml
Selenite-tungstate solution (see below)	2.0	ml
Resazurin solution (1 g per L)	1.0	ml
Distilled water	900.0	ml

Solution B:

$NaHCO_3$	2.5	g
Distilled water	50.0	ml

Solution C:

D,L-Dithiothreitol	385.0	mg
Distilled water	50.0	ml

Solution D ("red mix"):

L-Cysteine	37.5	mg
Na_2S x H_2O (dissolved separately in water)	40.0	mg
Distilled water	50.0	ml

Solution E (electron acceptor):

Disodium fumarate	4.0	g
Distilled water	50.0	ml

Solution F: Vitamin solution according to Wolin and coauthors

Biotin	2.0	mg
Folic acid	2.0	mg
Pyridoxine-HCl	10.0	mg
Thiamine-HCl x 2 H_2O	5.0	mg
Riboflavin	5.0	mg
Nicotinic acid	5.0	mg
D-Ca-pantothenate	5.0	mg
Vitamin B_{12}	0.1	mg
p-Aminobenzoic acid	5.0	mg
Lipoic acid	5.0	mg
Distilled water	1000.0	ml

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Prepare solutions A, B, and D separately and anaerobically under 80% N_2 + 20% CO_2 and autoclave. Filter-sterilize solutions C, E and F and outgas with N_2. After cooling, add about 1 ml solution B per 10 ml solution A, by this way adjusting pH to 7.2. Add 0.2 ml solution C, 0.2 ml solution D, 0.2 ml solution F and 0.05 ml solution E, each per 10 ml solution A.

Trace element solution SL-10 B:

Distilled water	1000.0	ml
HCl (25%)	7.7	ml
$FeSO_4$ x 7 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	300.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg

Selenite and tungstate solution

NaOH	0.5	g
Na_2SeO_3 x 5 H_2O	3.0	mg
Na_2WO_4 x 2 H_2O	4.0	mg
Distilled water	1000.0	ml

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1201. ALICYCLOBACILLUS FERROOXYDANS MEDIUM

Solution A:

$MgSO_4$ x 7 H_20	0.50	g
$(NH4)_2SO_4$	0.40	g
K_2HPO_4	0.20	g
$K_2S_4O_6$	0.15	g
KCl	0.10	g
$MnSO_4$ x H_2O	0.01	g
Yeast extract	0.20	g
Distilled water	930.00	ml

Adjust pH to 1.8 – 2.5 with H_2SO_4.

Solution B:

$FeSO_4$ x 7 H_2O	20.00	g

Dissolve in 0.2 N H_2SO_4 and fill up to a volume of 100 ml. Filter sterilize.

Autoclave solution A, cool down to 50°C and combine solution A with 70 ml of solution B.

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1202: SOLIDESULFOVIBRIO MARRAKECHENSIS MEDIUM

K_2HPO_4	0.50	g
KH_2PO_4	0.50	g
NH_4Cl	0.50	g
NaCl	0.40	g
KCl	0.10	g
Na_2SO_4	2.80	g
$CaCl_2$ x 2 H_2O	0.05	g
$MgCl_2$ x 6 H_2O	0.30	g
Yeast extract	0.10	g
Na-lactate	1.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Sterilize vitamins by filtration. Adjust pH of the complete medium to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1203. FASTIDIOUS ANAEROBE AGAR

Fastidious Anaerobe Agar (acumedia)	45.7	g
add deionized water to	1000.0	ml

Allow to soak for 10 minutes, swirl to mix then sterilize by autoclaving at 121°C for 15 minutes. Cool to 47°C then aseptically add 5-10% of sterile defibrinated horse blood, mix well and pour plates. pH: 7.2 ± 0.2

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1204: SPHAEROCHAETA MEDIUM

NaCl	1.00	g
KCl	0.50	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
Na_2SO_4	0.15	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
Yeast extract	4.00	g
Casein peptone	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Maltose monohydrate	2.00	g
DL-Dithiothreitol (DTT)	0.80	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, maltose and DTT, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 20 minutes. Add maltose and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Check pH of complete medium and adjust to 7.4, if necessary.

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1205: 1/2 TRYPTIC SOY BROTH (ANAEROBIC)

Trypticase soy broth (BD BACTO)	13.75	g
Yeast extract	1.00	g
NaCl	2.50	g
D-Glucose	3.60	g
DL-Dithiothreitol (DTT)	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients except glucose and DTT, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Adjust pH to 7.2 with HCl, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 20 min. Add glucose and DTT from anoxic stock solutions prepared under 100% N_2 gas atmosphere and sterilized by filtration.

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1206: CALDANAEROVIRGA MEDIUM

KH_2PO_4	0.27	g
NH_4Cl	0.50	g
$(NH_4)_2SO_4$	0.50	g
TAPS sodium salt (SIGMA)	2.40	g
$MgCl_2$ x 6 H_2O	0.01	g
$CaCl_2$ x 2 H_2O	0.02	g
Yeast extract	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.50	g
D-Xylose	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, xylose, vitamins, cysteine and sulfide), adjust pH to 8.5, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add xylose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Sterilize stock solutions of xylose and vitamins by filtration. Adjust pH of the complete medium to 8.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1207. TGE

Tryptone	10.0	g
Beef extract	6.0	g
Glucose	2.0	g
(Agar	15.0	g)

Adjust pH to 7.0

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1208. BACILLUS ALKALIDIAZOTROPHICUS MEDIUM

$NaHCO_3$	8.00	g
Na_2CO_3	22.00	g
NaCl	6.00	g
K_2HPO_4	1.00	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Yeast extract	0.05	g
Na_2SO_4	2.80	g
Trace element solution SL-11 (see medium 722)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Vitamin solution (see medium 141)	10.00	ml
Glucose	2.00	g
Distilled water	1000.00	ml

Dissolve ingredients except ammonium chloride, magnesium chloride, yeast extract, trace elements, vitamins, boil medium for 1 min, then cool to room temperature under N_2 gas atmosphere. Dispense under same gas atmosphere in culture vessels and autoclave. After sterilization add ammonium chloride, magnesium chloride, yeast extract, trace elements, vitamins, from sterile, anoxic stock solution prepared under N_2. The vitamin solution should be sterilized by filtration. Adjust pH of final medium to 10.

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1209. ALICYCOBACILLUS POHLIAE MEDIUM

Tryptone	5.00	g
D-Glucose	0.50	g
$(NH_4)_2SO_4$	0.10	g
$FeSO_4$ x 7 H_2O	0.07	g
(Agar	15.00	g)
Distilled water	1000.00	ml

Adjust pH to 5.5.

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1210: GEOGLOBUS MEDIUM

NaCl	18.00	g
$MgCl_2$ x 6 H_2O	4.00	g
KCl	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$(NH_4)_2SO_4$	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Amorphous $Fe(OH)_3$	200.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Na-acetate	1.50	g
KH_2PO_4	0.33	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	800.00	ml

Dissolve ingredients except carbonate, acetate, hydrogenphosphate, yeast extract, vitamins and ferric iron hydroxide sludge. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Thereafter, dispense suspension under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add acetate, hydrogenphosphate, yeast extract and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 6.5 - 6.8.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Amorphous $Fe(OH)_3$ (from medium 1210)

$FeCl_3$ x 6 H_2O (60.00 g/l)	320.00	ml
NaOH (10% w/v), adjust if required	80.00	ml

Slowly titrate 320 ml of a $FeCl_3$ x 6 H_2O stock solution (60.00 g/l) with 10% (w/v) NaOH to pH 8.0-8.5 under agitation (use magnetic stirrer). Total amount of added NaOH approx. 80 - 100 ml. The precipitated $Fe(OH)_3$ should be stored at room temperature overnight with surface covered with water. Thereafter, centrifuge at 2000 rpm for 5 min and discard the supernatant. Wash several times with distilled water. Resuspend the pellet in medium as described above. For storage autoclave under 100% N_2 atmosphere.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1211: CALDISERICUM MEDIUM

K_2HPO_4	0.05	g
KH_2PO_4	0.09	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	5.00	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.25	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Wolin's vitamin solution	2.00	ml
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, thiosulfate, vitamins and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate, vitamin solution, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 6.5 - 6.8.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve

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mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1212. MARINOSPIRILLUM CELERE

NH_4Cl	0.5	g
KH_2PO_4	0.5	g
NaCl	25.0	g
Na_2SO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.2	g
KCl	0.5	g
Yeast extract	1.0	g
Peptone	1.0	g
Sodium DL-lactate	1.0	g
Vitamin B_{12}	15.0	µg
Na_2CO_3	5.0	g
$NaHCO_3$	5.0	g
Trace element solution (see below)	1.0	ml
Distilled water	1000.0	ml

Autoclave the Na_2CO_3, $NaHCO_3$ and the lactate separately from the medium and combine after cooling. Vitamin B12 is added from a sterile stock solution. The final pH of the medium should be 9.5. Add 15.0 g/l agar for solid media.

Trace element solution SL-10:

Distilled water	1000.0	ml
HCl (25%)	7.7	ml
$FeSO_4$ x 7 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	300.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg

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1213. THALASSOBACILLUS CYRI MEDIUM

NaCl	78.00	g
KCl	2.00	g
NaBr	0.23	g
$MgCl_2$ x 76 H_2O	13.00	g
$MgSO_4$ x 7 H_2O	20.30	g
$NaHCO_3$	0.06	g
$CaCl_2$ x 2 H_2O	0.33	g
Yeast extract	5.00	g
Distilled water	1000.00	ml

Final pH should be 7.4. For solid media add 15.0 g/l agar.

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1214. HALORUBRUM CHOAVIATOR MEDIUM

NaCl	200.0	g
KCl	2.0	g
$CaCl_2$ x 2 H_2O	0.2	g
$MgCl_2$ x 6 H_2O	20.0	g
Yeast extract	5.0	g
Casamino acids	5.0	g
Distilled water	1000.0	ml

Final pH should be 7.4.

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1215. BEIJERINCKIA DOEBEREINERAE MEDIUM

KH_2PO_4	0.80	g
K_2HPO_4	0.20	g
$MgSO_4$ x 7 H_2O	0.50	g
$FeCl_3$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.05	g
Na_2MoO_4 x 7 H_2O	5.00	mg
Glucose	20.00	g
Distilled water	1000.00	ml

Final pH is 6.5. Add 15.0 g/l agar for solid media.

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1216. SALINISPHAERA HYDROTHERMALIS MEDIUM

NaCl	24.0	g
KCl	0.7	g
$MgCl_2$ x 6 H_20	7.0	g
Bacto peptone	2.5	g
Yeast extract	3.0	g
Distilled water	1000.0	ml

Add the vitamin B_{12} solution from a filter sterilised stock solution to the autoclaved, cooled medium. Add 15.0 g/l agar for solid media.

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1217. ROSEOCOCCUS SUDUNTUYENSIS MEDIUM

NH_4Cl	0.4	g
KH_2PO_4	0.5	g
$MgCl_2$ x 6 H_2O	0.2	g
Na_2SO_4	0.5	g
NaCl	2.0	g
KCl	0.5	g
$NaHCO_3$	5.0	g
Na_2CO_3	0.5	g
$Na_2S_2O_3$ x 5 H_2O	1.0	g
Vitamin B_{12}	10.0	µg
Sodium pyruvate	1.0	g
Sodium acetate	1.0	g
Peptone	1.0	g
Yeast extract	1.0	g
Trace element solution (SL10)	1.0	ml
Distilled water	1000.0	ml

Trace element solution SL10

HCl (concentrated)	5.000	ml
$FeCl_2$ x 4 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.200	g
H_3BO_3	0.006	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled water	1000.000	ml

Autoclave the $NaHCO_3$ and Na_2CO_3 separately and add to the medium. Add the vitamin B_{12} solution from a filter sterilised stock solution to the autoclaved, cooled medium. Final is pH 8.5.

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1218. ROSEINATRONOBACTER MONICUS MEDIUM

NH_4Cl	0.4	g
KH_2PO_4	0.5	g
$MgCl_2$ x 6 H_2O	0.2	g
Na_2SO_4	0.5	g
$NaNO_3$	0.4	g
NaCl	40.0	g
KCl	0.5	g
$NaHCO_3$	10.0	g
Na_2CO_3	5.0	g
$Na_2S_2O_3$ x 5 H_2O	1.0	g
Vitamin B_{12}	10.0	µg
Sodium pyruvate	1.0	g
Sodium acetate	1.0	g
Peptone	1.0	g
Yeast extract	1.0	g
Trace element solution (SL10)	1.0	ml
Distilled water	1000.0	ml

Trace element solution SL10

HCl (concentrated)	5.000	ml
$FeCl_2$ x 4 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.200	g
H_3BO_3	0.006	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled water	1000.000	ml

Autoclave the $NaHCO_3$ and Na_2CO_3 separately and add to the medium. Add the vitamin B_{12} solution from a filter sterilised stock solution to the autoclaved, cooled medium. Final is pH 9.0 - 9.5.

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1219. METHYLOVIRGULA LIGNI MEDIUM

KH_2PO_4	0.10	g
$(NH_4)_2SO_4$	0.10	g
$CaCl_2$ x 2 H_2O	0.01	g
$MgSO_4$ x 7 H_2O	0.05	g
Trace elements (see medium 922)	1.00	ml
Yeast extract	0.10	g
Methanol	1.00	ml
Distilled water	1000.00	ml

Adjust to pH 5.0 – 5.8. Prepare the medium in flasks or tubes with screw caps. The methanol may either be filter sterilised or autoclaved in half full serum bottles sealed with a rubber stopper and a crimp closure. Add to the cooled autoclaved medium. Agar may be added at 15 g/l for solid media.

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1220. ACIDOSOMA MEDIUM

KH_2PO_4	0.10	g
$(NH_4)_2SO_4$	0.25	g
$CaCl_2$ x 2 H_2O	0.05	g
$MgSO_4$ x 7 H_2O	0.02	g
Trace elements (see medium 922)	1.00	ml
Yeast extract	0.10	g
Na gluconate	0.50	g
Distilled water	1000.00	ml

Adjust to pH 5.0 – 5.5. Agar may be added at 15 g/l for solid media.

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1221. SALISAETA LONGA MEDIUM

NaCl	100.0	g
K_2SO_4	5.0	g
$CaCl_2$ x 2 H_2O	0.1	g
$MgCl_2$ x 6 H_2O	50.0	g
Yeast extract	1.0	g
Casamino acids	1.0	g
Starch	2.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0. Agar may be added at 15 g/l for solid media. Plates should be sealed during incubation to prevent drying out.

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1223. TINDALLIA TEXCOCOENSIS MEDIUM

NaCl	75.00	g
Na_2CO_3	3.00	g
$NaHCO_3$	20.00	g
KCl	0.10	g
K_2HPO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.20	g
NH_4Cl	1.00	g
Na_2S x 9 H_2O	0.40	g
Yeast extract	0.50	g
Peptone	5.00	g
Cysteine-HCl	0.50	g
Trace element solution (see medium 141)	10.00	ml
Resazurin	10.00	mg
Distilled water	1000.00	ml

Adjust to pH 9.0

Prepare the medium omitting Na_2CO_3, $NaHCO_3$, Na_2S, Cysteine-HCl, $CaCl_2$ x 2 H_2O and $MgCl_2$ x 6 H_2O. Boil the medium and cool under nitrogen, add the Na_2CO_3 and $NaHCO_3$ and adjust the pH to 9.0 with 10 M KOH. Dispense into tubes or bottle fitted with rubber stoppers. Sterilize by autoclaving and add the Na_2S (neutralized), Cysteine-HCl, $CaCl_2$ x 2 H_2O and $MgCl_2$ x 6 H_2O from sterile stock solutions.

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1224. ECTOTHIORHODOSPIRA VARIABILIS MEDIUM

NaCl	50.00	g
Na_2CO_3	5.00	g
$NaHCO_3$	15.00	g
KCl	0.10	g
K_2HPO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.20	g
NH_4Cl	0.50	g
Na_2S x 9 H_2O	0.50	g
$Na_2S_2O_3$ x 5 H_2O	0.50	g
Yeast extract	0.10	g
Na acetate	0.50	g
Trace element solution (see medium 320)	1.00	ml
Vitamin B_{12}	20.00	µg
Distilled water	1000.00	ml

Final pH 9.0 – 9.5

Prepare and boil the medium in the absence of Na_2S x 9 H_2O, $NaHCO_3$, Vitamin B_{12}, $CaCl_2$ x 2 H_2O, $MgCl_2$ x 6 H_2O. Cool under a stream of nitrogen and add the remaining components, except vitamin B_{12} and $Na_2S_2O_3$ x 5 H_2O. Dispense into tubes or bottle fitted with rubber stoppers and autoclave. Add the vitamin B_{12} (filter sterilised) and $Na_2S_2O_3$ x 5 H_2O from sterile stock solutions.

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1225. THIOALKALIBACTER HALOPHILUS MEDIUM

Mineral base:

Na_2CO_3	95.0	g
$NaHCO_3$	15.0	g
NaCl	16.0	g
K_2HPO_4	1.0	g
Distilled water	1000.0	ml

Sterilize at 120°C 20 min in a closed vessel (i.e. a serum tube or bottle) with an air to liquid ratio of 10:1. pH after sterilization will be about 10.

After sterilization add:

Trace elements solution (see medium 925)	1.0	ml/l
$MgCl_2$ x 6 H_2O (200.0 g/l)	1.0	ml/l
(a white colloid will form which will rapidly dissolve after mixing)		
Sodium thiosulfate pentahydrate (1.0 M - 248.0 g/l)	20.0	ml/l
NH_4Cl - (1 M - 53.5 g/l)	4.0	ml/l

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1227. RHODOBACA BARGUZINENSIS MEDIUM

KH_2PO_4	0.5	g
NaCl	20.0	g
$NaNO_3$	0.4	g
NH_4Cl	0.4	g
Na_2SO_4	0.5	g
$MgCL_2$ x 6 H_2O	0.2	g
Yeast extract	1.0	g
$Na_2S_2O_3$ x 5 H_2O	1.0	g
KCl	0.5	g
Na_2CO_3	10.0	g
$NaHCO_3$	5.0	g
Trace element solution SL7	1.0	ml
Vitamin B_{12} (20mg/l)	0.5	ml
Distilled water	1000.0	ml
Adjust pH to 9.0-9.5		

Trace element solution SL7:

HCl (25%, v/v)	1.0	ml
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	60.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$CuCl_2$ x 2 H_2O	20.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	40.0	mg
Distilled water	1000.0	ml

Prepare and boil the medium in the absence of $Na_2S_2O_3$ x 5 H_2O, $NaHCO_3$, Vitamin B_{12}, $MgCl_2$ x 6 H_2O. Cool under a stream of nitrogen and add the remaining components, except vitamin B_{12} and $Na_2S_2O_3$ x 5 H_2O. Dispense into tubes or bottle fitted with rubber stoppers and autoclave. Add the vitamin B_{12} (filter sterilized) and $Na_2S_2O_3$ x 5 H_2O from sterile stock solutions.

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1228. THIOALKALIVIBRIO THIOCYANODENITRIFICANS MEDIUM

Mineral base:

Na_2CO_3	22.0	g
$NaHCO_3$	8.0	g
NaCl	5.0	g
K_2HPO_4	1.0	g
Distilled water	1000.0	ml

Sterilize at 120°C 20 min in a closed vessel (i.e. a serum tube or bottle) with a liquid to air ratio of about 1:10. pH after sterilization will about 10.

Trace element solution:

EDTA	5.0	mg
$FeSO_4$ x 7 H_2O	2.0	mg
$ZnSO_4$ x 7 H_2O	100.0	mg
$MnCl_2$ x 4 H_2O	30.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
H_3BO_3	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add from sterile stock solutions:

Trace elements solution	2	ml
$MgCl_2$ x 6 H_2O (200.0 g/l)	1	ml
(a white colloid will form which will rapidly dissolve after mixing)		
Sodium thiosulfate pentahydrate (4 M – 992 g/l) -	10	ml
KSCN solution (2 M - 194 g/l)	5	ml

Incubate statically until growth occurs, then shake at 100 rpm.

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1229. THIOHALOSPIRA ALKALIPHILA MEDIUM

Mineral base:

NaCl	120.0	g
K_2HPO_4	2.0	g
$(NH_4)_2SO_4$	0.5	g
Distilled water	1000.0	ml

Flush the gas phase with nitrogen and add 5% oxygen. Sterilize at 120°C 20 min in a closed vessel (i.e. a serum tube or bottle) with a liquid to air ratio of about 1:10.

Trace element solution:

EDTA	5.0	mg
$FeSO_4$ x 7 H_2O	2.0	mg
$ZnSO_4$ x 7 H_2O	100.0	mg
$MnCl_2$ x 4 H_2O	30.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
H_3BO_3	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add from sterile stock solutions:

Trace elements solution	1	ml
$MgCl_2$ x 6 H_2O (200.0 g/l)	2	ml
(a white colloid will form which will rapidly dissolve after mixing)		
Sodium thiosulfate pentahydrate (4 M – 992 g/l) -	5	ml
Vitamin B12 (filter sterilized 100mg/l)	1	ml
$NaHCO_3$ (84.0 g/l)	100	ml

Final pH is 8.0 – 8.5.

Incubate statically until growth occurs, then shake at 100 rpm.

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1230. THIOALKALIMICROBIUM MICROAEOPHILUM MEDIUM

Mineral base:

NaCl	6.0	g
K_2HPO_4	2.0	g
$NaHCO_3$	40.0	g
KNO_3	0.5	g
Distilled water	1000.0	ml

Adjust the pH to 9.0 – 9.5 with 2M KOH. Dispense into closed vessels (i.e. a serum tube or bottle) with a liquid to air ratio of about 1:10. Flush the gas phase with nitrogen and add 2% oxygen. Sterilize at 120°C 20 min.

Trace element solution:

EDTA	5.0	mg
$FeSO_4$ x 7 H_2O	2.0	mg
$ZnSO_4$ x 7 H_2O	100.0	mg
$MnCl_2$ x 4 H_2O	30.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
H_3BO_3	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add from sterile stock solutions:

Trace elements solution	1	ml
$MgCl_2$ x 6 H_2O (200.0 g/l)	1	ml
(a white colloid will form which will rapidly dissolve after mixing)		
Sodium thiosulfate pentahydrate (4 M – 992 g/l) -	5	ml

Incubate statically until growth occurs, then shake at 100 rpm.

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1231. HALOPLASMA CONTRACTILE MEDIUM

NaCl	80.0	g
KH_2PO_4	0.5	g
Yeast extract	0.2	g
Peptone	0.2	g
Starch	5.0	g
Na_2S x 9 H_2O	0.5	g
Resazurin	0.5	µg
Artificial seawater	250.0	ml
Distilled water	750.0	ml

Artificial seawater:

NaCl	27.70	g
$MgSO_4$ x 7 H_2O	7.00	g
$MgCl_2$ x 6 H_2O	5.50	g
KCl	0.60	g
NaBr	0.10	g
H_3BO_3	0.03	g
$CaCl_2$ x 2 H_2O	1.50	g
$SrCl_2$ x 6 H_2O	15.00	mg
KI	0.05	mg
Double distilled water	1000.00	ml

Adjust pH to 5.5 − 6.0.

Prepare the medium without the Na_2S x 9 H_2O by boiling and cooling under a nitrogen atmosphere. Adjust the pH to 6.5 − 7.0. Dispense into Hungate tubes or serum bottles fitted with butyl rubber stoppers. Autoclave and reduce the medium before use by adding the appropriate amount of Na_2S x 9 H_2O from a neutralised, sterile stock solution.

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1233: CALDICOPROBACTER MEDIUM

NaCl	0.50	g
KCl	0.20	g
$MgCl_2$ x 6 H_2O	0.18	g
NH_4Cl	0.10	g
HEPES (SIGMA)	4.80	g
Na_2HPO_4 x 7 H_2O	54.00	mg
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	4.00	ml
$CaCl_2$ x 2 H_2O (0.1% w/v)	2.00	ml
$MnCl_2$ x 4 H_2O (0.1% w/v)	1.00	ml
Yeast extract (OXOID)	5.00	g
Tryptone (BD Bacto)	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	1.00	g
D-Glucose	5.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine and glucose), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add solid cysteine and adjust pH to 7.3 - 7.5, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation, add glucose from a sterile anoxic stock solution prepared under 100% N_2 gas.

2. Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) can be used to completely reduce the medium and may stimulate growth at the beginning.

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1234: ACIDITHIOBACILLUS FERRIVORANS MEDIUM

Na_2SO_4	1.40	g
$(NH_4)_2SO_4$	3.00	g
KCl	0.10	g
$MgSO_4$ x 7 H_2O	0.50	g
K_2HPO_4	0.05	g
$Ca(NO_3)_2$ x 4 H_2O (0.1% w/v)	20.00	ml
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O (0.1% w/v)	20.00	ml
Modified Wolin's mineral solution	10.00	ml
$K_2S_4O_6$	0.80	g
$FeSO_4$ x 7 H_2O	0.03	g
Distilled water	950.00	ml

Dissolve ingredients (except tetrathionate and ferrous sulfate), adjust pH to 2.5 with H_2SO_4 and autoclave. After cooling, add tetrathionate from a 100 mM stock solution and $FeSO_4$ from a 10 mM stock solution (prepared in water adjusted to pH 2.0 with H_2SO_4). Both solutions should be freshly prepared and sterilized by filtration.

For DSM 27026: Omit tetrathionate and ferrous sulfate. Add 0.90 g/l D-fructose as substrate after autoclaving from a sterile stock solution. Adjust pH of complete medium to 3.5.

For DSM 104037: Omit tetrathionate and ferrous sulfate. Add 0.30 g/l D-glucose as substrate after autoclaving from a sterile stock solution. Adjust pH of complete medium to 4.5.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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1235. XYLAN MEDIUM

Xylan (oat spelt)	10.00	g
Peptone	5.00	g
Yeast extract	1.00	g
K_2HPO_4	4.00	g
$MgSO_4$ x 7 H_2O	1.00	g
KCl	0.20	g
$FeSO_4$ x 7 H_2O	0.02	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust to pH 7.0

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1236: SPOROLITUUS MEDIUM

KH_2PO_4	0.75	g
K_2HPO_4	1.50	g
NaCl	0.90	g
$MgCl_2$ x 6 H_2O	0.20	g
NH_4Cl	0.90	g
Modified Wolin's mineral solution	9.00	ml
Tryptone (BD Bacto)	2.00	g
Yeast extract (OXOID)	2.00	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sodium citrate	2.00	g
Wolin's vitamin solution	5.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferrous sulfate, citrate, vitamins and sulfide), adjust pH to 6.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add ferrous sulfate and dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add citrate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and adjust pH of complete medium to 7.0, if necessary.

For DSM 25808: Replace citrate with 2.00 g/l Casamino acids (DIFCO).

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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1237: TEPIDIMICROBIUM MEDIUM

KH_2PO_4	0.49	g
Na_2HPO_4	1.19	g
NaCl	25.00	g
NH_4Cl	0.30	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.01	g
Modified Wolin's mineral solution	10.00	ml
Tryptone (BD Bacto)	2.00	g
Proteose peptone (BD Difco)	2.00	g
Yeast extract (OXOID)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$FeCl_2$ (0.1% w/v in 0.2 N HCl)	1.50	ml
Na_2CO_3	2.50	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferrous chloride, carbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.5 with NaOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, ferrous chloride, cysteine and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of ferrous chloride and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 8.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1238. WOODSHOLEA MEDIUM

Sea salts (Sigma)	30.0	g
NH_4Cl	0.5	g
Distilled water	1000.0	ml

After autoclaving and cooling the medium, 20 ml 50 x PYE (100 g peptone and 50 g yeast extract in 1l distilled water, autoclaved), 2 ml 50 % glucose (autoclaved) and 5 ml filter-sterilized riboflavin (0.2 mg ml^{-1}) were added.

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1239. DESULFURISPIRILLUM MEDIUM

$NaHCO_3$	30.0	g
NaCl	6.0	g
K_2HPO_4	0.5	g
Na_2CO_3	5.3	g
Trace elements (see medium 28)	1.0	ml
Distilled water	1000.0	ml

Flush the medium with N_2 and dispense 20 ml into 100ml serum bottles under an N_2 atmosphere, seal with butyl rubber stoppers and autoclave. To the cooled. sterile medium add, from anaerobic, sterile stock solutions, $MgSO_4$ (1 mM), yeast extract (50 mg/l), sodium acetate (20 mM), KNO_3 (20 mM) and Na_2S (0.1 mM).

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1240. STARCH-MINERAL SALT-AGAR + 10% NACL

Starch, soluble	10.0	g
$(NH_4)_2SO_4$	2.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
NaCl	1.0	g
NaCl	100.0	g
$CaCO_3$	2.0	g
Trace element solution (see medium 84)	1.0	ml
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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1241. STARCH - MINERAL SALT - AGAR (STMS) + 15% NaCl

Starch, soluble	10.0	g
$(NH_4)_2SO_4$	2.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	1.0	g
NaCl	1.0	g
NaCl	150.0	g
$CaCO_3$	2.0	g
Trace element solution (see medium 84)	1.0	ml
Agar	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2.

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1242. ALGORIPHAGUS ALKALIPHILUS MEDIUM

Yeast extract	1.00	g
Proteose Peptone n°3	1.00	g
Casamino acids	1.00	g
Glucose	1.00	g
Pyruvic acid	0.05	g
$MgSO_4$ x $7H_2O$	0.10	g
K_2HPO_4	0.60	g
Mineralsolution 1 (see below)	50.00	ml
Traceelements solution (see medium 1033)	5.00	ml
Tris buffer (1 M pH 8.0)	100.00	ml
Agar	15.00	g
Distilled water	845.00	ml

Adjust pH 8 $\pm$ 0.2
Autoclave at 121°C for 15 minutes.

Mineralsolution 1

Nitrilotriacetic acid	1000	mg
$CaSO_4$ x $2H_2O$	600	mg
$MgSO_4$ x $7H_2O$	1000	mg
NaCl	800	mg
KNO_3	1030	mg
$NaNO_3$	6890	mg
Na2HPO4	1110	mg
Distilled water	1000	ml

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1243: PARADESULFITOBACTERIUM MEDIUM

Ferric citrate monohydrate	13.70	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Modified Wolin's mineral solution	10.00	ml
$NaHCO_3$	2.50	g
Na-pyruvate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

First dissolve ferric citrate by heating the water. After cooling to room temperature adjust the pH to 6.0 and dissolve the other ingredients (except bicarbonate, vitamins, pyruvate and sulfide). Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation add vitamins, pyruvate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.2, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1244. SCHLESNERIA PALUDICOLA MEDIUM

N-acetylglucosamine	1.00	g
KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_20	0.05	g
$CaCl_2$ x 2 H_2O	0.01	g
Hutners basal salts (see below)	20.00	ml
Na-ampicillin	0.20	g
Peptone	0.10	g
Yeast extract	0.10	g
Agar-agar (Difco)	18.00	g
Distilled water	1000.00	ml

Adjust pH to 5.5 – 5.6.

Hutners basal salts:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$(NH_4)MoO_7O_{24}$ x 4 H_2O	9.25	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.0	mg
$ZnSO_4$ x 7 H_2O	1095.0	mg
$FeSO_4$ x 7 H_2O	500.0	mg
$MnSO_4$ x H_2O	154.0	mg
$CuSO_4$ x 5 H_2O	39.2	mg
$Co(NO_3)_2$ x 6 H_2O	24.8	mg
$Na_2B_4O_7$ x 10 H_2O	17.7	mg
Distilled water	1000.0	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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1245. OTEB-MEDIUM

OTEB-medium is available from Anaerobe Systems (www.anaerobesystems.com)

The following text was taken from the above page:

ORAL TREPONEME ENRICHMENT BROTH (OTEB)
Products: AS-603 Oral Treponeme Enrichment Broth (OTEB) 10 tubes / pkg

Intended Use
OTEB is an enriched medium for the isolation, cultivation and maintenance of *Treponemes* found as part of the oral flora.

Summary
OTEB is a complex peptone-yeast extract medium containing volatile fatty acids and serum for a source of long chain fatty acids.
Volatile fatty acids and serum are necessary nutrients for recovery of *Treponemes* from the oral flora. Inoculation of the medium can be done using a gassing cannula or inside an anaerobic chamber. This medium is prepared, stored and dispensed under
oxygen-free conditions to prevent the formation of oxidized products prior to use.

Formula

Proteose Peptone #2	5.0 g
Heart Infusion Broth	5.0 g
Yeast Extract	5.0 g
Potassium Phosphate, dibasic	2.0 g
Sodium Chloride	5.0 g
Magnesium Sulfate	0.1 g
Acetic Acid	1.6 ml
Propionic Acid	0.6 ml
N-Butyric Acid	0.4 ml
N-Valeric Acid	0.1 ml
Isovaleric Acid	0.1 ml
Isobutyric Acid	0.1 ml
2-Methylbutyric Acid	0.1 ml
Vitamin K1 (1% soln)	0.1 ml
L-Cysteine	0.5 g
Calf Serum, inactivated	100.0 ml
Sugar Stock Soln w/ TPP	100.0 ml
Distilled Water	800.0 ml

Sugar Stock Solution	
Glucose	8.0 g

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Fructose	8.0 g
Sucrose	8.0 g
Maltose	8.0 g
Maltose	8.0 g
Ribose	8.0 g
Xylose	8.0 g
Pectin	8.0 g
Soluble Starch	8.0 g
Sodium Pyruvate	8.0 g
Thiamine Pyrophosphate	750.0 µg
Distilled Water	1000.0 ml

Final pH 7.0 +/- 0.2 at 25 degrees C.
Final volume 7.0 ml +/- 0.7.

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1246. NSC3-MEDIUM WITH ACETATE

Solution A:

Na_2S x 9 H_2O	1.00	g
NH_4Cl	0.25	g
K_2HPO_4	0.25	g
$NaHCO_3$	1.50	g
NaCl	10.00	g
Na-Acetate	0.44	g
Trace element solution SL-4	1.00	ml
Agar agar	15.00	g
Distilled water	999.00	ml

Adjust pH to 8.5-9.0 before autoclaving.

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6 (see below)	100.00	ml
Distilled water	900.00	ml

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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1247. TYB

Yeast extract	3.0	g
Beef extract	2.0	g
Tryptone	6.0	g
$FeCl_3$	0.1	g
NaCl	3.0	g
Distilled water	1000.0	ml

Medium may be solidified with 15.0 g/l agar. pH 7.0

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1248: KOSMOTOGA SHENGLIENSIS MEDIUM

NaCl	18.00	g
KCl	0.34	g
$MgSO_4$ x 7 H_2O	3.45	g
K_2HPO_4	0.14	g
NH_4Cl	0.25	g
Na-acetate	1.00	g
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
D-Glucose	10.00	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, thiosulfate and glucose. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then add cysteine and adjust pH to 6.8 - 7.2. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate and glucose from anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

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1249: GEOALKALIBACTER SUBTERRANEUS MEDIUM

NaCl	17.00	g
$NaNO_3$	2.55	g
$MgCl_2$ x 6 H_2O	4.50	g
$CaCl_2$ x 2 H_2O	0.35	g
NH_4Cl	1.00	g
KH_2PO_4	0.08	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.50	g
Na-acetate	1.00	g
Yeast extract	3.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, acetate and yeast extract), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add acetate and yeast extract from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 7.0 - 7.2.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg

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1249: GEOALKALIBACTER SUBTERRANEUS MEDIUM

Distilled water	1000.00	ml

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1250: DESULFOSPOROSINUS ACIDOPHILUS MEDIUM

$(NH_4)_2SO_4$	0.45	g
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.05	g
$Ca(NO_3)_2$ x 4 H_2O	0.02	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
KH_2PO_4	10.00	g
Na_2HPO_4	1.00	g
D-Fructose	3.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphates, vitamins, fructose and cysteine) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prepare a phosphate buffer stock solution with KH_2PO_4 (25% w/v) and Na_2HPO_4, adjust pH of the buffer to 5.8. Prior to inoculation add phosphate buffer, vitamins, fructose and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins and D-fructose should be sterilized by filtration. The pH of the complete medium should be at 5.8.

For DSM 27692, DSM 26589, DSM 29876: Replace fructose with 2.50 g/l Na-DL-lactate and adjust pH of complete medium to 5.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1250: DESULFOSPOROSINUS ACIDOPHILUS MEDIUM

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1251: CALDINITRATIRUPTOR MEDIUM

NH_4Cl	1.00	g
KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
KCl	0.10	g
NaCl	1.00	g
$NaNO_3$	1.50	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	0.50	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	1.00	g
Na_2CO_3	1.00	g
D-Glucose	3.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate and glucose), sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add glucose from a sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be 7.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust

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final to pH 7.0 with KOH.

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1253. LACTOBACILLUS KEFIRANOFACIENS

Man Rogosa Sharp broth (MRS, Difco)	55.0	g
Maltose	10.0	g
Distilled water	900.0	ml
White wine (filter sterilized)	100.0	ml
Hemin solution	10.0	ml
Cysteine hydrochloride solution (10%)	1.0	ml

Add all products except white wine, hemin and cysteine solutions. Adjust pH to 6.5 and autoclave for 15 minutes. Cool to 50°C and add aseptically white wine, hemin and cysteine solutions.

Hemin solution:
Dissolve 50 mg hemin in 1 ml 1N NaOH; make to 100 ml with distilled water. Autoclave at 121°C for 15 min.

Cysteine hydrochloride solution (10%):

Cysteine hydrochloride solution	10.0	g
Distilled water make up to	100.0	ml

Filter sterilize.

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1255: THERMOVENABULUM MEDIUM

NaCl	1.00	g
NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
KH_2PO_4	0.38	g
K_2HPO_4	0.75	g
Trace element solution SL-10	10.00	ml
Yeast extract (OXOID)	2.00	g
Tryptone (BD Bacto)	2.00	g
$FeSO_4$ (0.1% w/v in 0.1 N H_2SO_4)	0.50	ml
PIPES (SIGMA)	3.80	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, vitamins and sulfide), adjust pH to 7.0 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add glucose, vitamins and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas. Vitamins are sterilized by filtration. The pH of the complete medium should be 7.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1257: METHANOCELLA MEDIUM

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.10	g
KH_2PO_4	0.20	g
KCl	0.50	g
Trace element solution	1.00	ml
Yeast extract (OXOID)	0.10	g
Na-acetate	0.10	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins and reducing agents), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic and adjust pH to 6.0. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Prior to inoculation add vitamins, cysteine and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins are sterilized by filtration. If necessary, adjust the pH of the complete medium to 7.0.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g

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Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1258. NUTRIMENT AGAR WITH 150 G/L NACL

Peptone	5.0	g
Meat extract	1.0	g
Yeast extract	2.0	g
NaCl	150.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2
Autoclave at 121°C 15 min.

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1259. NUTRIMENT AGAR

Peptone	5.0	g
Meat extract	1.0	g
Yeast extract	2.0	g
Nacl	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2 ;
autoclave at 121°C 15 min.

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1260. MODIFIED BACTO TRYPTIC SOY BROTH WITHOUT DEXTROSE

Bacto Tryptic Soy Broth without Dextrose	27.5	g

To which the following ingredients were added:

Agar	20.0	g
NaCl	100.0	g
Distilled water	1000.0	ml

Adjust to pH 7.3;
autoclave at 121°C 15 min.

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1261. ISP 2 MEDIUM WITH 10% NACL

Yeast extract	4.0	g
Malt extract	10.0	g
Glucose	4.0	g
NaCl	50.0	g
Agar	20.0	g
Distilled water	1000.0	ml

pH 7.3 - 7.5

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1263: SPIROCHAETA DISSIPATITROPHA MEDIUM

NaCl	30.00	g
KCl	0.20	g
K_2HPO_4	0.20	g
$MgCl_2 \times 6\ H_2O$	0.10	g
NH_4Cl	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.76	g
$NaHCO_3$	24.00	g
Yeast extract	0.50	g
Cellobiose	5.00	g
Wolin's vitamin solution	2.00	ml
$Na_2S \times 9\ H_2O$	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, bicarbonate, yeast extract, cellobiose, vitamins and sulfide), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add solid carbonate and bicarbonate to the medium, dissolve and adjust to pH 9.4. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum bottles and autoclave. To the autoclaved medium add yeast extract, cellobiose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Stock solutions of vitamins and cellobiose should be sterilized by filtration.

For DSM 8901: For DSM 8901 exchange 'Modified Wolin's Mineral Solution' with 'Modified Wolin's Mineral Solution II'

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1265. BACTEROIDES GALACTURONICUS MEDIUM

Sodium polygalacturonate	4.00	g
Trypticase	5.00	g
Yeast Extract	2.50	g
$MgSO_4$ x 7 H_2O	2.50	g
$CaCl_2$ x 2 H_2O	0.15	g
$FeSO_4$ x 7 H_2O	20.00	mg
$(NH_4)_2SO_4$	1.40	g
L-Cysteine	1.00	g
Resazurin	1.00	mg
$NaHCO_3$	2.00	g
Distilled water	1000.00	ml

Adjust pH to 7.1 and prepare medium in a gas atmosphere of 100% N_2.
$NaHCO_3$ is prepared as 5% (w/v) solution, filter sterilized and added to the autoclaved medium.

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1266. MEDIUM VL55

Liquid media:

MES (2-Morpholinoethanesulfonic acid)	1.95	g
20 mM $MgSO_4$ x 7 H_2O	10.00	ml
30 mM $CaCl_2$ x 2 H_2O	10.00	ml
20 mM $(NH_4)_2HPO_4$	10.00	ml
Selenite-tungstate solution (see below)	1.00	ml
Trace element solution SL10 (see below)	1.00	ml
Distilled water	960.00	ml

Adjust to pH 5.5 with NaOH/KOH solution (see below).
After autoclaving add:

Vitamin solution (see below)	3.00	ml
0.2M Glucose (filter-sterilized)	10.0	ml

Solid media:

MES (2-Morpholinoethanesulfonic acid)	3.90	g
20 mM $MgSO_4$ x 7 H_2O	20.00	ml
30 mM $CaCl_2$ x 2 H_2O	20.00	ml
20 mM $(NH_4)_2HPO_4$	20.00	ml
Selenite-tungstate solution (see below)	2.00	ml
Trace element solution SL10	2.00	ml
Distilled water	920.00	ml

Adjust to pH 5.5 with NaOH/KOH solution (see below).

Autoclave in 250ml lots, with a magnetic stirring bar in each bottle.
After autoclaving, keep at about 60°C, then add to each 250 ml lot:

Vitamin solution (see below)	1.50	ml
0.2M Glucose (filter-sterilized)	10.00	ml
3% washed Agar (autoclaved, kept about 60°C)	250.00	ml

Stocks for the media:

20 mM $MgSO_4$ x 7 H_2O:

H_2O	100.00	ml
$MgSO_4$ x 7 H_2O	0.49	g

30 mM $CaCl_2$ x 2 H_2O:

H_2O	100.00	ml
$CaCl_2$ x 2 H_2O	0.44	g

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20mM $(NH_4)_2HPO_4$:

H_2O	100.00	ml
$(NH_4)_2HPO_4$	0.26	g

NaOH/KOH:

H_2O	100.00	ml
NaOH	0.80	g
KOH	0.56	g

Selenite-tungstate solution:

NaOH	0.500	g
Na_2SeO_3 x 5 H_2O	0.003	g
Na_2WO_4 x 2 H_2O	0.004	g
Distilled water	1000.000	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Vitamin solution:

Vitamin B_{12}	17.0	mg
4-aminobenzoate	13.0	mg
Biotin	3.0	mg
Nicotinic acid	33.0	mg
Hemicalcium D-(+)- pantothenate	17.0	mg
Pyridoxamine-HCl	50.0	mg
Thiamine-HCl x 2 H_2O	33.0	mg
D,L-6,8-thioctic acid	10.0	mg
Riboflavin	10.0	mg
Folic acid	4.0	mg
Distilled water	1000.0	ml

Filter sterilize the solution.

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1267: FERRIPHASELUS (ES) MEDIUM

Solution A	901.00	ml
Solution B	1.00	ml
Solution C	100.00	ml

1. Preparation of the bottom layer: Mix 1 volume of solution A (except MES buffer, $NaHCO_3$, and agarose) with 1 volume of solution C and add 1% (w/v) agarose type 1, low EEO. After autoclaving, aseptically fill 1 ml of the suspension in sterile Hungate-type tubes (15 ml total volume). The bottom layer solidifies in approx. 30 min.

2. Preparation of the top layer: Add MES buffer, bicarbonate and low melt agarose to solution A and autoclave. Let the sterile solution cool to 40°C and add 10.00 ml/l of solution B. Sparge solution with sterile CO_2 gas until a pH of 6.1 - 6.4 is reached. Then, aseptically pipette aliquots of 5.0 ml over the bottom layer of each tube under 100% N_2 gas atmosphere and let medium equilibrate for at least three hours, but not longer than 12 hours.

3. Inoculation: Inoculate the semisolid top layer under a stream of 80% N_2 and 20% CO_2 gas mixture using a sterile Pasteur pipette that is inserted just above the FeS layer; the pipette tip is drawn upward as the inoculum is dispensed. After inoculation close the tube and add 1.00 ml of sterile air.

4. Note: Some hints on the inoculation of Hungate-tubes under anoxic conditions can be found in the Video tutorial on the „Proper Handling of the Double-Vial Glass Ampoules (anaerobe)" (www.dsmz.de/shop-1/faqs/video-tutorials).

Solution A

NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
K_2HPO_4	0.05	g
Modified Wolin's mineral solution	1.00	ml
MES (SIGMA, for top layer)	1.95	g
$NaHCO_3$ (for top layer)	0.50	g
Agarose (for top layer, low melt)	1.50	g
Distilled water	900.00	ml

Solution B

Wolin's vitamin solution (10x)	1.00	ml

Sterilize by filtration and store at 4°C in the dark.

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Solution C

Ferrous sulfide sludge	100.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Ferrous sulfide sludge (from medium 1267)

$FeSO_4$ x 7 H_2O	15.40	g
Na_2S x 9 H_2O	12.30	g
Distilled water	100.00	ml

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Heat distilled water to 50°C in a 250 ml beaker with a stir bar present. While rapidly stirring the water, add the ferrous sulfate followed immediately by the sodium sulfide. The formed black FeS sludge is decanted into a glass bottle that can be stoppered. The FeS is allowed to settle for several hours and then the overlying water is decanted and replaced. This procedure is repeated at least five times to wash the FeS. After washing, the pH of the FeS solution should be close to neutrality. The FeS suspension can be kept in closed bottles or tubes under a nitrogen atmosphere for at least three months.

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1269. ISP 6 MEDIUM

Peptone	15.00	g
Proteose Peptone	5.00	g
Ferric Ammonium Citrate	0.50	g
Dipotassium Phosphate	1.00	g
Sodium Thiosulfate	0.08	g
Yeast Extract	1.00	g
Agar	20.00	g
Distilled water	1000.00	ml

Adjust to pH 7.2.

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1270. B12 - MEDIUM GUTTMAN

KH_2PO_4	6.0	g
K_2HPO_4	14.0	g
Na_2citrate x 2 H_2O	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
DL-Asparagine x H_2O	0.8	g
L-Arginine x HCl	0.2	g
L-Glutamic acid	0.2	g
Glycine	0.2	g
L-Histidine	0.2	g
L-Tryptophan	0.2	g
L-Proline	0.2	g
$(NH_4)_2SO_4$ (don't include in dry mix)	2.0	g
Glucose (added separately)	10.0	g
Distilled water	1000.0	ml

Adjust pH to 6.8 - 7.2.

Final concentration of vitamin B_{12}: 40 µg/L.

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1271: PLEOMORPHOCHAETA MEDIUM

NH_4Cl	0.50	g
KH_2PO_4	0.10	g
NaCl	25.00	g
$MgCl_2$ x 6 H_2O	4.00	g
$CaCl_2$ x 2 H_2O	1.00	g
Yeast extract	1.00	g
D-Glucose	1.70	g
Trace element solution	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.3 - 7.5, if necessary.

For DSM 23951: Replace glucose with 2.0 g/l sucrose.

For DSM 23952, DSM 104684: Replace glucose with 2.0 g/l maltose.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Trace element solution (from medium 732)

Na_2-EDTA	0.50	g
$FeCl_2$ x 4 H_2O	2.00	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg

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$CuCl_2$ x 2 H_2O	2.00	mg
AlCl3	10.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add remaining compounds.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1272. THIOALKALIVIBRIO JANNASCHI

Mineral base:

Na_2CO_3	0.5	g
$NaHCO_3$	15.0	g
NaCl	15.0	g
K_2HPO_4	0.5	g
Distilled water	1000.0	ml

Sterilize at 110°C 20 min in a closed vessel (i.e. a serum tube or bottle). pH after sterilization will about 10.

Trace element solution:

EDTA	5.0	mg
$FeSO_4$ x 7 H_2O	2.0	mg
$ZnSO_4$ x 7 H_2O	100.0	mg
$MnCl_2$ x 4 H_2O	30.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
H_3BO_3	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add:

Trace elements solution	2 ml/l
$MgCl_2$ x 6 H_2O (200.0 g/l)	1 ml/l

(a white colloid will form which will rapidly dissolve after mixing)

Prepare sterile stock solutions of the following

Sodium thiosulfate pentahydrate (40 mM - 9.92 g/l) - 20 ml/l

KNO_3 - (1 M - 101 g/l) – 5ml/l

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1273: METHANOSPIRILLUM LACUNAE MEDIUM

KH_2PO_4	0.14	g
NH_4Cl	0.54	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract (OXOID)	0.20	g
Na-acetate	0.80	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, cysteine and sulfide) and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1274. YEAST EXTRACT-STARCH AGAR (50% MARINE WATER)

Yeast extract	2.0	g
Soluble starch	10.0	g
Agar	15.0	g
Distilled water	500.0	ml
Artificial marine water	500.0	ml

Adjust to pH 7.2 (identical with NBRC medium 1077).

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1277: SULFURICELLA MEDIUM

NaCl	0.25	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
$NaNO_3$	1.70	g
$(NH_4)_2SO_4$	0.10	g
KH_2PO_4	0.10	g
KCl	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.50	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, thiosulfate and vitamins) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% volume and autoclave. Add thiosulfate and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.5, if necessary.

2. After inoculation add sterile air in an amount equivalent to 1% (v/v) of the total volume of the culture vessel.

For DSM 110711: Do not add air after inoculation.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1278: ARCHAEOGLOBUS SULFATICALLIDUS MEDIUM

NaCl	20.00	g
Na_2SO_4	4.00	g
$MgCl_2 \times 6\ H_2O$	4.30	g
NH_4Cl	0.25	g
KCl	0.34	g
$CaCl_2 \times 2\ H_2O$	0.14	g
KH_2PO_4	0.14	g
Modified Wolin's mineral solution	10.00	ml
Na-DL-lactate	2.00	g
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
DL-Dithiothreitol	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, and dithiothreitol), adjust pH to 6.8 - 7.0, and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate from a sterile anoxic stock solution prepared under an 80% N_2 and 20% CO_2 gas mixture. Add vitamins, and dithiothreitol from sterile anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration. Adjust the pH of the complete medium to 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1279. HELICOBACTER MEDIUM

Brucella Broth (Becton Dickinson)	2.8	g
Aqua dem.	80.0	ml

Adjust with HCl to pH 5.0, make anoxic under a stream of N_2/CO_2, distribute in Hungate tubes to 8 ml and autoclave.
Add aseptically to each tube:

Fetal Bovine Serum (heat inactivated)	2.00	ml
Vitox (Oxoid)	0.06	ml
Skirrow supplement (Oxoid)	0.02	ml
Amphotericin (10 mg/ml)	0.02	ml

Add 1 ml of sterile air to each tube.

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1280: METHANOREGULA (PEAT) MEDIUM

Solution A	954.00	ml
Solution B	10.00	ml
Solution C	13.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

1. Dissolve ingredients of solution A, adjust pH to 5.0 with 0.1 N HCl and sparge with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense solution A under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Solutions B - F are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Prior to inoculation complete the medium by adding appropriate amounts of solutions B to F to the sterile solution A in the sequence as indicated.

2. Adjust pH of the complete medium to 5.1 with a sterile anoxic solution of 1 N HCl. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

3. Note: Use 10-20% (v/v) as inoculum.

Solution A

NH_4Cl	27.00	mg
KH_2PO_4	14.00	mg
KCl (0.1% w/v)	2.00	ml
Na-acetate	17.00	mg
Trace elements solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Homo-PIPES (SIGMA)	1.58	g
NaOH (pellets)	0.18	g
Distilled water	10.00	ml

Adjust pH of buffer to 5.5.

Solution C

NaOH (0.38 M)	7.20	ml
Nitrilotriacetate (0.5 M, neutralized with 10 N NaOH)	4.80	ml
$TiCl_3$ (15% w/v in HCl, RIEDEL-de HAEN)	0.55	ml

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Solution D

Yeast extract	0.20	g
Distilled water	10.00	ml

Solution E

2-Mercaptoethanesulfonate (coenzyme M)	0.07	g
Distilled water	10.00	ml

Solution F

Wolin's vitamin solution (10x)	10.00	ml

Trace elements solution

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.34	g
$CoCl_2$ x 6 H_2O	24.00	mg
$ZnCl_2$	75.00	mg
H_3BO_3	19.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	24.00	mg
$MnSO_4$ x 4 H_2O	26.00	mg
$MgSO_4$ x 7 H_2O	1.56	g
$CaCl_2$ x 2 H_2O	2.34	g
$CuSO_4$ x 5 H_2O	9.00	mg
$AlK(SO_4)_2$ x 12 H_2O	3.45	g
Distilled water	1000.00	ml

First dissolve ferrous chloride in the HCl, then dilute in water and dissolve the other salts. Finally make up to 1000 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1283. SH SEAWATER MEDIUM

Peptone	0.20	g
Yeast extract	0.10	g
NH_4NO_3	3.20	mg
NaCl	24.32	g
$MgCl_2$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	1.50	g
KCl	0.66	g
Na_2SO_4	4.00	g
Na_2SO_4	4.00	g
Trace element solution	1.00	ml
HEPES	2.38	g
KBr (0.84M)	1.00	ml
H_3BO_3 (0.4M)	1.00	ml
$SrCl_2$ (0.15M)	1.00	ml
NH_4Cl (0.4M)	1.00	ml
KH_2PO_4 (0.04M)	1.00	ml
NaF (0.07M)	1.00	ml
Vitamin solution	1.00	ml
$NaHCO_3$	0.20	g
Distilled water	1000.00	ml

pH 7.5 – 8.5
To solid media add 15.0 g/l agar. Add the vitamin solution (filter sterilized) and the $NaHCO_3$ (0.2 g in 10 ml H_2O, filter sterilized) to the autoclaved, cooled medium.

Trace element solution:

25% HCl	10.000	ml
$FeCl_2$ x 4 H_2O	1.500	g
$CoCl_2$ x 6 H_2O	0.190	g
$MnCl_2$ x 2 H_2O	0.100	g
$ZnCl_2$	0.036	g
Na_2MoO_4 x 2 H_2O	10.000	g
$NiCl_2$ x 6 H_2O	0.024	g
H_3BO_3	0.600	mg
$CuCl_2$ x 2 H_2O	0.200	mg
Double distilled water	990.000	ml

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Vitamin solution:

para-Aminobenzoic acid	25.0	mg
Folic acid	10.0	mg
Biotin	10.0	mg
Nicotinic acid	25.0	mg
Ca pantothenate	25.0	mg
Riboflavin	25.0	mg
Thiamine hydrochloride	25.0	mg
Pyridoxine hydrochloride	25.0	mg
Cyanocobalamine	25.0	mg
Lipoic acid	25.0	mg
Double distilled water	1000.0	ml

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1283. KR Medium

Tryptone	1.000	g
Yeast extract	1.000	g
Pyromelitic acid	1.000	g
$(NH_4)_2\ HPO_4$	0.264	g
$MgSO_4$ x 7 H_2O	0.147	g
Distilled water	1000.000	ml

pH 7.2
To solid media add 15.0g/l agar.

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1284. GRANULICELLA MEDIUM

Glucose	0.50	g
Yeast extract	0.10	g
Casmino acids	0.10	g
$MgSO_4$ x 7 H_2O	0.04	g
CaCl2 x 2 H_2O	0.02	g
Distilled water	1000.00	ml

The medium may be solidified with 15 g/l agar. Adjust to pH 4.5 – 5.2.

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1285. GRANULICELLA PALUDICOLA MEDIUM

Fructose	0.50	g
Yeast extract	0.05	g
Casamino acids	0.05	g
$MgSO_4$ x 7 H_2O	0.04	g
CaCl2 x 2 H_2O	0.02	g
KH_2PO_4	0.10	g
$(NH_4)_2SO_4$	0.10	g
Distilled water	1000.00	ml

The medium may be solidified with 15 g/l agar. Adjust to pH 4.0 – 5.0 with alginic acid.

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1286. BRYOCELLA ELONGATA MEDIUM

Glucose (or fructose)	0.50	g
Yeast extract	0.05	g
$MgSO_4$ x 7 H_2O	0.05	g
CaCl2 x 2 H_2O	0.02	g
KH_2PO_4	0.10	g
$(NH_4)_2SO_4$	0.10	g
Distilled water	1000.00	ml

The medium may be solidified with 15 g/l agar. Adjust to pH 5.0 – 5.8.

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1288. RHIZOMICROBIUM MEDIUM

Trypticase (BBL)	10.00	g
Yeast extract	5.00	g
K_2HPO_4	0.45	g
KH_2PO_4	0.45	g
$(NH_4)_2SO_4$	0.90	g
NaCl	0.90	g
$MgSO_4$ x 7 H_2O	0.09	g
$CaCl_2$ x 2 H_2O	0.09	g
Na_2CO_3	0.20	g
L-cysteine HCl	0.30	g
Resazurin	1.00	mg
Glucose	0.25	g
Cellobiose	0.25	g
Maltose	0.25	g
Soluble starch	0.25	g

Prepare the medium without glucose, cellobiose, maltose, soluble starch and L-cysteine HCl. Boil the medium and cool under an atmosphere of N_2:CO_2 (80:20) and adjust the pH to 6.8. Dispense into Hungate tubes or serum bottles under the same gas atmosphere and seal with rubber stoppers. Add the glucose, cellobiose, maltose, soluble starch and L-cysteine HCl from sterile stock solutions.

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1289. ALCANIVORAX BALERICUS MEDIUM

Beef extract	5.0	g
Peptone	10.0	g
NaCl	5.0	g
Destilled water	1000.0	ml

For solid media add 15 g/l agar. Adjust pH to 7.2.

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1290: ALKALIPHILUS MEDIUM

Solution A	950.00	ml
Solution B	25.00	ml
Solution C	1.00	ml
Solution D	25.00	ml

Dissolve ingredients of solution A, adjust pH to 8.0 with 1 N NaOH and boil medium for 1 min, then cool to room temperature under 100% N_2 gas atmosphere. Dispense solution A under same gas atmosphere in anoxic culture vessels (e.g., Hungate-type tubes) and autoclave. Prior to inoculation complete the medium by adding appropriate amounts of solutions B, C and D prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of the completed medium to 8.0 with a sterile anoxic solution of 0.1 N HCl or 0.1 N NaOH, if necessary.

Solution A

NaCl	75.00	g
$MgSO_4$ x 7 H_2O	8.80	g
KCl	1.00	g
Yeast extract (OXOID)	10.00	g
Trypticase peptone (BD BBL)	10.00	g
Modified Wolin's mineral solution	10.00	ml
Distilled water	940.00	ml

Solution B

D-Glucose	2.50	g
Distilled water	25.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

Solution D

Na-thioglycolate	0.50	g
Na-ascorbate	0.50	g
Distilled water	25.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g

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$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1291: CHTHONOMONAS MEDIUM

Gellan Gum (Phytagel)	0.50	g
$(NH_4)_2SO_4$	4.00	g
K_2HPO_4	0.45	g
$CaCl_2$ x 2 H_2O	0.15	ml
Trace element solution SL-11	1.00	ml
$MgCl_2$ x 6 H_2O	1.00	g
Yeast extract	0.01	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Add gellan gum and boil to dissolve. Then add the remaining ingredients (except magnesium chloride, yeast extract and vitamins), adjust the pH value to 5.0 to 5.3 and fill the medium under an air atmosphere into tubes of the Hungate type or similar vials up to 30% their volume. After autoclaving add magnesium chloride, yeast extract and vitamins from steril stock solutions. Vitamins should be sterilized by filtration.

2. Note: Incubation with shaking may improve cell yield.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg

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1291: CHTHONOMONAS MEDIUM

Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1292: CALDICOPROBACTER ALGERIENSIS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
KCl	0.10	g
$MgCl_2 \times 6\ H_2O$	0.50	g
$CaCl_2 \times 2\ H_2O$	0.10	g
NaCl	0.50	g
Na-acetate	0.20	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.50	g
D-Glucose	2.50	g
L-Cysteine HCl $\times\ H_2O$	0.50	g
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, glucose, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, cysteine and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.2, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg

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1292: CALDICOPROBACTER ALGERIENSIS MEDIUM

Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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1293: SYMBIOBACTERIUM THERMOPHILUM MEDIUM

NaCl	5.00	g
$NaNO_3$	1.70	g
Yeast extract (OXOID)	5.00	g
Tryptone (BD Bacto)	10.00	g
NH_4HCO_3	3.20	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate) and adjust pH to 8.0, sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add bicarbonate from a sterile anoxic 3.2% (w/v) stock solution prepared under 80% N_2 and 20% CO_2 gas mixture (e.g., add 1 ml stock solution per 9 ml medium). Adjust pH of the complete medium to 7.5 - 7.8, if necessary.

2. After inoculation add sterile 100% H_2 gas to an overpressure of 0.5 bar.

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1294: DESULFURISPIRILLUM INDICUM MEDIUM

NaCl	23.00	g
KCl	1.30	g
KH_2PO_4	0.20	g
NH_4Cl	0.50	g
$NaNO_3$	0.90	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	3.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na-pyruvate	1.10	g
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, pyruvate and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, pyruvate and sulfide form anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration. Add carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1295. GYP MEDIUM

Peptone	3.0	g
Yeast extract	5.0	g
Glycerol	10.0	ml
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2.

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1296. M1DG-MEDIUM

Casitone	5.0	g
Glucose	3.5	g
$CaCl_2 \times 2\ H_2O$	0.5	g
$MgSO_4 \times 7\ H_2O$	2.0	g
Agar	14.0	g
Distilled water	1000.0	ml

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1297: NATRONIELLA SULFIDIGENA MEDIUM

$NaHCO_3$	25.00	g
Na_2CO_3	140.00	g
NaCl	15.00	g
K_2HPO_4	1.00	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Yeast extract	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Glycerol	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Sulfur (powdered)	2.00	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve hydrogen carbonate, carbonate, sodium chloride, and hydrogen phosphate, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense solution under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ammonium chloride, magnesium chloride, yeast extract, trace elements, glycerol, vitamins, and sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Sulfur is sterilized by steaming for 3 hours on each of 3 successive days (see medium 35) and added aseptically to the sterile medium as powder while retaining anoxic conditions. Adjust the pH of the complete medium to 9.8 - 10.0, if necessary.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1298: SULFUROSPIRILLUM ALKALITOLERANS MEDIUM

NaCl	1.00	g
K_2HPO_4	1.00	g
$NaHCO_3$	32.00	g
Na_2CO_3	10.60	g
KNO_3	2.00	g
$MgCl_2$ x 6 H_2O	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Yeast extract	0.02	g
Seven vitamins solution	1.00	ml
Na-DL-lactate	2.20	g
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

Dissolve sodium chloride and hydrogenphospate, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonates, then dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add nitrate, magnesium chloride, trace elements, yeast extract, vitamins, lactate and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 9.0, if necessary.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg

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1298: SULFUROSPIRILLUM ALKALITOLERANS MEDIUM

Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1299: THERMOSIPHO AFFECTUS MEDIUM

Sea Salt (SIGMA)	30.00	g
Yeast extract (OXOID)	0.10	g
Trace element solution SL-10	1.00	ml
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except glucose, vitamins and sulfide, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add glucose, vitamins and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.5 - 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg

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Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1301: TEPIDANAEROBACTER ACETATOXYDANS MEDIUM

KH_2PO_4	0.41	g
Na_2HPO_4	0.43	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.11	g
NaCl	0.30	g
NH_4Cl	0.30	g
Trace element solution	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
D-Glucose	1.80	g
Wolin's vitamin solution	2.00	ml
Na_2S x 9 H_2O	0.30	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, glucose, vitamins and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.0-7.2, if necessary.

Trace element solution (from medium 732)

Na_2-EDTA	0.50	g
$FeCl_2$ x 4 H_2O	2.00	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
AlCl3	10.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add remaining compounds.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1302. YIM MEDIUM + 10 % NaCl

Glucose	1.0	g
Yeast extract	2.0	g
Tryptone	0.5	g
$CaCO_3$	1.0	g
NaCl	100.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2 .

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1303. MARINE MEDIUM WITH PYRUVATE

Solution A:

Marine Broth (i.e. Difco)	3.7	g
Na-Pyruvate	5.0	g
Agar if required	16.0	g
Distilled water	250.0	ml

Solution B: Artificial sea water

NaCl	21.10	g
KCl	0.58	g
$CaCl_2$ x 2 H_2O	1.20	g
$MgCl_2$ x 6 H_2O	3.60	g
$NaHCO_3$	0.08	g
$MgSO_4$ x 7 H_2O	2.62	g
Distilled water	750.00	ml

Autoclave solution A and B separately and combine 250 ml Solution A and 750 ml Solution B after cooling. Adjust to pH 7.5.

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1304: SULFURITALEA MEDIUM

$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
$NaNO_3$	1.70	g
$(NH_4)_2SO_4$	0.10	g
KH_2PO_4	0.10	g
KCl	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$Na_2S_2O_3$ x 5 H_2O	0.40	g
$NaHCO_3$	5.00	g
Wolin's vitamin solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients except trace elements, thiosulfate, bicarbonate and vitamins, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to remove dissolved oxygen and to saturate the solution with CO_2. Dispense solution under 80% N_2 and 20% CO_2 gas atmosphere in vials suitable for anaerobic cultures (e.g., Balch-type tubes) to 30% of volume. Close vials with butyl rubber septa and autoclave. Add trace element solution, selenite-tungstate solution and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 6.7 - 7.0, if necessary.

2. After inoculation, add 1 bar overpressure of sterile 100% H_2 gas.

3. Note: Use at least 10% (v/v) as inoculum.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1304: SULFURITALEA MEDIUM

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1305. MMN

Malt extract powder	5.0	g
Sucrose	5.0	g
KH_2PO_4 x 7 H_2O	0.5	g
$MgSO_4$ x 7 H_2O	150.0	mg
$CaCl_2$	50.0	mg
$FeCl_3$ (1% sol)	1.2	ml
NaCl	25.0	mg
Ca-Pantothenate	25.0	mg
Agar	15.0	g
H_2O	1.0	l

pH not adjusted

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1306. DEINOCOCCUS MEDIUM

Tryptone	5.0	g
Yeast extract	2.5	g
Dextrose	1.0	g
Maltose	1.0	g
DL-Methionine	1.0	g
Trace elements (see below)	1.00	ml
Vitamin solution (see below)	10.00	ml
Distilled water, refill to	1000.0	ml

Adjust pH to 7.0.

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

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1307. BACILLUS MUCILAGINOSUS MEDIUM

Sucrose	10.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
CaCO3	5.0	g
Agar	15.0	g
Distilled water make up to	1000.0	ml

Adjust pH to 7.6. Autoclave at 121°C for 15 minutes.

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1308. PY4S

Trypticase peptone	10.00	g
Yeast extract	5.00	g
Glucose	0.25	g
Cellobiose	0.25	g
Maltose	0.25	g
Soluble starch	0.25	g
Cysteine-HCl x H_2O	0.50	g
Resazurin-Na (0.1% w/v)	1.00	ml
Salt solution I (see below)	75.00	ml
Salt solution II (see below)	75.00	ml
Na_2CO_3 (8% w/V)	2.50	ml
Vitamin B_{12}	10.00	µg
Distilled water	846.50	ml

The cysteine, Na_2CO_3, and vitamin B_{12} are added after the medium has been boiled and cooled under CO_2. Adjust pH to 7.3 Distribute under N_2 and autoclave.

Solution I:

K_2HPO_4	0.60	g
Distilled water	100.00	ml

Solution II:

$CaCl_2$ x 2 H_2O	0.12	g
$MgSO_4$ x 7 H_2O	0.12	g
KH_2PO_4	0.60	g
$(NH_4)_2SO_4$	1.20	g
NaCl	1.20	g
Distilled water	100.00	ml

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1309. BACILLUS LOCISALIS MEDIUM

Peptone	5.0	g
Yeast extract	5.0	g
Glucose	10.0	g
K_2HPO_4	1.0	g
$MgSO_4$ x 7 H_2O	0.2	g
Agar	20.0	g
NaCl	80.0	g
Na_2CO_3	20.0	g
Distilled water	1000.0	ml

The NaCl and Na_2CO_3 were dissolved and autoclaved separately and added to the organic components at 60°C before pouring the agar medium.

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1310: BUTYRIVIBRIO M2 MEDIUM

Casitone (BD Bacto)	10.00	g
Yeast extract (BD Bacto)	2.50	g
D-Glucose	2.00	g
Cellobiose	2.00	g
Maltose	2.00	g
Na-DL-lactate	5.00	g
Clarified rumen fluid	200.00	ml
K_2HPO_4	0.05	g
KH_2PO_4	0.05	g
$(NH_4)_2SO_4$	0.10	g
NaCl	0.10	g
$CaCl_2$ x 2 H_2O	0.01	g
$MgSO_4$ x 7 H_2O	0.01	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	800.00	ml

Dissolve ingredients (except carbonate, cysteine and sulfide), boil medium for 1 min, then cool to room temperature under 100% CO_2 gas atmosphere. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Add cysteine and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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1311. XTC-2 MEDIUM

Cultivation of *Diplorickettsia massiliensis*

Cultivation of XTC-2 (from *Xenopus laevis*) cells

Leibovitz 15-Medium	93.0	ml
Fetal Bovine Serum	5.0	ml
Tryptose-phosphate	2.0	ml
Glutamine	2.0	mM (final concentration)

Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

Prepare a 25 cm^2 flask and seed cells according to standard protocols. Incubate at 28 - 32°C in hermetic flasks. Cells are easily detached mechanically, no enzyme is necessary. Medium should be changed once a week. When a confluent layer has formed, infection can be carried out.

Infection medium:

Leibovitz 15-Medium	96.0	ml
Fetal Bovine Serum	2.0	ml
Tryptose-phosphate	2.0	ml
Glutamine	2.0	mM (final concentration)

Infect cells with 1 ml of *Diplorickettsia* stock solution (thawed quickly to 37°C). Incubate for 1 h at room temperature. **Cultivation temperature: 28°C.**

Up to 100% of the cells should be infected after several days. The infection level is evaluated after 3 days by Cytospin and Gimenez staining. Cytopathic effects may be visible after 3-5 days. Specific PCRs can be performed to verify the presence of diplorickettsiae.

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1312. FRESHWATER HD MEDIUM

Solution A:

$CaCO_3$	0.025	g
$NaHCO_3$	0.025	g
Peptone	0.500	g
D-Glucose	0.100	g
Yeast extract	0.250	g
HEPES	2.380	g
Agar (Oxoid No. 1) if appropriate	14.000	g
Freshwater Solution	100.000	ml
Distilled water	900.000	ml

Freshwater solution B:

NH_4Cl	5.3	mg
KH_2PO_4	14.0	mg
KNO_3	100.0	mg
$MgSO_4$ x 7 H_2O	493.0	mg
$CaCl_2$ x 2 H_2O	147.0	mg
Distilled water	1000.0	ml

Dissolve carbonates in 900 ml distilled water by stirring for 5 h or longer. Add other components while stirring, adjust pH to 7.2 with KOH. Autoclave.

Autoclave solution A and B separately and combine after cooling.

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1313. MARINE AMMONIUM MINERAL SALTS

Solution A:

NaCl	20.000	g
$(NH_4)_2SO_4$	1.000	g
$CaCl_2$ x 2 H_2O	0.200	g
$MgSO_4$ x 7 H_2O	1.000	g
$FeSO_4$ x 7 H_2O	0.002	g
Na_2WO_4 x 2 H_2O	0.003	g
Na_2MoO_4 x 2 H_2O	0.020	g
Trace element solution SL10	1.000	ml
Distilled water	900.000	ml

Adjust the pH to 7.2 if needed with NaOH/HCl

Solution B:

KH_2PO_4	0.36	g
K_2HPO_4	2.34	g
Distilled water	100.00	ml

Autoclave solution A and B separately and combine after cooling. Add 1ml/litre of filter sterilized vitamin solution V10

Trace element solution SL10

HCl (concentrated)	5.000	ml
$FeCl_2$ x 4 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.200	g
H_3BO_3	0.006	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled water	1000.000	ml

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Vitamin Solution V10

ρ-aminobenzoic acid	0.30	g
Biotin	0.02	g
Nicotinic acid	0.20	g
Calcium pentothenate	0.10	g
Pyridoxine dihydrochloride	0.50	g
Lipoic acid	0.05	g
Folic acid	0.05	g
Cyanocobalamin	0.10	g
Thiamine	0.20	g
Riboflavin	0.10	g
Distilled water	1000.00	ml

Filter sterilize and store in the dark at 4°C.

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1314. YEAST EXTRACT-STARCH AGAR (NBRC MED. 266)

Solution A:

Yeast extract	2.0	g
Soluble starch	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.3.

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1315. ANAEROBIC THAUERA MEDIUM

KNO_3	0.85	g
$NaHCO_3$	2.52	g
$CaCl_2$	0.01	g
$MgSO_4$ x 7 H_2O	0.50	g
$MnSO_4$	0.01	g
NH_4Cl	0.30	g
NaCl	0.05	g
Selenite and tungstate solution	2.00	ml
Distilled water	884.00	ml
Phosphate solution	100.00	ml
Acetate or capronate solution	10.00	ml
Vitamin solution	5.00	ml
Trace element solution SL-10	1.00	ml

Distribute to gas tight vessels, gas with 90% N_2 +10% CO_2 to achieve anaerobic conditions and autoclave. After cooling, add the phosphate solution, substrate solution, vitamin solution and trace element solution. After mixing, pH should be 7.1 - 7.5.

Phosphate solution

Na_2HPO_4 x 2 H_2O	1.45	g
KH_2PO_4	0.25	g
Distilled water	100.00	ml

Distribute to gas tight vessels under N_2, autoclave.

Acetate solution:

K-acetate	9.80	g
Distilled water	100.00	ml

Filter sterilize and gas with N_2. For strains DSM 12141, DSM 12142, DSM 11243 and DSM 12144, acetate may be replaced by Na-capronate (stock solution 2.76 g/100 ml).

Vitamin solution:

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg

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Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000 ml. Distribute to gas tight vessels under N_2, autoclave.

Selenite and tungstate solution:

Distilled water	1000.00	ml
NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg

First dissolve NaOH, subsequently the metal salts. Distribute to gas tight vessels under N_2, autoclave.

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1317. MODIFIED NUTRIENT AGAR

Solution :

Peptone	5.0	g
Meat extract	3.0	g
Agar	20.0	g
Seawater(40g / l seasalts)	1000.0	ml

Adjust to pH 7.2

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1318: METHANOCELLA CONRADII MEDIUM

Final pH: 7.0
Final volume: 1004 ml

$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.10	g
KH_2PO_4	0.20	g
KCl	0.50	g
Yeast extract	0.20	g
Na-acetate	0.09	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
DL-Dithiothreitol	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, cysteine, and DTT) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine, and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be 7.0.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 100% H_2 gas.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1320. HALF STRENGTH R2A MEDIUM IN 75% SEAWATER

Refer to the composition of DSMZ medium 830 and either make up the medium in 2 litres of 75% seawater or use half of the amounts of the components specified and make up the medium in 1 litre of 75% seawater. Liquid medium can be prepared by leaving out the agar.

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1321: ACIDIHALOBACTER FERROOXIDANS MEDIUM

Solution A	900.00	ml
Solution B	100.00	ml
Solution C	10.00	ml
Solution D	10.00	ml

1. Combine solution A and solution B after cooling and add solutions C and D to complete the medium.

2. Note: Use 10% (v/v) as inoculum.

For DSM 105917: Omit yeast extract and adjust pH of complete medium to 2.5.

Solution A

NaCl	15.00	g
$(NH_4)_2SO_4$	0.20	g
$MgSO_4$ x 7 H_2O	0.40	g
K_2HPO_4	0.10	g
Distilled water	900.00	ml

Adjust the pH to 1.7 with H_2SO_4 and autoclave at 121°C for 20 min.

Solution B

$FeSO_4$ x 7 H_2O	13.90	g
Distilled water	100.00	ml

Adjust the pH to 1.25 and autoclave solution at 115°C for 10 min or sterilize by filtration.

Solution C

$K_2S_4O_6$	0.30	g
Distilled water	10.00	ml

Sterilize by filtration.

Solution D

Yeast extract	0.10	g
Distilled water	10.00	ml

Autoclave at 121°C for 20 min.

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1322. METHYLOFERULA STELLATA MEDIUM

KH_2PO_4	100.00	mg
$(NH_4)_2SO_4$	100.00	mg
$MgSO_4$ x 7 H_2O	50.00	mg
$CaCl_2$ x 2 H_2O	10.00	mg
NaCl	20.00	mg
FeEDTA solution	3.00	ml
Trace elements	1.00	ml
Methanol	10.0	ml
Distilled water	1000.00	ml

Trace elements:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
Distilled water	1000.00	ml

FeEDTA solution:

$FeSO_4$ x 7 H_2O	1.54	g
Na_2EDTA	2.06	g
Distilled water	1000.00	ml

Final pH 5:0-5.8.
The medium is fairly weakly buffered so the pH should be checked before and after autoclaving. The pH should be adjusted with H_3PO_4 (sterilised if added to sterile medium). The strain should be grown with shaking.

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1323. METHYLOCAPSA AUREA MEDIUM

Solution A:

$NaNO_3$	200.00	mg
$MgSO_4$ x 6 H_2O	200.00	mg
$CaCl_2$ x 2 H_2O	40.00	mg
FeEDTA solution	3.00	ml
100mM Phosphate buffer pH 5.8	10.00	ml
Trace elements	1.00	ml
Distilled water	986.00	ml

Trace elements:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
Distilled water	1000.00	ml

FeEDTA solution:

$FeSO_4$ x 7 H_2O	1.54	g
Na_2EDTA	2.06	g
Distilled water	1000.00	ml

100mM Phosphate buffer:

K_2HPO_4 x 3 H_2O	1.71	g
KH_2PO_4	12.58	g
Distilled water	1000.00	ml

pH 5.8

Final pH 5:0-5.8.
20-30% methane is added to the gas phase. The strain should be grown with shaking.

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1324. MODIFIED METHYLOBACTERIUM MEDIUM

NH_4Cl	0.50	g
K_2HPO_4	0.70	g
KH_2PO_4	0.54	g
$MgSO_4$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.20	g
$FeSO_4$ x 7 H_2O	4.00	mg
Trace element solution (see below)	10.00	ml
Distilled water	1000.00	ml

Adjust pH to 6.8. Add sterile methanol to a concentration of 0.5% to the sterile medium. The medium may be solidified with 15 g/l agar

Trace element solution:

H_3BO_3	300.00	mg
$CoCl_2$ x 6 H_2O	200.00	mg
$ZnSO_4$ x 7 H_2O	100.00	mg
$MnCl_2$ x 4 H_2O	30.00	mg
$CuCl_2$ x 2 H_2O	10.00	mg
$NiCl_2$ x 6 H_2O	20.00	mg
Na_2MoO_4 x 2 H_2O	60.00	mg
Distilled water	1000.00	ml

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1325. PARACOCCUS BOGORIENSIS MEDIUM

Yeast extract	5.00	g
Peptone	5.00	g
Glucose	2.00	g
KH_2PO_4	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
Na_2CO_3 (7.5% w/v)	40.0	ml
Trace element solution SL10	1.00	ml
Distilled water	960.00	ml

Final pH to 9.5.

Autoclave the Na_2CO_3 solution separately and combine with the rest of the medium after cooling both to about 50°C. Agar may be added at 15g/l to solidify the medium

Trace element solution SL10

HCl (concentrated)	5.000	ml
$FeCl_2$ x 4 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.200	g
H_3BO_3	0.006	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled water	1000.000	ml

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1326. AURANTIMONAS MANGANOXYDANS MEDIUM

Yeast extract	0.5	g
Peptone	2.0	g
Artificial seawater	980.0	ml

After autoclaving add 20ml 1M HEPES sterile buffer (pH 7.8) and 0.1ml of a sterile 3mg/ml of ferric ammonium citrate. Final pH is 7.5. The medium may be solidified by adding 15 g/l agar.

Artificial seawater**:**

NaCl	17.55	g
KCl	0.75	g
$MgSO_4$ x 7 H_2O	12.35	g
$CaCl_2$ x 7 H_2O	1.46	g
Distilled water	1000.00	ml

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1327. LARKINELLA ARBORICOLA MEDIUM

KH_2PO_4	0.1	g
N-acetylglucosamine	1.0	g
Peptone	0.1	g
Yeast extract	0.1	g
Casamino acids	0.1	g
Glucose	0.5	g
Hutner's salts	20.0	ml
Distilled water	980.0	ml

Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

***"Metals 44"*:**

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

The medium may be solidified by adding 15 g/l agar. Final pH should be 6.0 − 6.5

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1328: DEFLUVIITOGA MEDIUM

KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
NH_4Cl	1.00	g
NaCl	1.00	g
KCl	0.10	g
$MgCl_2$ x 6 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
L-Cysteine HCl x H_2O	0.50	g
Trypticase peptone (BD BBL)	2.00	g
Na_2-fumarate	3.20	g
Na_2CO_3	1.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, cysteine, peptone, fumarate, carbonate and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissove cysteine, then dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and autoclave at 121°C for 20 min. Add peptone, fumarate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.0.

For DSM 24444: Omit Trypticase peptone and fumarate. Increase amount of yeast extract to 2.0 g/l and supplement medium with 0.2 g/l Na-acetate and 0.6 g/l DL-lactate added after autoclaving from sterile anoxic stock solutions prepared under 100% N_2 gas.

For DSM 25546: Omit Trypticase peptone and fumarate. Increase amount of yeast extract to 2.0 g/l, supplement medium with 0.2 g/l Na-acetate and add 3.0 g/l D-xylose after autoclaving from anoxic stock solutions prepared under 100% N_2 gas and sterilized by filtration.

For DSM 29926: Supplement medium with 6.0 g/l D-glucose added to the medium after autoclaving from a sterile anoxic stock solution prepared under 100% N_2 gas.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g

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$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1329: METHANOSAETA PELAGICA MEDIUM

$MgCl_2$ x 6 H_2O	3.05	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
NaCl	20.00	g
Na-acetate	6.56	g
Trace element solution	2.00	ml
Yeast extract (OXOID)	1.50	g
Trypticase peptone (BD BBL)	0.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
KH_2PO_4	1.19	g
K_2HPO_4	0.21	g
Na_2CO_3	1.50	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.14	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.36	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphates, carbonate, coenzyme M, vitamins and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add phosphates, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 7.5, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1330. ASAIA GYP MEDIUM

Glucose	10.0	g
Glycerol	10.0	g
Peptone	10.0	g
Yeast extract	5.0	g
$CaCO_3$	7.0	g
Distilled water	1000.0	ml

The medium may be solidified by adding 15 g/l agar. Calcium carbonate in the medium serves as a buffer. The calcium carbonate will settle in agar plates before the agar has set, producing an opaque layer in the bottom. As the strain grows and acid is produced this will react with the calcium carbonate, causing it to dissolve and form zones of clearing immediately below the colonies. pH 6.8

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1332. ALGORIPHAGUS AQUAEDUCTUS MEDIUM

Meat extract	20.00	g
NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.01	g
KH_2PO_4	0.50	g
Seven vitamins solution (see below)	3.00	ml
Trace element solution SL-8	1.00	ml
Distilled water	996.0	ml

Adjust pH to 8.0 before autoclaving. The medium may be solidified by adding 15 g/l agar.

Trace element solution SL-8:

Na_2-EDTA	5.20	g
$FeSO_4$ x 7 H_2O	2.00	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 2 H_2O	100.00	mg
$ZnSO_4$ x 7 H_2O	150.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
H_3BO_3	62.00	mg
$CuCl_2$ x 2 H_2O	17.00	mg
Distilled water	1000.00	ml

Adjust pH to 6.8.

Seven vitamins solution:

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D(+)-Biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Filter sterilise and add to the medium after it has been autoclaved.

Autoclave solution A and B separately and combine after cooling. Adjust to pH 8.1.

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1333. FLB MEDIUM

Yeast extract	3.00	g
Tryptone	5.00	g
$CaCl_2$ x 6 H_2O	0.09	g
Disteilled water	1000.00	ml

pH 6.5 – 7.0. The medium may be solidified by adding 15 g/l agar.

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1334. MODIFIED YMA MEDIUM

Yeast extract	0.5	g
Mannitol	7.0	g
K_2HPO_4	0.2	g
$MgSO_4$ x 7 H_2O	0.2	g
Distilled water	1000.0	ml

Adjust to pH 7.0. The medium may be solidified by adding 15 g/l agar.

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1335. MODIFIED T5 MEDIUM

Tryptone	0.5	g
Yeast extract	2.0	g
Glucose	1.0	g
Soluble starch	1.0	g
$CaCl_2$ x 2 H_2O	1.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0-7.5 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

For DSM 27250
Adjust to pH 7.5 $\pm$0.2

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1336. SEHGAL AND GIBBONS MEDIUM

NaCl	40.0	g
KCl	2.0	g
$MgSO_4$ x 7 H_2O	20.0	g
Yeast extract	10.0	g
Tryptone	5.0	g
Trisodium citrate dehydrate	3.0	g
Casein hydrolysate	5.0	g
Dest. Water	1000.0	ml

Adjust pH to 7.2-7.4.

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1337. METHYLOPHAGA MEDIUM

Mineral Salts Solution:

NaCl	24.0	g
$MgCl_2$ x 6 H_2O	3.0	g
$MgSO_4$ x 7 H_2O	2.0	g
KCl	0.5	g
$CaCl_2$	1.0	g
Distilled water	1000.0	ml

Adjust to pH 8.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

After autoclaving cool down and add 3 ml filter sterilized methanol, 20 ml of Solution T, 1 ml of vitamin B_{12} (0.1mg/ml) filter sterilized solution and 10 ml of Wolfe's Mineral Solution to 1 litre medium. Final pH 8.0.

Solution T:

KH_2PO_4	7.0	g
NH_4Cl	100.0	g
Bis-Tris	100.0	g
Ferric ammonium citrate	3.0	g
Distilled water	1000.0	ml

Adjust to pH 8.0 and sterilize by autoclaving.

Wolfe's Mineral Solution

Nitrilotriacetic acid	1.50	g
$MgSO_4$	3.00	g
$MnSO_4$	0.50	g
NaCl	1.00	g
$FeSO_4$	0.10	g
$CaCl_2$	0.10	g
$CoCl_2$	0.10	g
$ZnSO_4$	0.10	g
$CuSo_4$	0.01	g
$AlK(SO_4)_2$	0.01	g
H_3BO_3	0.01	g
Na_2MoO_4	0.01	g
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust to pH 8.0 with KOH and sterilize by autoclaving.

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1338. LB MEDIUM WITH CARBONATES

Mineral Salts Solution:

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	10.0	g
Distilled water	500.0	ml

Carbonate Salts Solution

$NaHCO_3$	5.0	g
Na_2CO_3	5.0	g
Distilled water	500.0	ml

Autoclave the Mineral Salts and the Carbonate Salts separately. Combine both solutions after cooling. The medium may be solidified by adding 20.0 g/l agar. The agar is added to Minerals Salts Solution and after autoclaving both the Mineral Salts Solution and the Carbonate Salts Solution are cooled to 50-55°C, mixed and poured into Petri dishes. Final pH 9.6.

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1340. SEHEL FOR HALANAEROBIUM

Mineral Salts Solution:

NH_4Cl	1.0	g
K_2HPO_4	0.3	g
KH_2PO_4	0.3	g
KCl	0.1	g
$MgCl_2$ x 7 H_2O	0.5	g
$CaCl_2$ x 2 H_2O	0.1	g
NaCl	200.0	g
KNO_3	2.0	g
Trace Elements Solution (see below)	10.0	ml
Resazurin	0.001	g
Distilled water	1000.0	ml

Trace Elements Solution:

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.03	mg
Distilled water	1000.0	ml

Prepare the liquid medium. Adjust to pH 8.3 with 10 M KOH solution. Boil the medium in a water bath and cool under N_2:CO_2 (80:20 v/v) to room temperature. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2:CO_2 (80:20 v/v). Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

$NaHCO_3$ (10% w/v)	20.0	ml/l
Na_2S x 9 H_2O (2% w/v)	20.0	ml/l
$MgCl_2$ x 6 H_2O (10% w/v)	20.0	ml/
Glucose 1M	20.0	ml/l
Yeast Extract (10% w/v)	10.0	ml/
Cysteine-HCl (10% w/v)	5.0	ml/

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1341. MIN E - METHYLOVERSATILIS MEDIUM

Mineral Salts Solution MIN E:

MIN E – Base (10x)	100.0	ml
Trace Metals (500x)	2.0	ml
Distilled water	900.0	ml

Add 100 ml of sterile phosphate buffer after autoclaving.

MIN E – Base (10x):

$CaCl_2$ x 6 H_2O	0.50	g
$MgSo_4$ x 7 H_2O	1.65	g
$(NH_4)_2SO_4$	5.00	g
Distilled water	1000.00	ml

Trace Metals (500x):
EDTA Solution

NaOH	9.00	g
EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
Distilled water	1000.00	ml

Dissolve the EDTA and NaOH in 800ml of distilled water then add the inorganic salts. Make up to 1000.0 ml with distilled water.

Phosphate buffer (10x):

K_2HPO_4	12.0	g
KH_2PO_4	6.0	g
Distilled water	1000.0	ml

pH 6.8 – 7.0

Autoclave the Mineral Salts Solution MIN E and phosphate buffer separately and combine after cooling. Add 20 ml/l filter sterilized methanol and 1 ml/l filter sterilized vitamin B_{12} (250 mg/l). The medium may be solidified by adding 15 g/l agar.

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1342. YIM MEDIUM WITH NaCl

Glucose	1.0	g
Yeast extract	2.0	g
Tryptone	0.5	g
$CaCO_3$	1.0	g
NaCl (see below)		
Distilled water	1000.0	ml

Adjust to pH 7.2.

The medium may be solidified by adding 20.0 g/l agar. For DSM 21387 add 60 g/l NaCl, DSM 21388 add 120g/l NaCl, DSM 21389 add 5g/l NaCl.

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1343. TISTLIA CONSOTENSIS MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
NaCl	20.00	g
NH_4Cl	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
Yeast extract	1.00	g
KCl	0.10	g
Zeikus trace elements solution (see below)	1.00	ml
Distilled water	1000.00	ml
pH 7.0		

Sterilise the medium by autoclaving. The medium is supplemented with 20mM sterile glucose. The medium may be solidified by adding 15 g/l agar.

Zeikus trace elements solution:

Nitrilotriacetic acid	12.500	g
$FeCl_3$x4 H_2O	0.200	g
$ZnCl_2$	0.100	g
$MnCl_2$ x 4 H_2O	0.100	g
H_3BO	0.010	g
$CoCl_2$ x 6 H_2O	0.017	g
$CuCl_2$ x 2 H_2O	0.020	g
$CaCl_2$ x 2 H_2O	0.100	g
Na_2MoO_4 x 2 H_2O	0.010	g
NaCl	1.000	g
Na_2SeO_3	0.020	g
Distilled water	1000.000	ml

Neutralise the nitrilotriacetic acid to a pH of 6.5 with KOH before adding the other trace elements.

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1346. AURANTIMONAS MEDIUM

Mineral Salts Solution:

Tryptone	10.000	g
Yeast Extract	5.000	g
NaCl	5.000	g
D-Glucose	1.000	g
$CaCl_2$ x 2 H_2O	0.345	g
Distilled Water	1000.000	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1347. M2-MEDIUM

Mineral Salts Solution:

KNO_3	0.25	g
KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_2O	0.05	g
$CaCl_2$ x 2 H_2O	0.01	g
NaCl	0.02	g
Trace Solution (see below)	1.00	ml
Distilled Water	1000.0	ml

Trace Solution:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
$MnCl_2$ x 4 H_2O		
Distilled water	1000.00	ml

Prepare the liquid medium. Final pH 5.5.The medium is fairly weakly buffered so the pH should be checked before and after autoclaving. Dispense the medium into serum bottles. Add 10-20% methane to the gas phase. Sterilize by autoclaving.
The strain should be grown with shaking.

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1348. SYP - SW MEDIUM

Soluble starch	10.0	g
Yeast extract	4.0	g
Peptone	2.0	g
Artifical sea salts (Sigma)	33.3	g
Distilled water	1000.0	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1349. TG 408 MEDIUM

Mineral Salts Solution:

Peptone	5.0	g
Yeast Extract	0.5	g
Na-Pyruvate	10.0	g
Vitamin Solution 141	10.0	ml
Artifical Sea Water	750.0	ml
Distilled Water	250.0	ml

Vitamine Solution 141:

Biotin	2.0	mg
Folic acid	2.0	mg
Pyridoxine-HCl	10.0	mg
Thiamine-HCl x 2 H_2O	5.0	mg
Riboflavin	5.0	mg
Nicotinic acid	5.0	mg
D-Ca-pantothenate	5.0	mg
Vitamin B_{12}	0.1	mg
p-Aminobenzoic acid	5.0	mg
Lipoic acid	5.0	mg
Distilled water	1000.0	ml

After autoclaving add 10 ml of Vitamine Solution 141 to 1 litre of the sterile Mineral Salts Solution. Final pH 7.5 - 8.0.

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1350. TRYPTICASE SOY AGAR NA-MG

Mineral Salts Solution:

Trypticase Soy Broth		
(BBL 11768, Oxoid CM129 or Merck 5459)	30.0	g
$MgSo_4$ x 7 H_2O	20.0	g
NaCl	30.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1351. R2A MEDIUM WITH SEAWATER

Mineral Salts Solution:

Yeast extract	0.50	g
Proteose Peptone (Difco no. 3)	0.50	g
Casamino acids	0.50	g
Glucose	0.50	g
Soluble starch	0.50	g
Na-pyruvate	0.30	g
K_2HPO_4	0.30	g
$MgSO_4$ x 7 H_2O	0.05	g
Seawater	750.00	ml
Distilled water	250.00	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1352. FUCHSIELLA ALKALIACETIGENA MEDIUM

KH_2PO_4	0.20	g
$MgCl_2$ x 6 H_2O	0.10	g
NH_4Cl	0.50	g
KCl	0.20	g
NaCl	60.00	g
Na_2CO_3	68.30	g
$NaHCO_3$	38.30	g
Trace element solution SL-4 (see below)	1.00	ml
Resazurin	0.50	mg
Vitamin solution (see medium 141)	10.00	ml
Yeast extract	0.05	g
Ethanol (50% v/v)	10.00	ml
Na_2S x 9 H_2O	1.00	g
$NaNO_3$	0.85	g
Distilled water	1000.00	ml

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4$ x 7 H_2O	0.20	g
Trace element solution SL-6 (see below)	100.00	ml
Distilled water	900.00	ml

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 8.8- 9.5; gas atmosphere: 100% N_2.
Dissolve ingredients except the sodium carbonate, sodium bicarbonate, ethanol, and sodium sulphide. Boil medium for a few minutes and cool to room temperature while flushing with N_2. Add and dissolve sodium carbonate, sodium bicarbonate and sodium sulphide while gassing the head space only. Dispense and autoclave under N_2. Before use add 10 ml/l (v/v) of 50% (v/v) ethanol solution (flushed and autoclaved under N_2).

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1353. YTBC - MEDIUM

Mineral salts solution:

Yeast Extract	1.00	g
Tryptone	5.00	g
Casamino Acids	5.00	g
Beef Extract (Lab-Lemco-Powder)	5.00	g
K_2HPO_4	1.00	g
NH_4NO_3	1.00	g
Na_2S x 9 H_2O	0.25	g
Cysteine HCl	0.25	g
$NiCl_2$ x 6 H_20	7.50	mg
Trace Element Solution (see below)	5.00	ml
Resazurin (0.1% v/v)	0.50	ml
Artifical Seawater (see below)	250.00	ml
Distilled Water	750.00	ml

Trace element solution:

$FePO_4$	1.00	g
Na_2WO_4 x 2 H_20	0.01	g
Na_2SO_3 x 5 H_20	0.01	g
Distilled Water	500.00	ml

Artifical seawater solution:

NaCl	20.00	g
$MgCl_2$ x 6 H_20	3.00	g
$MgSO_4$ x 7 H_20	6.00	g
$NaHCO_3$	0.20	g
$CaCl_2$ x 2 H_20	0.30	g
KCl	0.50	g
KH_2PO_4	0.42	g
NaBr	0.05	g
SrCl	0.02	g
$Fe(NH_4)$citrate	0.01	g
$(NH_4)_2SO_4$	1.00	g
Distilled Water	1000.00	ml

Prepare the liquid medium without Na_2S and cysteine-HCl. Boil the medium in a water bath and cool under N_2:CO_2 (80:20 v/v) to room temperature. Adjust to pH 7.0 with 10 M NaOH solution. Dispense the medium into Hungate tubes or serum bottles. Sterilize by autoclaving. Before inoculatiing add Na_2S and cysteine-HCl from sterile stock solutions.

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1354. BACTO MARINE BROTH WITH NA-ACETATE

Mineral Salts Solution:

Bacto peptone	5.00	g
Bacto yeast extract	1.00	g
Fe(III) citrate	0.10	g
NaCl	19.45	g
$MgCl_2$ (anhydrous)	5.90	g
Na_2SO_4	3.24	g
$CaCl_2$	1.80	g
KCl	0.55	g
$NaHCO_3$	0.16	g
KBr	0.08	g
$SrCl_2$	34.00	mg
H_3BO_3	22.00	mg
Na-silicate	4.00	mg
NaF	2.40	mg
$(NH_4)NO_3$	1.60	mg
Na_2HPO_4	8.00	mg
Na acetate	10.00	g
Distilled water	1000.00	ml

Final pH should be 6.8 − 8.5. If using the commercially available medium BD Difco Marine Broth 2216 add 10g/l Na acetate to the medium. The medium may be solidified by adding 15.0 g/l agar.

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1355. HYPHOMICROBIUM MEDIUM

Mineral Salts Solution:

K_2HPO_4	2.50	g
NaH_2PO_4	2.25	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	0.20	g
Methylamine (0.2% v/v)	2.00	ml
Visniac Trace Elements	1.00	ml
Distilled water	1000.00	ml

Visniac Trace Elements (1000x):

EDTA	5.000	g
$ZnSO_4$ x 7 H_2O	2.200	g
$CaCl_2$ x 2 H_2O	0.733	g
$MnCl_2$ x 4 H_2O	0.506	g
$FeSO_4$ x 7 H_2O	0.499	g
$(NH_4)6Mo_7O_4$ x 24 H_2O	0.110	g
$CuSO_4$ x 5 H_2O	0.157	g
$CoCl_2$ x 6 H_2O	0.161	g
Distilled water	500.000	ml

Adjust to pH 6 and sterilize by autoclaving. The medium may be solidified by adding 12.0 g/l agar. After autoclaving add sterile methylamine (0.2% v/v) to the sterile Mineral Salts Solution.

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1356. HMD MEDIUM

Mineral Salts Solution:

Yeast Extract	0.10	g
Casamino acids	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	20.00	g
K_2SO_4	5.00	g
NH_4Cl	0.50	g
KH_2PO_4	0.05	g
NaCl	180.00	g
Distilled Water	1000.00	ml

Adjust to pH 7.5 and sterilize by autoclaving. The medium may be solidified by adding 12.0 g/l agar.

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1357: MAB MEDIUM

Final pH: 9.8
Final volume: 1000 ml

NaCl	10.000	g
NH_4Cl	0.850	g
K_2HPO_4	0.480	g
$MgSO_4$ x 7 H_2O	0.120	g
$CaCl_2$ x 2 H_2O	0.061	g
$FeSO_4$ x 7 H_2O	0.021	g
$MnSO_4$ x H_2O	5.000	mg
$CoCl_2$ x 6 H_2O	1.000	mg
$ZnSO_4$ x 7 H_2O	1.000	mg
Trace element solution	1.000	ml
Vitamin solution	10.000	ml
Resazurin	1.000	µg
Na_2CO_3	10.600	g
$NaHCO_3$	13.600	g
Cysteine HCl	0.700	g
Distilled water	1000.000	ml

Prepare the liquid medium without Na_2CO_3, $NaHCO_3$, Vitamin solution and cysteine HCl. Boil and then cool under N_2 to room temperature. Add Na_2CO_3 and $NaHCO_3$. Dispense into suitable vessels for anaerobic growth (serum bottles or Hungate tubes sealed with rubber stoppers) and sterilize by autoclaving. After autoclaving add cysteine HCl from sterile stock solution and 10 ml of the vitamin solution to 1 litre of the sterile main solution. Final pH 9.8.

Trace element solution

$CuSO_4$ x 2 H_2O	0.10	g
Nitrilotriacetic acid	15.00	g
$AlK(SO_4)_2$ x 12 H_2O	0.10	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
Distilled water	1000.00	ml

Vitamin solution

Pyridoxine hydrochloride	0.10	mg
Thiamine HCl	0.05	mg
Riboflavin	0.05	mg

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Nicotinic acid	0.05	mg
p-Aminobenzoic acid	0.05	mg
Lipoic acid	0.05	mg
Biotin	0.02	mg
Folic acid	0.02	mg
Vitamin B_{12}	5.00	µg
Distilled water	1000.00	ml

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1358. THIORHODOCOCCUS MEDIUM

KH_2PO_4	0.50	g
NaCl	10.00	g
NH_4Cl	0.60	g
$CaCl_2$ x 2 H_2O	0.15	g
$MgSO_4$ x 7 H_2O	2.00	g
Na pyruvate	3.00	g
$NaHCO_3$ (10.0% w/v)	5.00	ml
Na_2S x 9 H_2O(24% w/v)	1.00	ml
$Na_2S_2O_3$ x 5 H_2O(24 % w/v)	5.00	ml
Yeast extract	0.30	g
Trace element solution SL-8	1.00	ml
Vitamin B_{12} (20mg/l)	1.00	ml
Distilled water	1000.00	ml

Bubble the medium with nitrogen gas and fill 10 ml in 15 ml tubes with a rubber septum under a stream of nitrogen gas.

Micronutrient solution SL8:

Na_2 x EDTA	5.2	g
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	62.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	17.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	1000.0	ml

Adjust to pH 6.5

Prepare the medium without the $NaHCO_3$, Na_2S x 9 H_2O, $Na_2S_2O_3$ x 5 H_2O and vitamin B_{12}, under a nitrogen atmosphere dispense into tubes or bottles fitted with rubber stoppers so that they are half full. Autoclave and add the vitamin B_{12} from a filter-sterilised stock solution and the $NaHCO_3$, Na_2S x 9 H_2O, $Na_2S_2O_3$ x 5 H_2O from sterile stock solutions. The final pH of the medium should be 7.5 $\pm$ 0.2.

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1360: HIPPEA JASONIAE MEDIUM

NaCl	26.33	g
$MgCl_2$ x 6 H_2O	1.50	g
KCl	0.45	g
$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Na-acetate	1.25	g
Trisodium citrate x 2 H_2O	2.94	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Yeast extract (DIFCO)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, vitamins, yeast extract and sulfide), adjust pH to 3.5 with 10 N H_2SO_4 and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium in 100 ml bottles) that contain already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1 - 2 hours on each of 3 successive days. Add vitamins (sterilized by filtration) and yeast extract from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to inoculation, add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH of complete medium to 4.5, if necessary.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1361. PLANCTOMYCETALES BACTERIUM MEDIUM

Mineral Salts Solution:

KH_2PO_4	0.14	g
$MgCl_2$ x $6H_20$	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
Trace Elements (see below)	1.00	ml
Glucose	0.18	g
Yeast Extract	0.20	g
Vitamin Solution (see medium141) (see below)	1.00	ml
Resazurin (0.1 v/v)	0.50	ml
$NaHCO_3$	2.50	g
Na_2S 9 H_2O	0.30	g
Cysteine HCl	0.30	g
Distilled Water	1000.00	ml

Vitamin Solution 141:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Trace elements Solution:

Nitrilotriacetic acid (NTA)	12.800	g
$FeCl_3$ x 6 H_2O	1.350	g
$MnCl_2$ x 4 H_2O	0.100	g
$CoCl_2$ x 6 H_2O	0.024	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnCl_2$	0.100	g
$CuCl_2$ x 2 H_2O	0.025	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.024	g
$NiCl_2$ x 6 H_2O	0.120	g
Na_2SeO_3 x 5 H_2O	0.004	g
Distilled water	1000.000	ml

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Prepare the medium without yeast extract, glucose, $NaHCO_3$, cysteine hydrochloride, vitamin solution and Na_2S x 9 H_2O. Boil and then cool under a gas atmosphere of N_2:CO_2 (80:20 v/v). Dispense the medium into Hungate tubes or serum bottles under gassing with N_2:CO_2 (80:20 v/v) and add the $NaHCO_3$. The final pH should be 7.0. Autoclave the medium and add from sterile stock solutions yeast extract, glucose, cysteine hydrochloride, vitamin solution and Na_2S x 9 H_2O.The final pH should be 7.0.

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1362. TRYPTONE SOYA BROTH (TSB) WITH ADDITIONAL SALTS

Peptone from casein	17.0	g
Peptone from soymeal	3.0	g
D(+)-Glucose	2.5	g
NaCl	100.0	g
K_2HPO_4	2.5	g
Distilled water	1000.0	ml

Adjust pH to 7.2 – 7.5.
The basic medium is the same as Caso Bouillon (Merck or Oxoid). The medium may be solidified by adding 15.0 g/l agar.

When using commercially available Caso Bouillon (Merck or Oxoid) for strains DSM 24264, DSM 24313, DSM 24197 increase the NaCl concentration to 100.0 g/L.

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1363. TUA ACETOBACTER MEDIUM

D-Glucose	10.0	g
Glycerol	10.0	g
Bacto Peptone (Difco)	10.0	g
Yeast Extract (Difco)	5.0	g
Ethanol	10.0	ml
Distilled Water	1000.0	ml

Make up the medium without the ethanol, adjust to pH 7.0 and sterilize by autoclaving. Add sterile ethanol to the sterile cooled medium. The medium may be solidified by adding 15.0 g/l agar.

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1364. NA SPHINGOBACTERIUM MEDIUM

Mineral Salts Solution:

Beef Extract	3.0	g
Peptone	10.0	g
NaCl	5.0	g
Distilled Water	1000.0	ml

Adjust to pH 7.0 – 8.0 and sterilize by autoclaving. The medium may be solidified by adding 15.0 g/l agar.

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1367. TELMATOCOLA MEDIUM

Mineral Salts Solution:

KH_2PO_4	0.10	g
$(NH_4)_2SO_4$	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
Hutner's basal salts (see below)	1.00	ml
Vitamin solution (see medium 621)(see below)	1.00	ml
Yeast Extract	0.10	g
Glucose	0.10	g
Distilled Water	1000.00	ml

Prepare the liquid medium without Staley´s vitamin solution and glucose. Adjust to pH 4.8 - 5.5. Dispense the medium into serum bottles. The gas phase is air:CO_2 (95:5) and the liquid:gas phase should be 1:9. Sterilize by autoclaving. After autoclaving add the Staley´s vitamin solution (filter sterilised) and glucose (autoclaved) from sterile stock solutions.

Hutner's basal salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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Vitamin solution:

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Distilled water	1000.00	ml

Filter-sterilized.

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1369. ECTOTHIORHODOSPIRA MAGNA MEDIUM

KH_2PO_4	0.50	g
NaCl	30.00	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	0.20	g
Na-acetate	1.00	g
$Na_2S_2O_3$	0.50	g
Na_2S x 9 H_2O	0.50	g
$NaHCO_3$	5.00	g
Na_2CO_3	5.00	g
Yeast extract	0.10	g
Vitamin B_{12}	10.00	µg
Trace elements solution (see below)	1.00	ml
Distilled water	1000.00	ml

pH 9.0-9.5

Prepare the medium without the $NaHCO_3$, Na_2S x 9 H_2O, Na_2CO_3 and vitamin B_{12}, under a nitrogen atmosphere.
Autoclave and add the vitamin B_{12} from a filter-sterilised stock solution and the $NaHCO_3$ (autoclaved in sealed, half full vessels), Na_2S x 9 H_2O, Na_2CO_3 from sterile stock solutions. The final pH of the medium should be 9.0-9.5.

Trace elements solution (Pfennig & Lippert,1966)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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1370. RICH ORGANIC MEDIUM

Mineral Salts Solution:

$MgSO_4$ x 7 H_2O	0.3	g
KH_2PO_4	0.3	g
NH_4Cl	0.1	g
$CaCL_2$ x 2 H_2O	1.0	g
Na-Acetat	1.0	g
Yeast Extract	0.5	g
Peptone	0.5	g
Casamino Acid	0.5	g
Trace elements N1 (see below)	2.0	ml
Vitamin solution N1 (see below)	2.0	ml
Distilled Water	1000.0	ml

Vitamin solution N1

Nicotinic acid	400.0	mg
Thiamine	400.0	mg
Biotin	80.0	mg
Vitamin B_{12}	20.0	mg
Distilled Water	1000.0	ml

Filter sterilised

Trace elements solution N1

$FeSO_4$ x 7 H_2O	300.0	mg
$ZnSO_4$ x 7 H_2O	5.0	mg
$MnCl_2$ x 4 H_2O	3.0	mg
H_3BO_3	2.0	mg
$CoCl_2$ x 6 H_2O	5.0	mg
$CuCl_2$ x 2 H_2O	1.0	mg
$NiCl_2$ x 6 H_2O	2.0	mg
Na_2MoO_4 x 2 H_2O	3.0	mg
Distilled Water	1000.0	ml

Adjust pH to 5.9. Add NaOH (0.1 mol/l) 16ml/l after sterilisation from sterile stock solution to readjust pH to 6.8.

Add the following from sterile stock solutions to the autoclaved medium:

Vitamin solution N1:	2.0	ml /l
Trace elements solution N1:	2.0	ml/l

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1372. SUCCINATE MINIMAL SALTS MEDIUM (SMS)

EDTA	0.010	g
KH_2PO_4	0.600	g
K_2HPO_4	0.900	g
NH_4Cl	1.000	g
$MgSO_4$ x 7 H_2O	0.200	g
CaCl2x6H2O	0.075	g
Na.succinate	2.200	g
Yeast extract	0.100	g
Trace elements solution (see below)	2.000	ml
Vitamin solution (see below)	2.000	ml
Distilled water	1000.000	ml

Adjust pH to 6.8.

Prepare the medium without the vitamin solution.
Autoclave and add the vitamin solution from a filter-sterilised stock solution.

Trace elements solution

$FeSO_4$ x 6 H_2O	0.300	g
$ZnSO_4$ x 7H_2O	0.005	g
$MnCl_2$ x 4 H_2O	0.003	g
H_3BO3	0.002	g
$CoCl_2$ x 6 H_2O	0.005	g
$CuCl_2$ x 2 H_2O	0.001	g
$NiCL_2$ x 6H_2O	0.002	g
Na_2MoO_4 x 2 H_2O	0.003	g
Distilled water	1000.000	ml

Vitamin solution:

Biotin	80	mg
Thiamine-HCl x 2 H_2O	400	mg
Nicotinic acid	400	mg
Vitamin B_{12}	20	mg
Distilled water	1000	ml

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1373. IRD MEDIUM

KH_2PO_4	0.3	g
K_2HPO_4	0.3	g
NaCl	1.0	g
NH_4Cl	1.0	g
$MgCl_2$ x 6 H_2O	0.5	g
$CaCl_2$ x 2 H_2O	0.1	g
KCl	0.1	g
Yeast extract	1.0	g
Tryptone	1.0	g
Cystein-HCL	0.5	g
Resazurin (0.1%)	0.5	ml
Trace elements solution (see Medium 141))	1.0	ml
Distilled water	1000.0	ml

pH 7.2

Prepare the medium under a N_2/CO_2 atmosphere.

Autoclave and add the following components after autoclaving from sterile stock solutions:

$NaHCO_3$ (10%)	0.2 ml / 10 ml
Na_2S (2%)	0.2 ml / 10 ml
Glucose 1 M	0.2 ml / 10 ml

The final pH of the medium should be 7.2.

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1374. IGNAVIBACTERIA MEDIUM

Solution A:

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
Na_2SO_4	4.00	g
Trace element solution (see below)	1.00	ml
Yeast extract	2.00	g
$NaHCO_3$	0.10	g
Distilled water	1000.00	ml

pH 7.2 – 7.8

Autoclave and add the following component after autoclaving from stock solutions: sterile solution B Na_2S (3%) end concentration 0.03% and filter-sterilised vitamin solution (see below) 0.1 ml / 10 ml.

Solution B:

Na_2S x 9 H_2O	0.3	g
Distilled water	10.0	ml

Trace element solution:

$FeSO_4(NH_4)_2$ x 6 H_2O	784.0	mg
$ZnSO_4$ x 7 H2O	143.6	mg
$MnCl_2$ x 4 H_2O	99.0	mg
Na_2SeO_4	94.6	mg
H_3BO_3	6.0	mg
Na_2WO_4 x 2 H_2O	33.0	mg
$CuCl_2$ x 2 H_2O	1.8	mg
$CoCl_2$ x 6 H_2O	238.0	mg
$NiSO_4(NH_4)_2SO_4$ x 6 H_2O	395.0	mg
Na_2MoO_4 x 2 H_2O	24.0	mg
conc. HCl	10.0	ml
Distilled water	990.0	ml

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Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1375. HALANAEROBACTER JERIDENSE MEDIUM

Mineral Salts Solution:

NH_4Cl	1.000	g
$MgCl_2$ x 6 H_2O	10.000	g
$MgSO_4$ x 7 H_2O	5.000	g
KCl	2.000	g
K_2HPO_4 x 3 H_2O	0.500	g
NaCl	150.000	g
Resazurin	0.001	g
Trace element solution SL 6 (see below)	10.000	ml
Distilled Water	1000.000	ml

Final pH 8.3

Prepare the medium without the Na_2S, Na_2CO_3, $NaHCO_3$, L-cysteine and substrates. Boil the medium and cool under a stream of nitrogen. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2. Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

Glucose (10% v/v)	0.40 ml/10 ml
Na_2CO_3 (10% v/v)	0.15 ml/10 ml
Na_2S x 9 H_2O (2% v/v)	0.05 ml/10 ml
Yeast extract (10% v/v)	0.05 ml/10 ml
L-Cysteine (10% v/v)	0.05 ml/10 ml

Trace element solution:

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

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1377. HALOGRANUM MEDIUM (NSSM)

Yeast extract	1.00	g
Proteose Peptone No. 3	1.00	g
Casamino acids	1.00	g
Glucose	1.00	g
Sodium pyruvate	0.05	g
K_2HPO_4	0.60	g
NaCl	132.00	g
Natural seawater	1000.00	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1378: MO-CFXI MEDIUM

NaCl	25.000	g
$MgCl_2$ x 6 H_2O	4.000	g
KCl	0.500	g
$CaCl_2$ x 2 H_2O	1.000	g
NH_4Cl	0.535	g
KH_2PO_4	0.100	g
Tryptone	1.000	g
Glucose	1.800	g
Sodium pyruvate	0.550	g
Yeast extract	1.000	g
$NaHCO_3$	2.000	g
Cysteine-HCl	0.300	g
Resazurin (1mg/ml)	1.000	ml
Trace element solution	1.000	ml
Distilled water	1000.000	ml
Na_2S	0.030	%
Vitamin solution	2.000	ml

1. pH 7.0

2. Prepare under N_2/CO_2 (80/20) gas.

3. Autoclave and add the following components after autoclaving from stock solutions:

4. sterile Na_2S solution end concentration 0.03%

5. and filter-sterile Vitamin solution (see below) 0.2 ml / 100 ml.

For DSM 29925: Supplement medium with 10 ml Na-crotonate solution (1 M) from medium 870. Omit Glucose, Tryptone, and Sodium pyruvate. Adjust amount of Yeast extract to 0.5 g.

Trace element solution

$FeCl_2$ x 4 H_2O	1.27	g
$ZnCl_2$	0.14	g
$MnCl_2$ x 4 H_2O	0.20	g
Na_2SeO_4	2.00	mg
H_3BO_3	6.00	mg
Na_2WO_4 x 2 H_2O	3.00	mg
$CuCl_2$ x 2 H_2O	1.00	mg
$CoCl_2$ x 6 H_2O	0.13	g
AlCl3	0.01	g
$NiCl_2$	0.01	g

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Na_2MoO_4 x 2 H_2O	0.02	g
Distilled water	1000.00	ml

Vitamin solution

Biotin	4.90	mg
Folic acid	8.80	mg
Pyridoxine hydrochloride	4.10	mg
Thiamine-HCl x 2 H_2O	6.70	mg
Riboflavin	7.50	mg
Nicotinic acid	2.40	mg
D-Calcium pantothenate	9.50	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	2.70	mg
Lipoic acid	4.10	mg
Distilled water	1000.00	ml

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1381: ½ S° HELIORESTIS MEDIUM

Final pH: 9.0 - 9.5
Final volume: 1000 ml

EDTA (1%)	1.000	ml
KH_2PO_4	0.500	g
NH_4Cl	0.500	g
Bicine	1.630	g
$MgSO_4$ x 7 H_2O	0.200	g
$CaCl_2$	0.075	g
Na_2CO_3	2.500	g
$NaHCO_3$	2.500	g
Na-acetate	1.000	g
Yeast extract	0.100	g
Na_2S x 9 H_2O	0.250	g
Na-pyruvate	1.000	g
Trace elements	1.000	ml
Vitamin B_{12}	20.000	µg
Distilled water	1000.000	ml

Prepare under N_2 gas and add vitamin B_{12}, $NaHCO_3$, Na_2CO_3 and sulfide as single sterile stock solutions to sterile medium after autoclaving. Adjust pH to 9 - 9.5.

Trace elements (from medium 1147)

EDTA	5.20	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 4 H_2O	0.10	g
$FeCl_2$ x 4 H_2O	1.50	g
H_3BO_3	6.00	mg
$CuCl_2$ x 2 H_2O	17.00	mg
Na_2MoO_4 x 2 H_2O	188.00	mg
$NiCl_2$ x 6 H_2O	25.00	mg
$ZnCl_2$	70.00	mg
$VOSO_4$ x 2 H_2O	30.00	mg
Na_2WO_4 x 2 H_2O	2.00	mg
$NaHSeO_3$	2.00	mg
Deionized water	1000.00	ml

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1382. MP MEDIUM

KH_2PO_4	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
NH_4Cl	0.20	g
Yeast extract	0.10	g
Glucose	0.50	g
Trace element solution SLA (see Medium 351)	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 5.0-6.5
Serum flasks are filled with Medium by 5/6 of the total volume.
Sterilize at 121°C for 15 minutes.

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1384. YTC MEDIUM

$CaCl_2$ x 2 H_2O	0.01	g
Tryptone	5.0	g
Yeast extract	0.10	g
Distilled water	1000.00	ml

Adjust pH to 6.8 – 7.2
Sterilize at 121°C for 15 minutes.

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1387. 23% MGM-MEDIUM

NaCl	200.0	g
KCl	4.7	g
$MgCl_2$ x 6 H_20	20.0	g
$MgSO_4$ x 7 H_2O	23.3	g
$CaCl_2$ x 2 H_2O	0.05	g
Peptone from meat	3.40	g
Peptone from soybean	3.3	g
Yeast extract	2.00	g
Proteose peptone	3.3	g
Meat extract	2.00	g
Glucose	0.5	g
Distilled water	1000.00	ml

Adjust to pH 7.0 and autoclave.

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1388. 15% MGM-MEDIUM

Mineral Salts Solution:

Salt Water Solution	500.00	ml
Peptone (Difco)	5.00	g
Yeast extract	1.00	g
Make up distilled water to	1000.00	ml

Adjust to pH 7.0 with 1 M Tris-Base and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

Salt Water Solution (30% Stock Solution):

NaCl	240.0	g
KCl	7.0	g
$MgCl_2$ x 6 H_20	30.0	g
$MgSO_2$ x 7 H_2O	35.0	g
CaCl 1M	5.0	ml
Distilled water	1000.0	ml

Adjust to pH 7.5 with 1 M Tris-HCL and sterilize by autoclaving.

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1389. LAMPROBACTER MEDIUM

KH_2PO_4	0.50	g
NaCl	40.00	g
NH_4Cl	0.50	g
$MgCL_2$ x 6 H_2O	0.20	g
$NaHCO_3$	1.50	g
KCL	0.33	g
Na_2S x 9 H_2O	0.50	g
$Na_2S_2O_3$ x 5 H_2O	0.50	g
Na-acetate	0.50	g
Yeast extract	0.50	g
Trace element solution (see Medium1369)	1.00	ml
Vitamin B_{12}	10	µg
Distilled water	1000.00	ml

Adjust pH to 7.5.

Prepare the medium without the $NaHCO_3$, Na_2S x 9 H_2O, $Na_2S_2O_3$ x 5 H_2O and vitamin B_{12}, under a nitrogen atmosphere. Autoclave and add the vitamin B_{12} from a filter-sterilised stock solution and the $NaHCO_3$, Na_2S x 9 H_2O, $Na_2S_2O_3$ x 5 H_2O from sterile stock solutions. The final pH of the medium should be 7.5.

For *Allochromatium phaeobacterium* DSM 19781 reduce the amount of NaCl to 10.0 g/l and increase the amount of NH_4Cl to 0.64 g/l.

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1391. MMJHSY MEDIUM (modified)

NaCl	20.000	g
K_2HPO_4	0.090	g
KH_2PO_4	0.070	g
$CaCl_2$ x 2 H_2O	0.800	g
NH_4Cl	1.000	g
$NaNO_3$	1.000	g
$MgSO_4$ x 7 H_2O	4.000	g
$MgCl_2$ x 6 H_2O	3.000	g
KCl	0.330	g
$FeSO_4$ x 7 H_2O	0.010	g
$Na_2S_2O_3$ x 5 H_2O	2.000	g
Fe-citrate	0.01	g
Yeast extract	2.00	g
Trace mineral solution (see Medium 1121)	5.000	ml
Distilled water	1000.000	ml

Prepare the medium under N2/CO2 (80%N_2, 20%CO_2) and fill in , the pH of the medium is adjusted with NaOH to 6.8. After autoclaving, the separately autoclaved, concentrated solutions including each of follows are added to the medium. Then a mix gas (80%H_2, 20%CO_2) is purged for 5 min.
Finally, the mix gas (77%H_2, 20%CO_2, 3%O_2) is compressed into gas phase (>80% volume of the tube or bottle) at 2 atm.

Vitamin solution (see below Medium 141)	final concentration 1.0	ml/l
$NaHCO_3$	final concentration 0.1	%

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1393. HALOMONAS WANG MEDIUM

Mineral Salts Solution:

Casamino Acid	5.0	g
Yeast Extract	5.0	g
NaCl	50.0	g
Distilled Water	1000.0	ml

Adjust to pH 9.0 – 9.5 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1395. CHROMOHALOBACTER MEDIUM

NaCl	41.000	g
$MgCl_2$ x 6 H_2O	7.000	g
MgSO x 7 H_2O	9.600	g
$CaCl_2$ x 2 H_2O	0.360	g
KCl	2.000	g
$NaHCO_3$	0.060	g
NaBr	0.026	g
Peptone	5.000	g
Yeast Extract	10.000	g
Glucose	1.000	g
Distilled Water	1000.000	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1396. HALOARCHAEAL MEDIUM MH-1

L-Glutamic Acid	2.000	g
Trisodium Citrate Dihydrate	2.000	g
KH_2PO_4	1.000	g
NH_4Cl	1.000	g
K_2SO_4	5.000	g
NaCl	200.000	g
Casamino Acids	4.000	g
Yeast Extract	2.000	g
$FeSO_4$ x 6H2O	0.004	g
Trace Elements (see below)	2.000	ml
Agar	20.000	g
Distilled Water	800.000	ml

Adjust pH to 4.5-5.0 with.

Adjust to pH 4.5 with 40% KOH and sterilize by autoclaving. The $MgCl_2$ x $6H_2O$ concentration of strain MH1-52-1 (DSM 22442) for well growth is 10.0 mM (20.0 g/l) and for strain MH1-16-3 (DSM 22443 is 500 mM (200.0 g /l).

Trace Element Solution:

$CoCl_2$ x 6 H_2O	0.30	g
$MnCl_2$ x 4 H_2O	0.10	g
$ZnCl_2$	0.10	g
H_3BO_4	0.02	g
Na_2MoO_4 x 2 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.10	g
$CuCl_2$ x 6 H_2O	0.01	g
$AlCl_3$	0.04	g
Na_2WO_4 x 2 H_2O	0.02	g
$CaCl_2$ x 2 H_2O	1.00	g
$BaCl_2$ x 2 H_2O	0.01	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
Distilled Water	1000.00	ml

Adjust to pH 4.0.

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1397. GS MEDIUM

KCl	0.355	g
KH_2PO_4	0.140	g
NH_4Cl	0.250	g
$MgCl_2$x 6 H_2O	4.000	g
$MgSO_4$ x 7 H_2O	3.450	g
$CaCl_{2\ x}$ 2 H_2O	0.140	g
NaCl	6.000	g
$NaHCO_3$	5.000	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	2.000	mg
Bacto Yeast extract (Difco)	2.000	g
Trypticase Peptone (BBL)	2.000	g
Glucose	1.800	g
Resazurin	1.000	mg
Na_2S-cysteine solution (see below)	10.00	ml
Trace elements (see below)	10.00	ml
Vitamin solution (see below)	10.00	ml
Distilled water	1000.00	ml

pH 7.0

Prepare medium except vitamin solution, $NaHCO_3$ und Na_2S-cysteine solution under N_2/CO_2 gas. Add vitamin solution (filter sterilized), $NaHCO_3$ (filter sterilized) and Na_2S-cysteine solution(autoclaved) as sterile stock solutions to sterile medium after autoclaving. Adjust pH to 7.0.

<u>Na_2S-cysteine solution</u>

Cysteine-HCl	5.0	g
Na_2S x 9 H_2O	5.0	g
Distilled water	100.0	ml

Prepare under N_2 gas.

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Trace element solution:

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1398. 1/10 PYGV MEDIUM modified

S I (see below)	75.00	ml
S II (see below)	75.00	ml
Trypticase	1.00	g
Yeast extract	0.50	mg
Glucose	1.00	g
L-cysteine HCl	0.30	mg
0.1% Resazurin	0.50	ml
100x Vitamin solution (see below)	10.00	ml
Distilled water make up to	1000.00	ml

pH 7.0

Prepare the medium without the vitamin solution under an atmosphere of N_2 and dispense into Hungate tubes. Seal under an atmosphere of nitrogen with rubber stoppers and autoclave. Aseptically add the vitamin solution from a filter sterilised stock solution to the sterile medium.

S I

K_2HPO_4	6.0	g
Distilled water	1000.0	ml

SII

KH_2PO_4	6.0	g
$(NH_4)_2SO_4$	12.0	g
NaCl	12.0	g
$MgSO_4$ x 7 H_2O	1.2	g
$CaCl_2$ x 2 H_2O	1.2	g
Distilled water	1000.0	ml

100x Vitamin solution

Biotin	0.1	g
Cobalamine	0.1	g
Thiamine-HCl x 2 H_2O	0.5	g
p-Aminobenzoic acid	0.3	g
Pyridoxine	1.5	g
Riboflavin	0.5	g
Folic acid	0.5	g
Distilled water	1000.0	ml

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1399. HALOPHILIC MEDIUM

Mineral Salts Solution:

Proteose Peptone No. 3 (Difco)	5.0	g
Yeast Extract	5.0	g
Glucose	1.0	g
Total Salts (see below)	666.0	ml
Distilled Water	333.0	ml

Total Salts:

NaCl	234.0	g
$MgCl_2$ x 6 H_2O	42.0	g
$MgSO_4$ x 7 H_2O	60.0	g
$CaCl_2$ x 2 H_2O	1.0	g
KCl	6.0	g
NaBr	0.7	g
$NaHCO_3$	0.2	g
$FeCl_3$ x 6 H_2O (0,5 %)	9	drops
Distilled Water	1000.0	ml

Make up to 1000.0 ml. Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1400. SALINIBACTER HALOPHILIC MEDIUM

Mineral Salts Solution:

NaCl	200.0	g
$MgSO_4$ x 7 H_2O	20.0	g
KH_2PO_4	0.3	g
Yeast Extract	0.5	g
Bacto Peptone	0.5	g
Casamino Acids	0.5	g
Glucose	0.5	ml
Starch	0.5	g
Sodium Pyruvate	0.3	g
Distilled Water	1000.0	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1401. MSYS MEDIUM

KH_2PO_4	1.0	g
NaCl	0.2	g
$MgCl_2$ x 6 H_2O	0.2	g
$CaCl_2$ x 2 H_2O	0.045	g
$(NH_4)_2SO_4$	1.0	g
Yeast extract	0.5	g
Malate	1.0	g
Succinate	1.0	g
Trace elements solution (see below)	1.0	ml
Distilled water	1000.0	ml
pH 6.5		

Prepare the medium under a N_2 atmosphere.

Trace elements solution

Distilled water	1000.00	ml
Na_2-EDTA	5.00	g
$FeCl_2$ x 4 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	110.00	mg
$MnCl_2$ x 2 H_2O	30.00	mg
$ZnCl_2$	50.00	mg
$NiSO_4$ x 6 H_2O	30.00	mg
Na_2MoO_4 x 2 H_2O	30.00	mg
H_3BO_3	300.00	mg
$CuCl_2$ x 2 H_2O	10.00	mg

Adjust pH to 6.0.

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1403. METHYLOROSULA MEDIUM V-022

Mineral Salts Solution:

$(NH_4)_2SO_4$	0.05	g
$MgCl_2$ x 6 H_2O	0.04	g
KH_2PO_4	0.07	g
Trace Element Solution (see below)	1.00	ml
Distilled Water	1000.00	ml

Trace Element Solution:

Na_2EDTA	500.0	mg
$FeSO_4$ x 7 H_2O	200.0	mg
$ZnSO_4$ x 7 H_2O	10.0	mg
$MgCl_2$ x 6 H_2O	3.0	mg
H_3BO_3	30.0	mg
$CoCl_2$ x 6 H_2O	20.0	mg
$CaCl_2$ x 2 H_2O	1.0	mg
$NiCl_2$ x 6 H_2O	2.0	mg
Na_2MoO_4 x 2 H_2O	3.0	mg
Distilled Water	1000.0	ml

Prepare the liquid medium. Adjust to pH 6.0. Dispense the medium in tubes or flasks fitted with screw caps. Sterilize by autoclaving. Before inoculation add filter sterilized CH_3OH to a final concentration of 0.5% v/v.

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1404. MEDIUM FOR STRAIN 2M70-1

Mineral Salts Solution:

HEPES (10M)	2.38	g
NaCl	30.00	g
$MgSO_4$ x 7 H_2O	2.70	g
$MgCl_2$ x 6 H_2O	4.30	g
NH_4Cl	0.25	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4 x 3H_2O	0.14	g
Peptone	2.00	g
Yeast Extract	0.20	g
Trace Elements (see below)	1.00	ml
Vitamin Solution DSM 141 (see below)	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (2% v/v)	1.00	ml
Distilled Water	1000.00	ml

Trace Element Solution:

Na_2EDTA	29.00	g
$MnSO_4$ x H_2O	3.26	g
$CoCl_2$ x 6 H_2O	1.80	g
$ZnSO_4$ 7 H_2O	1.00	g
$NiSO_4$ x 6 H_2O	0.11	g
$CuSO_4$ x 5H_2O	0.10	g
H_3BO_3	0.10	g
$KAI(SO_4)_2$ x 12 H_2O	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_3	0.05	g
Distilled Water	1000.00	ml

Dissolve Na_2EDTA x 2 H_2O and adjust pH 8.0 with KOH before adding the minerals to the solution.

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Vitamin Solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Prepare the medium without the vitamin solution. Adjust to pH 7.25. Dispense the medium into Hungate tubes or serum bottles. Sterilize by autoclaving. Before inoculation add the sterile vitamin solution.

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1405. PYG MEDIUM MODIFIED

Mineral Salts Solution:

Peptone	5.0	g
Meat Extract	3.0	g
Yeast Extract	1.0	g
Glucose	5.0	g
Distilled Water	1000.0	ml

Adjust to pH 7.2 and sterilize by autoclaving. The medium may be solidified by adding 15.0 g/l agar.

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1406. HALARSENATIBACTER MEDIUM

Solution A:

NaCl	151.0	g
Na_2SO_4	84.0	g
K_2SO_4	25.0	g
$(NH_4)_2SO_4$	0.05	g
KH_2PO_4	0.08	g
K_2HPO_4	0.15	g
$MgSO_4$ x 7 H_2O	0.025	g
Na_2WO_4	0.075	g
H_3BO_3	4.0	g
Na_2SeO_4	0.01	mg
Trace Element Solution SL10 (see below)	3.0	ml
Na_2CO_3	36.0	g
$NaHCO_3$	6.0	g
Yeast extract (after autoclaving)	1.0	g
Na-lactate (after autoclaving)	10.0	mM
Arsenate (after autoclaving)	5	mM
Cysteine HCl	0.025	%
Vitamin solution med. 141 (after autoclaving)	10.0	ml
Distilled Water	1000.0	ml

Vitamin Solution Med. 141:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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Trace Element Solution SL10:

HCl (25%)	7.700	ml
$FeSO_4$ x 7 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.100	g
H_3BO_3	0.300	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled Water	1000.0	ml

Prepare the liquid medium without the following. $NaHCO_3$, yeast extract, Na-lactate, arsenate. Adjust to pH 9.5. Boil the medium in a water bath and cool under N_2. to room temperature. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2. Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

$NaHCO_3$	6.0	g/l
Yeast extract (after autoclaving)	1.0	g/l
Na-lactate (after autoclaving)	10.0	mM
Arsenate (after autoclaving)	5	mM
Cysteine HCl	0.025	%
Vitamin Solution Med. 141 (after autoclaving)	10.0	ml/l

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1407. PSYCHROBACTER MEDIUM

Mineral Salts Solution:

Na_2HPO_4	11.2	g
KH_2PO_4	4.0	g
NaCl	5.0	g
NH_4Cl	2.0	g
$MgSO_4$ x 7 H_2O	1.0	g
$CaCl_2$ (1% v/v)	1.0	ml
Peptone	2.0	g
Yeast Extract	1.0	g
Trace Element Solution SL10 (see below)	10.0	ml
Distilled Water	1000.0	ml

Trace Element Solution SL10:

HCl (25%)	7.700	ml
$FeSO_4$ x 7 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.100	g
H_3BO_3	0.300	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled Water	1000.0	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1409. METHYLOCYSTIS MEDIUM

Minaeral Salts Solution:

KNO_3	0.20	g
KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_2O	0.05	g
$CaCl_2$ x 2 H_2O	0.01	g
NaCl	0.01	g
Trace Element Solution (see below)	1.00	ml
Distilled Water	1000.00	ml

Trace Element Solution:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
Distilled water	1000.00	ml

Prepare the liquid medium. Final pH 5.8-6.2. Dispense the medium into serum bottles with a medium to headspace ratio of about 1:4. Add 20% methane to the gas phase. Sterilize by autoclaving. Incubate the cultures with shaking.

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1410. HALOBACTEROIDES MEDIUM

Mineral Salts Solution:

NH_4Cl	1.0	g
K_2HPO_4	0.3	g
KH_2PO_4	0.3	g
$CaCl_2$ x 2 H_2O	0.1	g
NaCl	250.0	g
KCl	0.1	g
KNO_3	2.0	g
Trace Element Solution (see medium 141) (see below)	10.0	ml
Distilled Water	1000.0	ml

Trace Element Solution

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Distilled water	1000.00	ml

Prepare the liquid medium. Adjust to pH 6.3 with 10 M KOH solution. Boil the medium in a water bath and cool under N_2:CO_2 (80:20 v/v) to room temperature. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2:CO_2 (80:20 v/v). Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

Glucose (10%)	40.0 ml/l
Yeast Extract (10%)	10.0 ml/l
$MgCl_2$x $6H_2O$ (10%)	10.0 ml/l
$NaHCO_3$ (10%)	20.0 ml/l

Final pH 7.2 – 7.4

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1411. HALORUBRUM MEDIUM

Mineral Salts Solution:

NaCl	195.00	g
$MgCl_2$	32.50	g
$MgSO_4$ x 7 H_2O	50.80	g
$CaCl_2$ x $2H_2O$	0.80	g
KCl	5.00	g
$NaHCO_3$	0.16	g
NaBr	0.60	g
Yeast extract	5.00	g
Distilled water	1000.00	ml

Adjust to pH 7.0 – 7.5 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1412. METHYLOTHERMUS MEDIUM

Mineral Salts Solution for strain HTM55:

NaCl	3.00	g
KH_2PO_4	14.00	mg
CaCl x 2 H_2O	80.00	mg
$MgSO_4$ x 7 H_2O	0.34	g
$MgCl_2$ x 6 H_2O	0.42	g
KCl	33.00	g
NH_4Cl	0.25	g
$NaNO_3$	0.25	g
Na_2SiO_3 x 9 H_2O	0.20	mg
$Fe_2(SO_4)3$ x n H_2O	20.00	mg
Na_2SeO_3 5 H_2O	0.50	mg
$NiCl_2$ x 6 H_2O	0.50	mg
Trace Element Solution (see below)	3.00	ml
Vitamin Solution (see below)	1.00	ml
Distilled Water	1000.00	ml

Mineral Salts Solution for strain IT-4 and IT-9:

NaCl	30.00	g
KH_2PO_4	140.00	mg
CaCl x 2 H_2O	800.00	mg
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
NH_4Cl	0.25	g
$NaNO_3$	0.25	g
Na_2SiO_3 x 9 H_2O	0.20	mg
$Fe_2(SO_4)3$ x n H_2O	20.00	mg
Na_2SeO_3 5 H_2O	0.50	mg
$NiCl_2$ x 6 H_2O	0.50	mg
Trace Element Solution (see below)	3.00	ml
Vitamin Solution (see below)	1.00	ml
Distilled Water	1000.00	ml

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Trace Element Solution:

Nitrilotriacetic Acid	1.50	g
$MnSO_4$ x 2 H_2O	0.50	g
$CoSO_4$ x 7 H_2O	0.18	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)$ x 12 H_2O	0.02	g
H_3BO_4	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
Distilled Water	1000.00	ml

Vitamin Solution:

Biotin	2.0	mg
Folic Acid	2.0	mg
Pyridoxine-HCl	10.0	mg
Thiamine-HCl	5.0	mg
Riboflavin	5.0	mg
Nicotinic Acid	5.0	mg
D-Ca-Pantothenate	5.0	mg
Vitamin B_{12}	0.1	mg
p-Aminobenzoic Acid	5.0	mg
Lipoic Acid	5.0	mg
Distilled Water	1000.000	ml

Prepare the liquid medium. Adjust to pH 6.2 with HCl or NaOH. Dispense the medium into serum bottles under gassing with N_2:CO_2 (80:20 v/v) for one minute. 20% methane and 5% air is added to the gas phase
Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

Vitamin solution	1.0	ml/l
$NaHCO_3$ (5% w/v)	50.0	ml/l
$CuSO_4$ x 5 H_2O (0.1 M)	10.0	ml/l

For DSM 109509 use Medium 1412 for strain IT4:

Before inoculation add from sterile stock solutions:

Vitamin solution	1.0	ml/l
$NaHCO_3$ (10% w/v)	1.0	ml/l
$CuSO_4$ x 5 H_2O (1 mM)	1.0	ml/l

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1414. PORPHYROBACTER MEDIUM

Mineral Salts Solution:

$MgSO_4$ x 7 H_2O	2.0	g
NH_4Cl	0.3	g
KCl	0.3	g
Na_2SO_4	15.0	g
Na Acetate	1.0	g
Malic Acid	1.0	g
Yeast Extract	1.0	g
Bactopeptone	0.5	g
Trace Element Solution (see below)	2.0	ml
Vitamine Solution (see below)	2.0	ml
Distilled Water	1000.0	ml

Adjust to pH 5.9 before autoclaving

Trace Element Solution:

$FeSO_4$ x $7H_2O$	300.0	mg
$MnCl_2$ x 4 H_2O	3.0	mg
$CoCl_2$ x 6 H_2O	5.0	mg
$CuCl_2$ x 2 H_2O	1.0	mg
$NiCl_2$ x 6 H_2O	2.0	mg
Na_2MoO_4 x 2 H_2O	3.0	mg
$ZnSO_4$ x 7 H_2O	5.0	mg
H_3BO_3	2.0	mg
Distilled Water	1000.0	ml

Adjust to pH 3.0

Vitamine Solution:

Nicotinic Acid	400.0	mg
Thiamine	400.0	mg
Biotine	80.0	mg
Vitamin B_{12}	20.0	mg
Distilled Water	1000.0	ml

Filtersterilized

Prepare the liquid medium. Before inoculation add from sterile stock solutions:

$NaHCO_3$ (10% w/v)	5.0	ml/l
$CaCl_2$ (10% w/v)	20.0	ml/l
KH2PO4 (10% w/v)	3.0	ml/l
Vitamine Solution	2.0	ml/l

Adjust to pH 8.0 - 8.5 with 0.5 N NaOH.

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1415. LYALIKOVIELLA MEDIUM

Mineral Salts Solution:

NH_4Cl	0.5	g
KH_2PO_4	0.5	g
NaCl	25.0	g
KCl	0.5	g
$MgSO_4$ x 7 H_2O	0.2	g
Na_2SO_4	0.5	g
Yeast Extract	1.0	g
Soluble Starch	1.0	g
Trace Element Solution SL10 (see below)	1.0	ml
Distilled Water	1000.0	ml

Trace Element Solution SL10:

HCl (25%)	7.700	ml
$FeSO_4$ x 7 H_2O	1.500	g
$ZnCl_2$	0.070	g
$MnCl_2$ x 4 H_2O	0.100	g
H_3BO_3	0.300	g
$CoCl_2$ x 6 H_2O	0.190	g
$CuCl_2$ x 2 H_2O	0.002	g
$NiCl_2$ x 6 H_2O	0.024	g
Na_2MoO_4 x 2 H_2O	0.036	g
Distilled Water	1000.000	ml

Prepare the liquid medium. Adjust to pH 8.5. Before inoculation add from sterile stock solutions:

$NaHCO_3$	50.000	g/l
CaCl2 x 2 H2O	0.500	g/l
Vitamine B_{12} (0.1mg/mL)	0.150	ml/l

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1416. MST-MEDIUM, AUTOTROPHIC GROWTH

Mineral Salts Solution:

$Na_2S_2O_3$ x 5 H_2O	5.0	g
NH_4Cl	1.0	g
$MgSO_4$ x 7 H_2O	0.5	g
K_2HPO_4	4.0	g
KH_2PO_4	1.5	g
Trace element solution SL-10 (see below)	1.0	ml
Distilled Water	1000.0	ml

Prepare separate solutions of sodium thiosulfate and magnesium sulfate. Adjust solutions to pH 7.5 -8.0 and sterilize by autoclaving. Add sterile trace element solution after autoclving and combining the solutions.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.0	ml
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	6.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	990.0	ml

First dissolve $FeCl_2$ in the hydrochloric acid, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

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1417. MMB MEDIUM

Mineral Salts Solution:

Peptone	0.15	g
Yeast Extract	0.15	g
Ammonium Sulfate	0.25	g
Glucose	1.00	g
Modified Hutner`s Basal Salts Solution (see below)	20.00	ml
Vitamine Solution (see below)	10.00	ml
Distilled Water	1000.00	ml

Vitamine Solution:

Biotin	2.0	mg
Folic Acid	2.0	mg
Thiamine HCl	5.0	mg
D Calcium Panthothenate	5.0	mg
Vitamin B_{12}	0.1	mg
Riboflavin	5.0	mg
Distilled Water	1000.0	ml

Modified Hutner`s Basal Salts Solution:

Nitrilotriacetic Acid	10.00	g
$MgSO_4 \times 7H_2O$	29.70	g
$CaCl_2 \times 2H_2O$	3.34	g
Ammonium Molybdate	9.25	mg
$FeSO_4 \times 7H_2O$	99.00	mg
Metalls 44 (see below)	50.00	ml
Distilled Water	1000.00	ml

Metalls 44:

EDTA	0.250	g
$ZnSO_4 \times 7H_2O$	1.100	g
$FeSO_4 \times 7H_2O$	0.500	g
$MnSO_4 \times 7H_2O$	0.154	g
$CuSo_4 \times 5H_2O$	0.040	g
$Co(NO_3)_2 \times 6H_2O$	0.025	g
$Na_2B_4O_7 \times 10H_2O$	0.018	g
Distilled Water	1000.000	ml

Initially add a few drops of H_2SO_4 to the distilled water to retard precipitation. Dissolve the nitrilotriacetic acid first and neutralize the solution with KOH. Add other

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ingredencients and readjust the pH with KOH and/or H_2SO_4 to 7.2. There may be a slight precipate. The medium may be solidified by adding 15.0 g/l agar.

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1419. HYPHOMICROBIUM MEDIUM

Mineral Salts Solution A:

KH_2PO_4	1.36	g
Na_2HPO_4 x $2H_2O$	2.68	g
Methylamine-HCl	3.38	g
Distilled Water	50.00	ml

Adjust pH to 7.5

Mineral Salts Solution B:

$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x $7H_2O$	0.20	g
Bacto Peptone	0.25	g
Trace Element Solution (see below)	2.00	ml
Hutner`s Basal Salts Solution (see below)	20.00	ml
Vitamine Solution (add after autoclaving, see below)	10.00	ml
Vitamin B_{12} (0.1%)(add after autoclaving)	2.50	µl
Distilled Water	1000.00	ml

Adjust pH to 7.5
For solid media add 15.0 g agar to solution B
Autoclave solution A and B separately and combine after cooling.

Trace Element Solution:

$CaCl_2$ x $2H_2O$	0.199	g
$FeSO_4$ x $7H_2O$	0.100	g
$MnSO_4$ x H_2O	0.035	g
Na_2MoO_4 x $2H_2O$	0.050	g
Distilled Water	100.00	ml

Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

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***"Metals 44"*:**

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

Vitamin solution:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine-HCl	10.000	mg
Thiamine-HCl x 2 H_2O	5.000	mg
Riboflavin	5.000	mg
Nicotinic acid	5.000	mg
D-Ca-pantothenate	5.000	mg
Vitamin B_{12}	0.100	mg
p-Aminobenzoic acid	5.000	mg
Distilled water	1000.000	ml

Prepare the liquid medium. Before inoculation add from sterile stock solutions:

Vitamin Solution	10.00	ml
Vitamin B_{12} (0.1%)	2.50	µl

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1421. NATRONORUBRUM THIOOXIDANS MEDIUM

Mineral Salts Solution:

NaCl	240.0	g
K_2HPO_4	3.5	g
$(NH_4)_2SO_4$	0.5	g
Trace Element Solution (see below)	1.0	ml
Distilled Water	1000.0	ml

Trace Element Solution (Pfennig & Lippert):

EDTA (Trilon B)	5.00	g
$FeSO_4$ x $7H_2O$	2.00	g
$ZnSO_4$	0.10	g
$MnCl_2$	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ 6 H_2O	0.20	g
$CuCl_2$	0.01	g
$NiCl_2$ x $2H_2O$	0.02	g
Na_2MoO_4 x $2H_2O$	0.02	g
Distilled Water	1000.00	ml

Prepare the liquid medium. Adjust to pH 7.5. Before inoculation add from sterile stock solutions:

Na-acetate (20 mM)	16.0	ml/l
$MgSO_4$ (2mM)	6.0	ml/l
Yeast Extract	1.0	g/l

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1423. METHYLOSINUS MEDIUM MM2

Mineral Salts Solution:

KNO_3	0.2	g
KH_2PO_4	0.04	g
$MgSO_4$ x 7 H_2O	0.02	g
$CaCl_2$ x 2 H_2O	0.004	g
Trace Elements	0.2	ml
Distilled Water	1000.0	ml

Trace elements Solution:

EDTA	5.00	g
$CuCl_2$ x 5 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	2.00	g
$ZnSO_4$ x 7 H_2O	0.10	g
H_3BO_3	0.30	g
$NiCl_2$ x 6 H_2O	0.02	g
$CoCl_2$ x 6 H_2O	0.20	g
Na_2MoO_4	0.03	g
$MnCl_2$ x 4 H_2O	0.03	g
Distilled Water	1000.00	ml

Prepare the liquid medium. Adjust to pH 5.0. Dispense the medium into serum bottles under gassing with CH_4 (20% v/v). Sterilize by autoclaving.

For **DSM 29914** use 1 mL of trace elements, 20-50% methane in the headspace, and pH 6.0 (5.2-6.5)

For **DSM 13967** use 1 mL of trace elements, 20% methane in the headspace, and pH 4.5-5.0

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1424. YED ACETOBACTER MEDIUM

Glucose	7.0	g
Yeast Extract	4.0	g
Distilled Water	1000.0	ml

Adjust to pH 7.0. The medium may be solidified by adding 20.0 g/l agar.

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1426. SSE/HD 1:10 MEDIUM

Peptone	0.50	g
Yeast extract	0.25	g
Glucose	0.10	g
MES (10 mM final concentration)	1.95	g
SSE (double concentrated) (see below)	500.00	ml
Distilled water	500.00	ml

adjust pH to 5.5

Add to 1000 ml of medium after autoclaving:

Trace Element Solution SL-10 (see below)	1.0	ml
Vitamin Solution (see below)	1.0	ml

SSE (double concentrated):

$CaCl_2$ x 2 H_2O	0.2938	g
NH_4Cl	0.1069	g
$MgCl_2$ x 6 H_2O	0.2036	g
$(NH_4)_2SO_4$	0.1983	g
$MgSO_4$ x 7 H_2O	0.7390	g
$CaSO_4$ x 2 H_2O	0.8606	g
$Ca(NO_3)_2$ x 4 H_2O	0.2360	g
Na NO_3	0.4240	g
KH_2PO_4 (100 mM solution)	0.5000	ml
$FeSO_4$ x 7 H_2O	0.0111	g
K_2SO_4	0.0870	g
add distilled water	1000.0000	ml

Dissolve by shaking overnight. Sterilize by autoclaving.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in HCl, then dilute with water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1427. DILUTE FILTERED MALT EXTRACT AGAR

Solution A:

Malt extract (food grade)	5	g
Dist. Water	100	ml

Autoclave for 15 min. and filter the cooled liquid until clear; fill up to 1 L.

Agar	15	g

Autoclave at 121°C for 15 min.

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1428. HALOMONAS MEDIUM FOR STRAIN MA-C

NaCl	100.0	g
MgCl x 6 H_2O	10.0	g
KCl	1.0	g
Na_2SO_4	0.5	g
Yeast extract	5.0	g
Tryptone	5.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0. The medium may be solidified by adding 20.0 g/l agar.

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1429. SABOURAUD GLUCOSE MEDIUM

SABOURAUD-2% Glucose-Bouillon (Merck 108339)	30.0	g
Agar	15.0	g
Distilled water	1000.0	ml

For DSM 6160, DSM 13536 after autoclaving add 3.2 ml/L of filter sterilized acetic acid (99% w/v).
The pH should be 3-4.

For DSM 5432 after autoclaving add 1.0 ml/L of filter sterilized acetic acid (99% w/v).
The pH should be 4-5.

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1430. GLUCONACETOBACTER MEDIUM

Yeast extract	3.0	g
Peptone	4.0	g
Glucose	5.0	g
Acetic acid	30.0	ml
Ethanol	30.0	ml
Distilled water	940.0	ml

The ethanol and acetic acid are sterilized separately by filtration (use filters resistant to these solvents) and added aseptically to the sterile medium. The medium may be solidified by adding 15.0 g/l agar.

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1431. ALKALOPHILIC HALOPHILE MEDIUM

Yeast extract	10.0	g
Casamino acid	7.5	g
Trisodium citrate	3.0	g
KCl	2.0	g
$MgSO_4$ x 7 H_2O	1.0	g
$MnCl_2$ x 4 H_2O	0.36	mg
$FeSO_4$ x 7 H_2O	50.0	mg
NaCl	200.0	g
Na_2CO_3	50.0	g

Liquid medium may be prepared by adding all components together. Agar (2%) medium should be prepared without Na_2CO_3 and sterilised by autoclaving. Add 20-30 ml of a sterile 10% Na_2CO_3 solution to the autoclaved medium containing agar so that the final pH is 9.0.

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1432. ENHYDROBACTER MEDIUM

Mineral salt sol. ("Hutner/Cohen-Bazire")	20.0	ml
Casamino acids (BD 223050)	1.0	g
Glucose	1.0	g
Agar (Bacto)	15.0	g
Distilled water	980.0	ml

Sterilize 20 min. /121°C. After cooling to 60°C add to the medium:

Biotin	0.02	mg

Mineral salt solution:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_2O	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
Na_2MoO_4 x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
Metal salt sol. "Metals 44"	50.00	ml
Distilled water	900.00	ml

Dissolve NTA first by neutralizing with KOH, then add other salts. Adjust pH to 7.2 with KOH or H_2SO_4. Adjust volume to 1000.0 ml with distilled water.

"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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1433. ACIDO-THERMOPHILE MEDIUM

NH_4Cl	1.0	g
KH_2PO_4	1.0	g
Na_2HPO_4 x 7 H_2O	0.1	g
$MgSO_4$ x 7 H_2O	0.2	g
$CaCl_2$ x $2H_2O$	0.02	ml
Yeast extract (Oxoid)	1.0	g
Sigmacell alpha Type 50 (Sigma S5504)	5.0	g
Cellobiose	0.5	g
Agar	20.0	g
Distilled water	1000.00	ml

Adjust to pH 5.2 with H_3PO_4 prior to addition of carbon sources.
Autoclave at 121°C for 15 minutes.

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1434. ALTEROMONAS MEDIUM

$(NH_4)_2SO_4$	2.0	g
K_2HPO_4	2.0	g
KH_2PO_4	1.0	g
NaCl	20.0	g
$MgSO_4$ x 7 H_2O	0.3	g
Trace elements (see below)	1.0	ml
Vitamin B_{12}	0.1	mg
Methanol	10.0	ml
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.0

Prepare the medium without methanol and vitamin B_{12} in closed vessels with a medium to headspace ratio of 1:5 to 1:10. Adjust pH to 7.0 and sterilize. Allow to cool to about 45°C before adding 1mL filter-sterilized Vitamin B_{12} stock solution (0.1mg/100ml) and 10 mL filter-sterilized methanol per liter medium.

Trace elements:

$MnSO_4$ x H_2O	76.00	mg
$CuSO_4$ x 5 H_2O	25.00	mg
$FeSO_4$ x 7 H_2O	28.00	mg
$ZnSO_4$ x 7 H_2O	0.14	g
$CaCl_2$ x 2 H_2O	15.00	mg
$CoCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x $2H_2O$	24.00	mg
Distilled water	100.00	ml

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1437. ALKALIPHILES PIRELLULA MEDIUM (M1PY)

Solution 1:

Peptone	0.25	g
Yeast extract	0.25	g
$CaCO_3$	5.00	g
Na_2HPO_4 x H_2O	0.10	g
$MgSO_4$ x $7H_2O$	0.50	g
Hutner's basal salts (see medium 590)	20.00	ml
Gellan gum Gelrite	9.00	g
Distilled water	930.00	ml

Adjust pH to 9.0 with NaOH, autoclave at 121°C for 20 min.

Solution 2:

N-acetylglucosamine	2.0	g
Ampicillin sodium salt	0.2	g
Cycloheximid	0.2	g
Vitamin solution (see below) (see medium 621)	10	ml
Distilled water	40	ml

Adjust pH to 9.0 with NaOH, filter-sterilized

After autoclaving add solution 2 to solution 1.

Vitamin solution:

Vitamin B_{12}	0.2	mg
Biotin	4.0	mg
Thiamine-HCl x 2 H_2O	10.0	mg
Ca-pantothenate	10.0	mg
Folic acid	4.0	mg
Riboflavin	10.0	mg
Nicotinamide	10.0	mg
p-Aminobenzoic acid	10.0	mg
Pyridoxine hydrochloride	20.0	mg
Distilled water	1000.0	ml

Filter-sterilized.

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1441. ALKALIPHILES PIRELLULA MEDIUM (M1)

Solution 1:

$CaCO_3$	5.00	g
Na_2HPO_4 x H_2O	0.10	g
$MgSO_4$ x $7H_2O$	0.50	g
Hutner's basal salts (see below) – (medium 590)	20.00	ml
Gellan gum Gelrite	8.00	g
Distilled water	930.00	ml

Adjust pH to 9.0, autoclave at 121°C for 20 min.

Solution 2:

N-acetylglucosamine	2.0	g
Vitamin solution No.6 (see below) - (see medium 621)	10.0	ml
Distilled water	40.0	ml

Adjust pH to 9.0, filter-sterilize, and add to **solution 1**.

Vitamin solution:

Vitamin B_{12}	0.2	mg
Biotin	4.0	mg
Thiamine-HCl x 2 H_2O	10.0	mg
Ca-pantothenate	10.0	mg
Folic acid	4.0	mg
Riboflavin	10.0	mg
Nicotinamide	10.0	mg
p-Aminobenzoic acid	10.0	mg
Pyridoxine hydrochloride	20.0	mg
Distilled water	1000.0	ml

Filter-sterilized.

Continued on next page

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Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44" (see below)	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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1445. ZYMOMONAS MOBILIS MEDIUM

KH_2PO_4	1.0	g
$(NH_4)_2SO_4$	1.0	g
$MgSO_4$ x 7 H_2O	0.5	g
Yeast extract	5.0	g
Glucose	20.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0. Autoclave 20 min at 121°C.

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1446. ALKALISPIRILLUM MOBILE MEDIUM

NaCl	20.00	g
$Na_2S_2O_3$	1.00	g
KH_2PO_4	0.80	g
Na-acetate	2.00	g
Yeast extract	1.00	g
Trace elements (see medium 253)	1.00	ml
$MgCl_2$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.05	g
NH_4Cl	0.80	g
Na_2CO_3	21.20	g
$NaHCO_3$ (8 %)	168.00	ml
Distilled water	832.00	ml

We recommend that liquid and solid media be made by autoclaving the Na_2CO_3 separately, otherwise the high pH will cause ammonia to be lost. When making media solidified with agar (20 g/l) the agar should also not be autoclaved with the Na_2CO_3 otherwise the agar will be hydrolysed. When mixing the ingredients for media cool all components to about 55°C otherwise hydrolysis of the agar may occur. Adjust pH after autoclaving by adding the $NaHCO_3$ (8 %) to pH 9.0. Please note that the $NaHCO_3$ solution should be filter sterilized.

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LENTIBACILLUS MEDIUM

NaCl	100.0	g
Casamino acids	5.0	g
Yeast extract	5.0	g
Glutamic acid	1.0	g
Trisodium citrate	3.0	g
$MgSO_4$ x 7 H_2O	20.0	g
$FeCl_2$ x 4 H_2O	36.0	mg
$MnCl_2$ x 4 H_2O	0.4	mg
Agar, if required	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.2 and autoclave.

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1451: DEFERRISOMA MEDIUM

KH_2PO_4	0.33	g
NH_4Cl	0.33	g
KCl	0.33	g
NaCl	18.00	g
$CaCl_2$ x 2 H_2O	0.33	g
$MgCl_2$ x 6 H_2O	4.00	g
Yeast extract	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Ferric citrate monohydrate	2.50	g
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except ferric citrate, carbonate and vitamins) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferric citrate (dissolve by boiling and adjust pH to 6.5 - 7.0) and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Sterilize vitamins by filtration. Adjust pH of the complete medium to 6.5, if necessary.

For DSM 103037: Supplement autoclaved medium with 0.41 g/l Na-acetate, 0.34 g/l Na -formate, 1.00 g/l D-glucose and 0.10 g/l Na_2S x 9 H_2O added from sterile anoxic stock solutions. Reduce amount of carbonate to 0.50 g/l and adjust pH of complete medium to 5.5 - 6.0.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1452. AQUISALIMONAS MEDIUM

Tryptone	0.5	g
Yeast Extract	2.0	g
Glucose	5.0	g
NaCl	100.0	g
$CaCO_3$	1.0	g
Distilled Water	1000.0	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1453. ALTERYTHROBACTER MEDIUM 216L

Mineral Salts Solution:

Sodium Acetate	1.0	g
Tryptone	10.0	g
Yeast Extract	2.0	g
Sodium Citrate	0.5	g
NH_4NO_3	0.2	g
NaCl	20.0	g
Distilled Water	1000.0	ml

Adjust to pH 7.5 and sterilize by autoclaving. The medium may be solidified by adding 12.0 g/l agar.

Note: Distilled water can be replaced by seawater (e.g., Biomaris MeeresTIEFwasser) if NaCl is omitted.

DSM 25986 shows better growth with seawater than with NaCl.

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1454. MEIOTHERMUS HYPOGAEUS MEDIUM

Bacto Yeast Extract (Difco)	1.00	g
Bacto Tryptone (Difco)	1.00	g
Sodium glutamate monohydrate	1.00	g
$CaSO_4$ x 2 H_2O	0.06	g
$MgSO_4$ x 7 H_2O	0.10	g
NaCl	8.00	mg
KNO_3	0.10	g
$NaNO_3$	0.69	g
Na_2HPO_4	0.11	g
Trace elements solution (see below)(see medium 318)	10.00	ml
Agar (if needed)	20.00	g
Distilled water	1000.00	ml

Adjust pH to 7.5 with NaOH.

Trace element solution:

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_3$ x 6 H_2O	1.35	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	24.00	mg
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$ x 2 H_2O	25.00	mg
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	24.00	mg
NaCl	1.00	g
$NiCl_2$ x 6 H_2O	0.12	g
Na_2SeO_4	4.00	mg
Na_2WO_4	4.00	mg
Distilled water	1000.00	ml

First dissolve the nitrilotriacetic acid and adjust pH to 6.5 with NaOH, and then add minerals. Final pH 7.0.

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1456: DESULFOSOMA MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
NaCl	1.00	g
NH_4Cl	1.00	g
KCl	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
$MgCl_2$ x 6 H_2O	0.50	g
Na_2SO_4	2.80	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-pyruvate	2.50	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, pyruvate, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.0 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1457. ALCALILIMNICOLA EHRLICHII MHLE-1 MEDIUM

Solution A:

$(NH_4)_2SO_4$	0.10	g
$MgSO_4$	25.00	mg
NaCl	60.00	g
K_2HPO_4	0.15	g
KH_2PO_4	0.08	g
Trace element solution SL-10	5.00	ml
0.1 mM Tungstate	0.30	ml
Vitamin solution	1.00	ml
Distilled water	490.00	ml

Solution B:

Na_2CO_3	10.60	g
$NaHCO_3$	4.20	g
Distilled water	500.00	ml

Solution C:

1M Na-acetate	10.00	ml

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Make up to a final volume of 1000.0 ml.

Autoclave solution A without the vitamin solution. Autoclave solution B in half full, closed bottles. Autoclave solution C. Combine the sterile solutions A, B, and C after cooling. Aseptically add 1.0 ml of the vitamin solution (filter sterilized) to 1 litre of the sterile medium.

Adjust to pH 9.8. The medium may be solidified by adding 20 g/l agar, but care should be taken that the temperature of the solutions to be mixed are about 50°C, otherwise the agar will darken and degrade.

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Glucose minerals salts medium

$NaNO_3$	2.0	g
K_2HPO_4	0.5	g
$MgSO_4$ x 7 H_2O	0.2	g
$MnSO_4$ x 5 H_2O	0.02	g
$FeSO_4$ x 7 H_2O	0.02	g
$CaCl_2$ x 7 H_2O	0.02	g
Glucose	5.0	g
Distilled water	1000.0	ml
Agar, if needed	15.0	g

Autoclave glucose separately. Adjust to pH 7.0

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1459: THERMOHALOBACTER MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
NH_4Cl	0.10	g
$MgCl_2$ x 6 H_2O	2.00	g
$CaCl_2$ x 2 H_2O	0.20	g
NaCl	100.00	g
KCl	4.00	g
Na-acetate	0.50	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
D-Glucose	10.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, glucose, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.0 - 7.2, if necessary.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g

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Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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1460. 28% MH MEDIUM

NaCl	195.00	g
$MgCl_2$	32.50	g
$MgSO_4$	50.80	g
$CaCl_2$	0.80	g
KCl	5.00	g
$NaHCO_3$	0.16	g
NaBr	0.60	g
Yeast extract (Difco)	5.00	g
Distilled water	1000.00	ml

Final pH 7.5
Add all the components together, except $NaHCO_3$ which is added from a sterilized stock solution when the autoclaved medium has cooled. Agar is added at a concentration of 20 g/l for solid media.

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1461. SALINIVIBRIO SHARMENSIS MEDIUM

NaCl	100.00	g
Tri-Na citrate	3.00	g
$MgSO_4$ x 7 H_2O	1.00	g
KCl	2.00	g
Na_2CO_3	3.00	g
$MnCl_2$ x 4 H_2O (0.36 g/l)	1.00	ml
$FeSO_4$ x 7 H_2O (50 g/l)	1.00	ml
Yeast extract (Difco)	10.00	g
Distilled water	1000.00	ml

Final pH 9.0

Add all the components together, except Na_2CO_3 which is added from a sterilized stock solution when the autoclaved medium has cooled. Agar is added at a concentration of 20 g/l for solid media and should be cooled to about 55°C before the Na_2CO_3.is added.

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1462: CAULOBACTER PYE MEDIUM

Peptone	2.00	g
Yeast extract (Difco)	1.00	g
$CaCl_2$ x 2 H_2O	0.07	g
Agar, for solid medium (optional)	12.00	g
Distilled water	1000.00	ml

1. Add all the components together, except calcium chloride which is added from a sterilized stock solution when the autoclaved medium has cooled. Agar is added at a concentration of 12 g/l for solid media. For agar media, do not add supplemental calcium.

2. Adjust final pH to 7.0.

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1463: YE/10 + Ca MEDIUM

Yeast extract (Difco)	0.30	g
Peptone	0.06	g
Tris-HCl buffer	0.61	g
$CaCl_2$ x 2 H_2O	0.30	g
Distilled water	1000.00	ml

1. Add all the components together, except calcium chloride which is added from a sterilized stock solution when the autoclaved medium has cooled. Adjust final pH to 7.5.

2. **Propagation of DSM 25223 in liquid cultures:** Add 3 ml of a *Caulobacter vibrioides* DSM 25117 culture (grown for 24 h in DSMZ medium 1462) to 20 ml of medium and inoculate with 1 ml of a liquid culture of DSM 25223 or transfer some material with an inoculation loop from the lysis zone of a double layer agar plate. Incubate with shaking (ca. 150 rpm) for approximately 48 h.

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1464. CHITINIVIBRIO MEDIUM

Mineral Base:

$NaHCO_3$	15.0 g/l
Na_2CO_3	95.0 g/l
NaCl	6.0 g/l
K_2HPO_4	1.0 g/l

Adjust pH to 10.0 with NaOH or HCl. Make anoxic under nitrogen stream and autoclave at 121°C.

After autoclaving add sterile, anoxic solutions of:

Chitin (amorphous) or Sigma C7170	1 – 2g/l (from 5% solution)
$MgSO_4$	1 mM (final concentration)
Yeast extract	20 mg/l
NH_4Cl	4 mM (final concentration)
Na_2S	1 mM (final concentration)
Acidic trace metals (Pfennig & Lippert, 1965)	1 ml/l
Vitamine mixture (Pfennig & Lippert, 1965)	1 ml/l

Acidic trace metals (Pfennig & Lippert, 1965)

1. EDTA	5000 mg/l
2. FeSO4 x 7H2O	2000 mg/l
3. ZnSO4 x 7H2O	100 mg/l
4. MnCl2	30 mg/l
5. H3BO3	300 mg/l
6. CoCl2 x 6H2O	200 mg/l
7. CuCl2	10 mg/l
8. NiCl2 x 2H2O	20 mg/l
9. Na2MoO4 x 2H2O or NH4MoO4	20 mg/l

adjust pH to 3-4 with HCl; autoclave in closed bottles, 120°C, 20 min.

Vitamine mixture (Pfennig & Lippert, 1965)

1: acidic solution (HCl, pH 3; 100 ml)

Thiamin	5 mg
Ca pantotenate	5 mg
B12	0.5 mg

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2: basic solution (100 ml 0.1 N NaOH)	
Biotin	2 mg
PABA	5 mg
Nicotinic acid	5 mg
Pyridoxine	10 mg

3: Neutral solution (100 ml Aqua dem.)	
Folic acid	2 mg
Riboflavin	5 mg

Sterilize by filtration and keep at 4°C. Before addition to the medium mix 1:1:1 (vol:vol:vol).

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1465. CHITINISPIRILLUM MEDIUM

Mineral Base:

$NaHCO_3$	8.0	g
Na_2CO_3	22.0	g
NaCl	6.0	g
K_2HPO_4	1.0	g

Adjust pH to 10.0 with NaOH or HCl. Make anoxic under nitrogen stream and autoclave at 121°C.

After autoclaving add sterile, anoxic solutions of:

Chitin (amorphous)	1 – 2g/l (from 5% solution)
$MgSO_4$	1 mM (final concentration)
Yeast extract	20 mg/l
NH_4Cl	4 mM (final concentration)
Na_2S	1 mM (final concentration)
Acidic trace metals (Pfennig & Lippert, 1965)	1 ml/l
Vitamine mixture (Pfennig & Lippert, 1965)	1 ml/l

Acidic trace metals (Pfennig & Lippert, 1965)

1. EDTA	5000 mg/l
2. FeSO4 x 7H2O	2000 mg/l
3. ZnSO4 x 7H2O	100 mg/l
4. MnCl2	30 mg/l
5. H3BO3	300 mg/l
6. CoCl2 x 6H2O	200 mg/l
7. CuCl2	10 mg/l
8. NiCl2 x 2H2O	20 mg/l
9. Na2MoO4 x 2H2O or NH4MoO4	20 mg/l

adjust pH to 3-4 with HCl; autoclave in closed bottles, 120°C, 20 min.

Vitamine mixture (Pfennig & Lippert, 1965)

1: acidic solution (HCl, pH 3; 100 ml)

Thiamin	5 mg
Ca pantotenate	5 mg
B12	0.5 mg

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2: basic solution (100 ml 0.1 N NaOH)

Biotin	2 mg
PABA	5 mg
Nicotinic acid	5 mg
Pyridoxine	10 mg

3: Neutral solution (100 ml Aqua dem.)

Folic acid	2 mg
Riboflavin	5 mg

Sterilize by filtration and keep at 4°C. Before addition to the medium mix 1:1:1 (vol:vol:vol).

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1467. T5 MEDIUM

Tryptone	0.5	g
Yeast extract	2.0	g
Glucose	5.0	g
NaCl	100.0	g
$CaCO_3$	1.0	g
Agar, if needed	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0

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1468. HALORHABDUS SEGORENSIS S21 MEDIUM

NaCl	41.00	g
$MgCl_2$ x 6 H_2O	203.00	g
$CaCl_2$ x 2 H_2O	29.4	g
KCl	7.5	g
Yeast extract	1.00	g
Casamino acids (with vitamins)	1.00	g
Soluble starch	2.00	g
Agar, if necessary	20.00	g
Distilled water	1000.00	ml

Before autoclaving adjust pH to 7.0 with NaOH.
For solid medium sterilize the $MgCl_2$ x 6 H_2O dissolved in 500 ml distilled water by autoclaving. Dissolve the remaining ingredients in 500 ml distilled water (including 20.0 g agar), sterilise by autoclaving, cool to about 50°C and then mix with the warm sterile $MgCl_2$ solution before pouring the plates.

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1470. MODIFIED CASTENHOLZ MEDIUM

Nitrilotriacetic acid (Titriplex I)	100.00	mg
$MgSO_4$ x 7 H_2O	100.00	mg
$CaSO_4$ x 2 H_2O	60.00	mg
NaCl	8.00	mg
$NaNO_3$	689.00	mg
KNO_3	103.00	mg
Na_2HPO_4 x 2 H_2O	111.00	mg
Tryptone (Oxoid)	5.00	g
Peptone (BD)	5.00	g
Sucrose	2.00	g
$FeCl_3$ x 6 H_2O (w/v=1%)	2.80	mL
Nitsch Element Solution	1.00	mL
Distilled water	1000.00	ml

Adjust pH to 8.2 with NaOH.

Nitsch Element Solution:

H_2SO_4	5.00	mL
$MnSO_4$ x H_2O	2.20	mg
$ZnSO_4$ x 7 H_2O	0.50	mg
H_3BO_3	0.50	mg
$CuSO_4$ x 5 H_2O	16.00	µg
Na_2MoO_4 x 2 H_2O	25.00	µg
$CoCl_2$ x 6 H_2O	46.00	µg
Distilled water	1000.00	ml

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1472. MODIFIED TRYPTONE SOYA BROTH

Trypticase Soy Broth	30.0	g
NaCl	40.0	g
$NaCO_3$	10.0	g
Distilled water	1000.0	ml

Adjust pH to 10.5

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1473. PLANTOMYCETACEAE MEDIUM

KH_2PO_4	0.33	g
NH_4Cl	0.33	g
$MgCl_2$	0.33	g
$CaCl_2$	0.33	g
KCl	0.33	g
Trace Element Solution (see below)	1.00	ml
Distilled water	1000.00	ml

Trace Element Solution:

$(NH_4)_2SO_4FeSO_4 \times 6\ H_2O$	784.0	mg
$NiCl_2 \times 2\ H_2O$	284.0	mg
$CoCl_2 \times 6\ H_2O$	38.0	mg
$MnCl_2 \times 4H_2O$	99.0	mg
$ZnSO_4 \times 7\ H_2O$	144.0	mg
Na_2SeO_4	95.0	mg
$Na_2WO_4 \times 2H_2O$	33.0	mg
$Na_2MoO_4 \times 2\ H_2O$	24.0	mg
H_3BO_3	6.0	mg
$CuCl_2 \times 2\ H_2O$	2.0	mg
Distilled water	1000.0	ml

Prepare the liquid medium. Boil the medium in a water bath and cool under N_2 to room temperature. Adjust to pH 5.8-6.2. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2. Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

KNO_3	1.00	g
Glucose	2.00	g
Yeast Extract (Sigma)	0.05	g
Vitamin Solution (see medium 141)	1.00	ml

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1474. PROLIXIBACTER MEDIUM GYSW

NH_4Cl	0.54	g
KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace elements (see below)	10.00	ml
Vitamin solution (see medium 141)	10.00	ml
Resazurin solution (see below)	1.00	ml
D-Glucose	1.80	g
Yeast extract (Difco)	1.00	g
$NaHCO_3$	2.52	g
Cysteine-HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Prepare the liquid medium without Vitamin solution, Cysteine-HCl x H_2O and Na_2S x 9 H_2O. Adjust to pH 7.0 with 1 M NaOH. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2:CO_2 (80:20 v/v). Sterilize by autoclaving. Before inoculation add from sterile stock solutions Cysteine-HCl x H_2O, Vitamin solution (filter sterilized) and Na_2S x 9 H_2O.

Trace element solution:

nitrilotriacetic acid	12.800	g
$FeCl_3$ x 6 H_2O	1.350	g
$MnSO_4$ x H_2O	0.100	g
$CoCl_2$ x 6 H_2O	0.024	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnCl_2$	0.100	g
$CuCl_2$ x 5 H_2O	0.025	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.024	g
NaCl	1.000	g
$NiCl_2$ x 6 H_2O	0.120	g
Na_2SeO_3 x 5 H_2O	0.004	g
Na_2WO_4	0.004	g
Distilled water	1000.000	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Continued on next page

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Resazurin solution:

Resazurin	1.0	mg
Distilled Water	1.0	L

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1475: METALLIBACTERIUM MEDIUM

$(NH_4)_2SO_4$	132.00	mg
$MgCl_2$ x 6 H_2O	53.00	mg
KH_2PO_4	27.00	mg
$CaCl_2$ x 2 H_2O	147.00	mg
$VOSO_4$ x 5 H_2O	0.50	mg
Wolfe's mineral elixir	1.00	ml
$FeSO_4$ x 7 H_2O	10.00	mg
Trypticase peptone (BD BBL)	1.40	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except vitamins and ferrous sulfate and adjust pH of solution to 5.0. Dissolve ferrous sulfate (1% w/v) in 0.1 N H_2SO_4 and add to the medium prior to autoclaving. After autoclaving add vitamins from a stock solution that has been sterilized by filtration.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg

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Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1476: SALIMESOPHILOBACTER MEDIUM

K_2HPO_4	0.40	g
KH_2PO_4	0.40	g
NH_4Cl	0.50	g
Na_2SO_4	1.00	g
$MgCl_2$ x 6 H_2O	5.00	g
$CaCl_2$ x 2 H_2O	1.00	g
NaCl	30.00	g
KCl	0.70	g
K-acetate	5.00	g
Yeast extract	5.00	g
Trypticase peptone (BD BBL)	5.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1477: CALORAMATOR QUIMBAYENSIS MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	1.50	g
$CaCl_2$ x 2 H_2O	0.10	g
NaCl	20.00	g
KCl	0.10	g
Trace element solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	0.50	g
D-Glucose	3.60	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, glucose and vitamins, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.0 - 7.2, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve

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mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1480. MODIFIED TRYPTONE SOYA BROTH

Trypticase Soy Broth	30.0	g
NaCl	40.0	g
$NaCO_3$	2.5	g
Agar	15.0	g
Distilled water	1000.0	ml

The pH should be 10.5 after autoclaving

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1481. HALOFERAX MEDIUM RS75

NaCl	160.0	g
$MgCl_2$ x 6 H_2O	50.0	g
K_2SO_4	5.0	g
$CaCl_2$ x 2 H_2O	0.1	g
Na pyruvate	2.0	g
Na acetate x 3 H_2O	0.5	g
Na malate	0.5	g
Yeast extract	1.0	g
Tryptone	0.5	g
Glucose	0.5	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 6.8

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1482. ARHODOMONAS MEDIUM RS91

NaCl	100.0	g
$MgCl_2$ x 6 H_2O	10.0	g
KCl	1.5	g
$CaCl_2$ x 2 H_2O	0.2	g
$MgSO_4$ x 7 H_2O	2.0	g
$(NH_4)_2HPO_4$	0.5	g
Na pyruvate	0.4	g
Na acetate	0.8	g
Casein hydrolysate (Oxoid LP0041)	0.4	g
Yeast extract (BD212750)	0.3	g
Tryptone (BD211921)	0.6	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 6.5-7.5

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1483. HALARCHAEUM MEDIUM RS94

NaCl	200.0	g
$MgCl_2$ x 6 H_2O	25.0	g
K_2SO_4	5.0	g
$CaCl_2$ x 2 H_2O	0.1	g
NaH_2PO_4 x 2 H_2O	0.2	g
Na_2HPO_4 x 12 H_2O	0.4	g
Na pyruvate	2.0	g
Na acetate	0.5	g
Na malate	0.5	g
Casein hydrolysate (Oxoid LP0041)	2.0	g
Yeast extract (Fisher BP1422-500)	1.0	g
Tryptone (BD211921)	0.5	g
Glucose	0.5	g
Trace elements solution (see below, see also medium 1369)	1.0	ml
Agar	20.0	g
Distilled water	1000.0	ml

Adjust to pH 7.1

Trace elements solution (Pfennig & Lippert,1966)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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1484. Glycerol-Yeast extract-Peptone Medium

Bacto-peptone	3.0	g
Yeast extract	5.0	g
Betaine	1.25	g
Sodium pyruvate	1.25	g
(Agar, if needed	15.0	g
Trace element solution (see medium 141)	10.0	ml
Vitamin solution (see medium 141)	10.0	ml
Glycerol	10.0	ml
Distilled water	970.0	ml

Adjust to pH 7.2

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1485: DESULFONATRONUM ALKALITOLERANS MEDIUM

Solution A	950.00	ml
Solution B	1.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml
Solution H	10.00	ml

1. Dissolve ingredients of solution A except carbonates, sparge with 100% N_2 gas for 30 - 45 min to make it anoxic, then add bicarbonate and carbonate, adjust pH to 9.0, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

2. Prior to inoculation complete the medium by adding appropriate amounts of sterile solutions B to H prepared under 100% N_2 gas atmosphere. Adjust pH of the complete medium to 8.7 - 9.0, if necessary.

Solution A

NaCl	6.00	g
K_2HPO_4	1.00	g
$NaHCO_3$	32.00	g
Na_2CO_3	10.60	g
Na_2SO_4	2.80	g
Na-acetate	0.20	g
Distilled water	950.00	ml

Solution B

Trace elements solution (Pfennig & Lippert,1966)	1.00	ml

Solution C

Selenite-tungstate solution	1.00	ml

Solution D

NH_4Cl	0.20	g
Distilled water	10.00	ml

Solution E

$MgCl_2$ x 6 H_2O	0.20	g

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Distilled water	10.00	ml

Solution F

Yeast extract	20.00	mg
Distilled water	10.00	ml

Solution G

Na-formate	3.40	g
Distilled water	10.00	ml

Solution H

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	ml

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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1486. GBS salts modified for Thermocrinis jamiesonii

NaH_2PO_4	1.380	g
NaCl	3.000	g
NH_4Cl	0.107	g
$CaCl_2$ x 2 H_2O	0.015	g
$MgSO_4$ x 7 H_2O	0.123	g
KCl	0.150	g
Na_2SO_4	0.300	g
Mineral solution	5.00	ml
Distilled water	1000.00	ml

Final pH 7.25

Mineral solution :

EDTA-Na_2	3.7000	g
$FeSO_2$ x 7 H_2O	1.1000	g
$ZnSO_4$ x 7 H_2O	0.0288	g
$MnCl_2$ x 4 H_2O	0.0984	g
H_3BO_3	0.0310	g
$CoCl_2$ x 6 H_2O	0.0238	g
$CuCl_2$ x 2 H_2O	0.0172	g
$NaMoO_4$ x 2 H_2O	0.0242	g
Distilled water	1000.0	ml

Adjust pH to 6.0 with KOH

Prepare the medium under a nitrogen atmosphere. Dispense 5 ml into 25 ml tubes fitted with rubber stoppers.
Autoclave 121°C 20 min and add 2mM (final concentration) sodium acetate trihydrate, 2mM (final concentration)sodium thiosulfate pentahydrate and 1mM (final concentration) sodium bicarbonate from anaerobic, autoclaved stock solutions(100x).
Before inoculation 2% sterile O2 or 10% sterile air is added to the headspace.

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1487: MAGNETOVIBRIO MEDIUM

NaCl	16.50	g
$MgCl_2$ x 6 H_2O	3.50	g
Na_2SO_4	2.75	g
KCl	0.50	g
$NaNO_3$	0.50	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	0.25	g
Trace element solution	10.00	ml
Na-acetate	0.25	g
Disodium succinate	0.50	g
Casamino acids (BD Bacto)	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
K_2HPO_4	0.15	g
Na_2CO_3	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except hydrogenphosphate, carbonate, vitamins and cysteine), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add hydrogenphosphate, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1488. METHYLOCEANUS CENITEPIDUS MEDIUM

Solution 1 (10x NMS salts):

NH_4Cl	10.00	g
$MgSO_4$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	2.00	g
2% NaCl in distilled water	1000.00	ml

Dissolve the ingredients listed above (in that order) in about 700 ml of 2% NaCl, and then make up to 1 litre with 2% NaCl.

Solution 2 (Fe EDTA):

Fe EDTA	3.80	g
Distilled water	100.00	ml

Solution 3 (Sodium molybdate):

Na_2MoO_4 x 2 H_2O	0.26	g
Distilled water	1000.00	ml

Trace elements:

EDTA di sodium salt	25.00	mg
$CuSO_4$ x 5 H_2O	20.00	mg
$FeSO_4$ x 7 H_2O	50.00	mg
$ZnSO_4$ x 7 H_2O	40.00	mg
H_3BO_3	1.50	mg
$CoCl_2$ x 6 H_2O	5.00	mg
$MnCl_2$ x 4 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	1.00	mg
Distilled water	100.00	ml

May be stored at 4°C in the dark

Phosphate buffer:

Na_2HPO_4 x 12 H_2O	71.60	g
KH_2PO_4	26.00	g
Distilled water	1000.00	ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Prepare the growth medium as follows:
Dilute 100 ml solution 1 to 1 litre with 2% NaCl and then add 1 ml of solution 3, 1 ml of the trace elements, and 0.1 ml of solution 2. If solid media is required add 1.5% agar. Autoclave the phosphate buffer separately. When the growth medium is cool 10 ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate.
Add filter-sterilized methanol to produce 1% (final concentration).

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1489. TSB-GLUCOSE MEDIUM

Trypton Soja Broth (Oxoid)	3.0	g
α-D-Glucose	1.0	g
Agar if appropriate	15.0	g
Distilled water	1000.0	ml

Adjust to pH 6.5 and autoclave.

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1490: DESULFOVECTIS MEDIUM

NaCl	20.00	g
NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
KCl	0.10	g
Na_2SO_4	2.00	g
$CaCl_2$ x 2 H_2O	0.10	g
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
$Na_2S_2O_3$ x 5 H_2O	3.00	g
$MgCl_2$ x 6 H_2O	3.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, thiosulfate, magnesium chloride, cysteine and sulfide), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add magnesium chloride, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

2. After inoculation pressurize cultivation vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

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1492. HALF STRENGTH R2A

Yeast extract	0.250	g
Proteose Peptone (Difco no. 3)	0.250	g
Casamino acids	0.250	g
Glucose	0.250	g
Soluble starch	0.250	g
Na-pyruvate	0.250	g
K_2HPO_4	0.150	g
$MgSO_4$ x 7 H_2O	0.0250	g
Agar	15.00	g
Distilled water	1000.00	ml

Final pH 7.2; adjust with crystalline K_2HPO_4 or KH_2PO_4 before adding agar. Add agar, heat medium to boiling to dissolve agar, and autoclave for 15 min at 121°C.

For DSM 25439 use 2% (w/v) gellan gum as solidifying agent.

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1493 . VERRUCOMICROBIA MEDIUM

$NaHCO_3$	0.50	g
NaCl	0.50	g
KH_2PO_4	0.40	g
$(NH_4)_2SO_4$	0.20	g
$MgSO_4$ x 6 H_2O	0.10	g
CaCl2	0.01	g
$Li_2B_4O_7$	0.01	g
FeEDTA solution (see below)	1.00	ml
Trace element solution (see below)	1.00	ml
Distilled water	1000.00	ml

Do not adjust the pH.

For plates add 8g/l Gelrite or 15g Phytagel and 1g/L MgCl. A carbon source is not required because the organism degrades the polysaccharides in Gelrite or Phytagel
For liquid medium use 0.8-2.0g/l Gelrite as carbon source (the medium remains liquid). Alternatively mannose (1g/l) may be used instead of Gelrite in liquid medium. Do not add mannose to solid media, which will inhibit growth.

FeEDTA solution:

FeSO4 x7 H2O	1.54	g
NaEDTA	2.06	g
Distilled water	1000.00	ml

Trace element solution:

Nitrilotriacetic acid	1.500	g
$Fe(NH_4)_2(SO_4)_2$ x $6H_2O$	0.200	g
Na_2SeO_4 x 10 H_2O	0.440	g
$CoCl_2$ x 6 H_2O	0.180	g
$MnSO_4$ x H_2O	0.120	g
Na_2MoO_4	0.160	g
Na_2WO_4	0.100	g
$ZnSO_4$ x 7 H_2O	0.540	g
$AlCl_3$	0.040	g
$NiCl_2$ x 6 H_2O	0.025	g
H_3BO_3	0.110	g
$CuSO_4$	0.210	g
MnCl	0.190	g
Distilled water	1000.000	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

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1494 . OMIZ-PAT Medium (modified)

This recipe describes the preparation of 200 ml medium, which can be used for about 4 weeks when kept at 4°C.

Prepare **Solutions A – E**, flush each with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution A:

L-Alanine	90	mg
L-Arginine	350	mg
L-Asparagine	300	mg
L-Aspartic acid	236	mg
L-Cystein Hcl	700	mg
L-Glutamine	1360	mg
L-Glutamic acid	600	mg
Glycine	150	mg
L-Histidine	1240	mg
L-Isoleucine	262	mg
L-Leucine	262	mg
L-Lysine HCl	364	mg
L-Methionine	300	mg
L-Ornithine HCl	340	mg
L-Phenylalanine	330	mg
L-Proline	230	mg
L-Serine	1050	mg
L-Threonine	240	mg
L-Tryptophan	204	mg
L-Tyrosine	180	mg
L-Valine	234	mg
Distilled Water	100	ml

Solution B:

KCl	1936	mg
$MgSO_4$ x $7H_2O$	500	mg
NaH_2PO_4 x H_2O	280	mg
NH_4Cl	3200	mg
Citric acid, trisodium salt	400	mg
Distilled water	100	ml

Solution C:

Calcium D-(+)-panthothenate	10	mg
Choline chloride	100	mg
Myo-Inositol	100	mg
Thiamine dichloride	10	mg
Thiamin pyrophosphate	10	mg
Coenzym A, sodium salt	2	mg
Flavin adenine dinucleotide (FAD)	2	mg
ß-nicotinamide adenine dinucleotide (NAD)	2	mg
2-mercaptoethanesulfonic acid, sodium salt (MES)	20	mg
N-acetylmuramic acid	50	mg
N-acetylglucosamin	400	mg
D-glucuronic acid, sodium salt	1600	mg
D-galacturonic acid	1600	mg
Pyruvic acid, sodium salt	1100	mg
Fumaric acid, disodium salt	1000	mg
Formic acid, sodium salt	600	mg
Distilled water	100	ml

Solution D:

Pyridoxal - HCl	10	mg
Pyridoxal - Phosphat	10	mg
D,L-lactic acid, sodium salt	1120	mg
Hypoxanthine	2.8	mg
Uracil	2.2	mg
Thymidine	4.8	mg
N-(2-acetamido)-2-aminoethanesulfonic acid	3.6	mg
D,L-carnithine	400	mg
Putrescine 2 HCl	10	mg
Phenol red, sodium salt	20	mg
Distilled water	100	ml

Solution E:

D-Glucose	4	g
D-Fructose	1.6	g
D-Maltose	1.6	g
D-Mannitol	1.6	g

D-Mannose	4	g
D-Arabinose	4	g
L-Fucose	4	g
D-Trehalose	4	g
D-Sucrose	4	g
L-Rhamnose	4	g
Distilled water	100	ml

Prepare **Solution F**, flush with nitrogen to make it anoxic, filter-sterilize and store at 4°C

Solution F:

$CaCl_2$ x H_2O	300	mg
Distilled water	100	ml

Prepare **Solutions G and H**, flush each with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution G:

Asialofetuin (1 mg/ml) in distilled water

Solution H:

Vitamine B12	5	mg
D-(+)-biotin	5	mg
Folic acid	5	mg
Folinic acid, calcium salt	10	mg
Nicotinamide	5	mg
Nicotinic acid	10	mg
Riboflavine	1	mg
Distilled water	100	ml

Prepare **Solution I** and store at -20°C

Solution I:

Isobutyric acid	10	µl
2-methylbutyric acid	10	µl
Valeric acid	10	µl

Isovaleric acid	10	µl

Solution J:
D,L-α-lipoic acid (1 mg/ml); in 2-mercaptoethanol-ethanol (1:10); store at -20°C

Solution K:
Hemin (0.2 mM) in NaOH (10 mM)

Solution L:
Homogenize 42 g fresh baker yeast in 140 ml distilled water, incubate for 24h at 56°C and centrifuge to harvest the supernatant. Adjust pH to 7 with NaOH, make anoxic, filter-sterilize and store at -80°C

Prepare **Trace elements I – III**, flush each with nitrogen to make them anoxic, filter-sterilize and store at 4°C

Trace elements I

$CuSO_4$	7.89	mg
$MnSO_4$ x H_2O	169	mg
$ZnSO_4$ x $7H_2O$	2.87	g
10 mM HCl	100	ml

Trace elements II

Na_2SeO_3	173	mg
10mM HCl	100	ml

Trace elements III

$NiSO_4$ x $6H_2O$	13.1	mg
$SnCl_2$ x $2H_2O$	11.8	mg
$NaVO_3$	61	mg
$(NH_4)_6Mo_7O_{24}$ x H_2O	1240	mg
10 mM HCl	100	ml

Prepare **Supplements 1 - 3**, flush each with nitrogen to make them anoxic, filter-

sterilize and store at -20°C

Supplement 1:
$FeSO_4$ x $7H_2O$ (10 mM) in HCl (10 mM)

Supplement 2:
Ascorbic acid (10%; w/v); in distilled water, neutralize with NaOH

Supplement 3:
Glutathion (0.5 M); in distilled water, neutralize with NaOH

Supplement 4:
Cholesterol (1 mg/ml); in Ethanol (100%); store at -20°C

Supplement 5:
$NaHCO_3$ (10%; w/v) in distilled water, store at 4°C

Preparation of medium

Distilled water		100	ml
Solution A-G		10	ml of each
Solution H		200	µl
Solution I		8	µl
Solution J		2	µl
Solution K		40	µl
Solution L		2	ml
Trace elements I and II	pre-dilute 1:1000; use	2	ml of each
Trace elements III	pre-dilute 1:1000; use	200	µl

Add distilled water to 200 ml

Supplements 2, 3, 5	2	ml of each
Supplements 1, 4	200	µl of each

Add 10 ml rabbit serum, flush the medium with N_2CO_2, adjust the pH to 6.9 and filter-

sterilize the completed medium into tubes.

1495. AQUASPIRILLUM MEDIUM II

Peptone (BD)	2.00	g
Succinic acid	1.00	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_20	1.00	g
$CaCl_2$ x 2 H_2O	0.03	g
Vitamin solution (see below)	1.00	ml
Trace elements (see below)	1.00	ml
Distilled water	1000.0	ml

Adjust pH to 7.4-7.6
Autoclave and add 1.0 ml/l vitamin solution from a filter sterilised stock solution (see below). The medium may be solidified by adding 15.0 g/l agar.

Trace elements solution (Pfennig & Lippert, 1966)

EDTA	5.00	g
$FeSO_4$ x 6 H_2O	2.20	g
$ZnSO_4$ x 7H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Vitamin solution

Biotin	200.0	mg
Folic acid	20.0	mg
Pyridoxine-HCl	100.0	mg
Thiamine-HCl	50.0	mg
Riboflavin	100.0	mg
Nicotinic acid	50.0	mg
DL-Pantothenic acid	50.0	mg
Vitamin B_{12}	1.0	mg
p-Aminobenzoic acid	50.0	mg
Distilled water	1000.00	ml

Filter sterilise.

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1496. PGY MEDIUM

Tryptone	5.0	g
Yeast extract	3.0	g
D-Glucose	1.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0

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1497. HALOARCHAEUM SALINUM MEDIUM

Mineral Salts Solution for solid Medium:

L-Glutamic Acid	1.000	g
Trisodium Citrate Dihydrate	1.000	g
KH_2PO_4	0.500	g
NH_4Cl	0.500	g
K_2SO_4	2.500	g
MgCl x 7 H_2O	20.00	g
Casamino Acids	2.000	g
Yeast Extract (Oxoid)	1.000	g
$FeSO_4$	0.002	g
Trace Elements (see below) (see medium 1396)	1.000	ml
Distilled Water	300.000	ml

NaCl	100.00	g
Agar	20.00	g
Distilled Water	200.00	ml

Mineral Salts Solution for liquid Medium:

L-Glutamic Acid	1.000	g
Trisodium Citrate Dihydrate	1.000	g
KH_2PO_4	0.500	g
NH_4Cl	0.500	g
K_2SO_4	2.500	g
MgCl x 7 H_2O	20.000	g
NaCl	100.000	g
Casamino Acids	2.000	g
Yeast Extract	1.000	g
$FeSO_4$	0.002	g
Trace Elements (see below) (see medium 1396)	1.000	ml
Distilled Water	500.000	ml

Adjust to pH 5.5 with 10% KOH and sterilize by autoclaving.

Trace Element Solution:

$CoCl_2$ x 6 H_2O	0.30	g
$MnCl_2$ x 4 H_2O	0.10	g
$ZnCl_2$	0.10	g

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H_3BO_4	0.02	g
Na_2MoO_4 x 2 H_2O	0.10	g
$NiCl_2$ x 6 H_2O	0.10	g
$CuCl_2$ x 6 H_2O	0.01	g
$AlCl_3$	0.04	g
Na_2WO_4 x 2 H_2O	0.02	g
$CaCl_2$ x 2 H_2O	1.00	g
$BaCl_2$ x 2 H_2O	0.01	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
Distilled Water	1000.00	ml

Adjust to pH 4.0.

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1499. GW3 MEDIUM

Starch	5.0	g
Casein hydrolysate acid	0.5	g
KNO3	0.5	g
CaCO3	0.1	g
KCl	20.0	g
NaCl	150.0	g
MgCl2	2.0	g
Agar	18.0	g
Distilled water	1000.0	ml

pH 7.0 − 7.5

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ACANTHAMOEBA MEDIUM

Media for maintenance of infected amoeba cultures

Trypticase Soy Broth with Yeast Extract (TSY)

Trypticase Soy Broth	30	g
Yeast extract	10	g
Distilled water	1000	ml

Adjust pH to 7.3, autoclave at 121°C

OR

Peptone-yeast-glucose medium (PYG)

Proteose peptone	20	g
Glucose	18	g
Yeast extract	2	g
Sodium citrate dihydrate	1	g
$MgSO_4$ x $7H_2O$	0.98	g
Na_2HPO_4 x $7H_2O$	0.355	g
KH_2PO_4	0.34	g
$Fe(NH_4)_2(SO_4)_2$ x $6H_2O$	0.02	g
Distilled water	1000	ml

Adjust pH to 6.5, autoclave at 110°C or filter-sterilize

Maintenance conditions

Infected amoeba cultures can be maintained e.g. in 25 cm^2 flasks (e.g. Nunc flasks, VWR, 734-2081, Thermo-Scientific, 156340) at 20-30°C in an incubator (no special requirements concerning CO_2 or light).

Maintenance/Passage of infected amoeba cultures

Infected Acanthamoeba cultures don't have to be transferred to fresh culture flasks as frequently as usually done for mammalian cell cultures. Amoeba cultures can be maintained in the same flask for several weeks or months. However, if the cultures reach a high cell density, medium should be exchanged. For that purpose amoebae are detached from the surface of the flask by knocking at the culture flask (no trypsin required, amoebae are only weakly adherent compared to most mammalian cells) and the medium (containing the majority of cells) is then completely removed. Fresh medium is added and the few remaining attached amoebae will continue to grow.
Alternatively, cultures can also be passaged regularly (e.g. weekly) by transfer of a small aliquot of a densely grown culture (after cell detachment, as described above) to a fresh flask containing medium.
If cultures contain few attached cells, but high amounts of cell debris, medium should be exchanged without prior cell detachment. Cultures usually recover fast.
Depending on the host cell strain, the bacteria may cause host cell lysis. To maintain

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such cultures, bacteria have to be transferred by adding culture supernatant (approximately 0.5 ml) to fresh culture flasks containing uninfected host cells (approximately semi-confluent culture) each 7-10 days.

Storage
Bacteria released from host cells can be stored in

Sucrose-Phosphate-Glutamate buffer

Sucrose	75	g
KH_2PO_4	0.52	g
Na_2HPO_4 x $2H_2O$	1.53	g
Glutamic acid	0.75	g
Distilled water	1000	ml

at -80°C for several months.

Infected cell cultures (supplemented with 10% DMSO) are best stored in liquid nitrogen.

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1501. METHYLOCALDUM MARINUM MEDIUM

Solution 1 (10x NMS salts):

KNO_3	10.00	g
$MgSO_4$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	2.00	g
Distilled water	1000.00	ml

Dissolve the ingredients listed above (in that order) in about 700 ml of 2% NaCl, and then make up to 1 litre with 2% NaCl.

Solution 2 (Fe EDTA):

Fe EDTA	3.80	g
Distilled water	100.00	ml

Solution 3 (Sodium molybdate):

Na_2MoO_4 x 2 H_2O	0.26	g
Distilled water	1000.00	ml

Trace elements:

EDTA di sodium salt	25.00	mg
$CuSO_4$ x 5 H_2O	20.00	mg
$FeSO_4$ x 7 H_2O	50.00	mg
$ZnSO_4$ x 7 H_2O	40.00	mg
H_3BO_3	1.50	mg
$CoCl_2$ x 6 H_2O	5.00	mg
$MnCl_2$ x 4 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	1.00	mg
Distilled water	100.00	ml

May be stored at 4°C in the dark

Phosphate buffer:

Na_2HPO_4 x 12 H_2O	71.60	g
KH_2PO_4	26.00	g
Distilled water	1000.00	ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Prepare the growth medium as follows:
Dilute 100 ml solution 1 to 1 litre with 2% NaCl and then add 1 ml of solution 3, 1 ml of the trace elements, and 0.1 ml of solution 2. If solid media is required add 1.5% agar. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 25% methane to the gas phase and autoclave at 121°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. When the growth medium is cool 10 ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.

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1503. CULTIVATION MEDIUM FOR CHLAMYDOPHILA PECORUM

Cultivation of CACO-2 cells (ACC 169)

MEM-Medium	45.0	ml
Fetal Bovine Serum	5.0	ml
Aminoacids (100 x)	0.5	ml

Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

Prepare a 25 cm^2 flask and seed cells according to standard protocols (see DSMZ catalogue for ACC 169). Incubate at 37°C plus 5% CO_2. When a confluent layer has formed, infection can be carried out.

Exchange medium with 6 ml of Infection Medium (as above with the addition of 1 µg/ml cycloheximide (final concentration)) and add 500 − 1000 µl of EB stock solution (thawed quickly to 37°C).

Centrifuge for 1 h onto the cell layer at 1600 rpm at 20°C.

Incubate at 37°C + 5% CO_2. Control cells daily and look for inclusions. Not all Chlamydiae form well-visible inclusions, ultimately, immunofluorescence or in situ-hybridization techniques are necessary to visualize inclusions.

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1504. CM MEDIUM

Peptone	7.5	g
Yeast extract	10.0	g
$MgSO_4$ x 7 H_2O	10.0	g
Na_3Citrat	3.0	g
KCl	2.0	g
NaCl	150.0	g
Fe*	1.0	ml
Agar	20.0	g
Distilled water	1000.0	ml

pH 7.4 (NaOH)

* Prepare 4.98% $FeSO_4$ x 7 H_2O (= 10 g Fe in 1l 0.001 M HCl); add 1 ml per liter medium.

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1505: PROTEINIVORAX MEDIUM

KH_2PO_4	0.30	g
$MgSO_4$ x 7 H_2O	0.12	g
$CaCl_2$ x 2 H_2O	0.03	g
NaCl	20.00	g
Tryptone (BD Bacto)	3.00	g
Trace element solution SL-10	1.00	ml
Na_2CO_3	60.00	g
$NaHCO_3$	50.00	g
Na-thioglycolate	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonates and thioglycolate), then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add carbonate and hydrogencarbonate, adjust pH to 8.5 - 9.0, dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add thioglycolate from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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HIGH MAGNESIUM MEDIUM

$MgSO4 \times 7\ H_2O$	20.0	g
KCl	2.0	g
Yeast extract	10.0	g
Tryptone	5.0	g
Na_3-Citrate x 2 H_2O	3.0	g
Casein hydrolysate	5.0	g
Distilled water	1000.0	ml

Adjust to pH 7.3.

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1508: CALDERIHABITANS MEDIUM

Sea Salt (SIGMA)	21.00	g
K_2HPO_4	0.10	g
NH_4Cl	0.10	g
$NaNO_3$	0.05	g
Yeast extract	0.05	g
Ferric citrate monohydrate	0.01	g
Trace element solution SL-4	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-pyruvate	1.00	g
$Na_2S_2O_3 \times 5\ H_2O$	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9\ H_2O$	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, pyruvate, thiosulfate, vitamins and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, thiosulfate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of pyruvate, thiosulfate and vitamins should be sterilized by filtration. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.

Trace element solution SL-4 (from medium 14)

Na_2-EDTA	0.50	g
$FeSO_4 \times 7\ H_2O$	0.20	g
$ZnSO_4 \times 7\ H_2O$	0.10	g
$MnCl_2 \times 4\ H_2O$	0.03	g
H_3BO_3	0.30	g
$CoCl_2 \times 6\ H_2O$	0.20	g
$CuCl_2 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.02	g
$Na_2MoO_4 \times 2\ H_2O$	0.03	g
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.

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1508: CALDERIHABITANS MEDIUM

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1509. NB-PYRUVATE AGAR

Solution A:

Nutrient Broth	6.0	g
NaCl	1.5	g
Na-Pyruvate	1.0	g
Agar	14.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0.

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1510. MODIFIED MEDIUM 514 FOR HALOMONAS SP.

Difco Marine Broth 2216	37.4	g
pancreatic digest of casein (BD Trypticase Peptone)	1.0	g
soya peptone (BD Soytone)	1.0	g
malt extract (BD)	1.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5

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1512. SCGYEM- MEDIUM

Medium for maintenance of infected amoeba cultures

Solution A		
Casein	10	g
Na_2HPO_4 x $7H_2O$	0.355	g
Distilled water	800	ml

Adjust pH to > 8.0, autoclave at 110°C

Solution B		
Glucose	2.5	g
KH_2PO_4	0.8	g
Distilled water	50	ml

Filter-sterilize

Solution C		
Yeast extract	5	g
Distilled water	50	ml

Filter-sterilize

Working Medium		
Combine Solutions A, B and C and add		
Fetal Bovine Serum (heat-inactivated, 56°C, 30 min)	100	ml

Maintenance conditions
Infected amoeba cultures can be maintained e.g. in 25 cm^2 flasks (e.g. Nunc flasks, VWR, 734-2081, Thermo-Scientific, 156340) at 20-30°C in an incubator (no special requirements concerning CO_2 or light).

Maintenance/Passage of infected amoeba cultures
Infected Acanthamoeba cultures don't have to be transferred to fresh culture flasks as frequently as usually done for mammalian cell cultures. Amoeba cultures can be maintained in the same flask for several weeks or months. However, if the cultures reach a high cell density, medium should be exchanged. For that purpose amoebae are detached from the surface of the flask by knocking at the culture flask (no trypsin required, amoebae are only weakly adherent compared to most mammalian cells) and the medium (containing the majority of cells) is then completely removed. Fresh medium is added and the few remaining attached amoebae will continue to grow.
Alternatively, cultures can also be passaged regularly (e.g. weekly) by transfer of a small aliquot of a densely grown culture (after cell detachment, as described above) to a fresh flask containing medium.

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If cultures contain few attached cells, but high amounts of cell debris, medium should be exchanged without prior cell detachment. Cultures usually recover fast.
Depending on the host cell strain, the bacteria may cause host cell lysis. To maintain such cultures, bacteria have to be transferred by adding culture supernatant (approximately 0.5 ml) to fresh culture flasks containing uninfected host cells (approximately semi-confluent culture) each 7-10 days.

Storage
Bacteria released from host cells can be stored in

Sucrose-Phosphate-Glutamate buffer

Sucrose	75	g
KH_2PO_4	0.52	g
Na_2HPO_4 x $2H_2O$	1.53	g
Glutamic acid	0.75	g
Distilled water	1000	ml

at -80°C for several months.

Infected cell cultures (supplemented with 10% DMSO) are best stored in liquid nitrogen.

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1513: SULFURIMONAS GOTLANDICA MEDIUM

NaCl	5.55	g
$MgCl_2$ x 6 H_2O	2.29	g
$CaCl_2$ x 2 H_2O	0.34	g
KCl	0.15	g
Na_2SO_4	0.91	g
KNO_3	1.01	g
HEPES buffer (SIGMA)	2.38	g
NH_4Cl	0.05	g
SrCl2 (0.1% w/v)	8.00	ml
H_3BO_3 (0.1% w/v)	6.00	ml
NaF (0.1% w/v)	4.00	ml
K_2HPO_4 (0.1% w/v)	1.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$NaHCO_3$	0.20	g
$Na_2S_2O_3$ x 5 H_2O	2.48	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	980.00	ml

1. Dissolve ingredients (except bicarbonate, thiosulfate and vitamins) and adjust pH to 7.3. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes) and autoclave. Add thiosulfate and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of vitamins and thiosulftae should be sterilized by filtration. Adjust pH of the complete medium to 7.3 - 7.5, if necessary.

2. After inoculation pressurize vials to 0.7 bar overpressure with sterile 100% N_2 gas.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1514. Grace's Insect Medium

Medium for maintenance of SF-9 cells (ACC 125) for cultivation of Rhabdochlamydiae.

Grace's Insect Medium (Sigma Aldrich, G8142)	900	ml
Fetal Bovine Serum (heat-inactivated, 56°C, 30 min)	100	ml

Filter-sterilize

Maintenance conditions of SF-9 cells (see also DSMZ catalogue for ACC 125)
SF-9 cells are cultivated at 25 – 29°C without the addition of CO_2 in flasks (25 cm^2) with tightly closed lids. When a confluent layer has formed, infection can be carried out.

Infection with Rhabdochlamydiae
Exchange medium and add 500 – 1000 µl of EB stock solution (thawed quickly to 37°C). Centrifuge for 1 h onto the cell layer at 1600 rpm at 20°C.

Maintenance/Passage of infected insect cell cultures
Infected cultures can be maintained e.g. in 25 cm2 flasks (e.g. Nunc flasks, VWR, 734-2081) at 27°C in an incubator (no special requirements concerning CO_2 or light).

If the percentage of infected cells is still low (which might occur initially after inoculation of cultures with bacteria from a frozen stock) infected cultures may be maintained by weekly passage (1:5). Infection of yet uninfected cells by added bacteria or bacteria released from neighboring infected cells is facilitated by centrifugation (130 x g, 15 min) of the culture flask.
In well-infected cultures host cell lysis will be visible (the bacteria cause host cell lysis within approximately 5-7 days after invasion of host cells). To maintain such cultures, bacteria have to be transferred by adding culture supernatant (approximately 0.5-1 ml) to fresh culture flasks containing uninfected insect cells (approximately semi-confluent culture) each 7-10 days.

Storage
Bacteria released from host cells can be stored in

Sucrose-Phosphate-Glutamate buffer

Sucrose	75	g
KH_2PO_4	0.52	g
Na_2HPO_4 x $2H_2O$	1.53	g
Glutamic acid	0.75	g
Distilled water	1000	ml

at -80°C for several months.

Infected cell cultures (supplemented with 10% DMSO) are best stored in liquid nitrogen.

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1515: OCEANIRHABDUS MEDIUM

Na_2SO_4	1.00	g
NaCl	30.00	g
KCl	0.70	g
$MgCl_2$ x 6 H_2O	5.00	g
$CaCl_2$ x 2 H_2O	1.00	g
K_2HPO_4	0.40	g
KH_2PO_4	0.40	g
NH_4Cl	0.50	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	3.00	g
Tryptone (BD Bacto)	4.00	g
Na-acetate	6.80	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.60	g
$NaHCO_3$	2.50	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine and bicarbonate, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve cysteine, adjust pH to 6.5, then dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 6.5 - 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1516. THERMOFILUM MEDIUM

Mineral Salts Solution:

KH2PO4	0.33	g
NH4Cl	0.33	g
MgCl2	0.33	g
CaCl2	0.33	g
KCl	0.33	g
Glucose	2.00	g
Yeast extract	0.10	g
Trace Element Solution (see below)	1.00	ml
Distilled water	1000.00	ml

Trace Element Solution:

(NH4)2SO4FeSO4 x 6 H2O	784.0	mg
NiCl2 x 2 H2O	225.0	mg
$CoCl_2$ x 6 H_2O	38.0	mg
MnCl2 x 4H2O	99.0	mg
ZnSO4 x 7 H2O	144.0	mg
Na2SeO4	95.0	mg
Na2WO4 x 2H2O	33.0	mg
Na2MoO4 x 2 H2O	24.0	mg
H3BO3	6.0	mg
CuCl2 x 2 H2O	2.0	mg
Distilled water	1000.0	ml

Prepare the liquid medium. Boil the medium in a water bath and cool under N_2 to room temperature. Adjust to pH 5.8-6.2. Dispense the medium into Hungate tubes or serum bottles under gassing with N_2. Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

Filtrate of Desulfurococcus kamchatkensis (DSM18924)(see below)	10.0 ml
Vitamin Solution (see medium 141)	10.0 ml
Na_2SxH_2O	0.3 g

Filtrate:

Strain DSM 18924 was inoculated into anaerobic tube, containing 10ml of the medium mentioned above. Glucose (2g/l) and yeast extract (0,6g/l) were added as substrate. After 3 days of incubation culture was filtered throuth 0,22nm filter. Filtered culture broth is used for the cultivation of the strain at a concentration of 1% as a source of growth factor

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1517. MODIFIED BACTO MARINE BROTH (DIFCO 2216)

Bacto peptone (BD 212750)	5.00	g
Bacto yeast extract (BD 211921)	1.00	g
Fe(III) citrate	0.10	g
NaCl	19.45	g
$MgCl_2$ (anhydrous)	5.90	g
Na_2SO_4	3.24	g
$CaCl_2$	1.80	g
KCl	0.55	g
$NaHCO_3$	0.16	g
KBr	0.08	g
$SrCl_2$	34.00	mg
H_3BO_3	22.00	mg
Na-silicate	4.00	mg
NaF	2.40	mg
$(NH_4)NO_3$	1.60	mg
Na_2HPO_4	8.00	mg
Distilled water	1000.00	ml

Final pH should be 7.6 ± 0.2 at 25°C.
It is strongly suggested that Bacto peptone (BD 212750) and Bacto yeast extract (BD 211921) should be used for the incubation

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1518. TRYPTONE SOYA BROTH (TSB) WITH ADDITIONAL SODIUM CHLORIDE

Peptone from casein	17.0	g
Peptone from soymeal	3.0	g
D(+)-Glucose	2.5	g
NaCl	55.0	g
K_2HPO_4	2.5	g
Distilled water	1000.0	ml

Adjust pH to 7.2 – 7.5.
The medium may be solidified by adding 15.0 g/l agar.

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1519. HALLOBELLUS RUBER MEDIUM

Glucose	0.90	g
Yeast extract	4.50	g
Peptone protease	4.50	g
$NaHCO_3$	0.12	g
NaBr	0.42	g
$CaCl_2 \times 2H_2O$	0.66	g
KCl	32.43	g
$MgCl_2 \times 6H_2O$	25.20	g
$MgSO_4 \times 6H_2O$	35.60	g
NaCl	140.40	g
$FeCl_3 \times 6H_2O$ (0.5%)	0.30	ml
Agar	20.00	g

Add distilled water to give a final volume of 1000 ml.
Adjust pH to 7.3 with NaOH. Autoclave at 121°C for 15 min.

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1520. HALOPIGER MEDIUM (SG)

NaCl	250.0	g
KCl	3.0	g
Trisodium citrate	3.0	g
$MgSO_4$ x 7 H_2O	20.0	g
Yeast extract (Difco)	1.0	g
Casamino acids (Difco)	7.5	g
Agar, if necessary	20.0	g

Add distilled water to give a final volume of 1000 ml.
Adjust pH to 8.0 before autoclaving!
If solid medium is prepared, autoclave agar and sodium chloride separately.

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1522. ACETOBACTER LAMBICUS MEDIUM

Yeast extract	8.0	g
Peptone	15.0	g
Glucose	10.0	g
Acetic acid	3.0	ml
Ethanol	5.0	ml
Distilled water	992.0	ml

The ethanol and acetic acid are sterilized separately by filtration (use filter resistant to these solvents), and added aseptically to the sterile medium.
Adjust pH to 3,5 with concentrated HCl after autoclaving.

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1523: MODIFIED METHANOBACTERIUM MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1002 ml

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Brain heart infusion (BD Bacto)	6.00	g
Proteose peptone (BD Difco)	6.00	g
Yeast extract (OXOID)	2.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, vitamins, cysteine, and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.5. Then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Before use, check the complete medium's pH and adjust it to 6.8 - 7.0, if necessary.

2. After growth has started and the culture is becoming turbid add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 - 1 bar overpressure.

For DSM 1093, DSM 1125, DSM 16643: Supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) and 0.30 g/l DTT (DL-Dithiothreitol) added from filter-sterilized anoxic stock solutions prepared under N_2. Omit sulfide and cysteine from the medium.

For DSM 1535: Adjust pH of complete medium to 7.6.

For DSM 2640, DSM 2702, DSM 3823, DSM 4140, DSM 4179, DSM 4273, DSM 4274, DSM 10196: Increase amount of Na-acetate to 4.00 g/l.

For DSM 15163: Adjust pH of complete medium to 6.0.

For DSM 16632: Supplement medium after autoclaving with 50 ml/l clarified rumen fluid

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and 0.3 g/l DTT (DL-dithiothreitol). Omit Na_2S x 9 H_2O.

For DSM 115764, DSM 115765: Supplement medium after autoclaving with 0.30 g/l DTT (DL-dithiothreitol), 10.00 ml/l methanol (50 % v/v), and 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from filter-sterilized anoxic stock solutions prepared under N_2. Omit Na_2S x 9 H_2O and L-cysteine HCl x H_2O.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1524. PLANCTOMYCETES MEDIUM NH11

Hutner's basal salts (see below)	20.00	ml
Peptone (Bacto)	0.50	g
Glucose	0.25	g
HEPES (10mM)	2.38	g
Atificial seawater (see below)(see medium 600a)	500.00	ml
Agar (Bacto)	15.00	g
Distilled water	475.00	ml

Sterilize 20 min/121°C. After cooling to 60°C add to the medium:

Vitamin sol. (see medium 621) (see below)	5.00	ml

Adjust pH to 7.5 (the medium is only weakly buffered; one needs approx. 10 drops/l medium of 6 N KOH).

Hutner's basal salts:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_2O	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
Na_2MoO_4 x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
Metall salt sol. "44" (see medium 590)	50.00	ml
Distilled water	900.00	ml

Dissolve NTA first by neutralizing with KOH, then add other salts. Adjust pH to 7.2 with KOH or H_2SO_4. Adjust volume to 1000.0 ml with distilled water.

Artifical sea water:

NaCl	23.47	g
Na_2SO_4	3.92	g
$MgCl_2$ x 6 H_2O	4.98	g
$CaCl_2$	1.10	g
$NaHCO_3$	192.0	mg
KCl	664.0	mg
KBr	96.0	mg
H_3BO_3	26.0	mg
$SrCl_2$	24.0	mg
NaF	3.0	mg
Distilled water	1000.0	ml

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Vitamin solution (double conc.):

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Distilled water	1000.00	ml

Store in the dark and cold (5°C).

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1525: KALLOTENUE MEDIUM

HEPES buffer (SIGMA)	3.57	g
NH_4Cl	27.00	mg
$CaSO_4$ x 2 H_2O	60.00	mg
$MgSO_4$ x 7 H_2O	0.10	g
NaCl	8.00	mg
KNO_3	0.10	g
$NaNO_3$	0.69	g
Na_2HPO_4	0.11	g
Modified Wolin's mineral solution	10.00	ml
D-Glucose	2.00	g
Trypticase peptone (BD BBL)	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, peptone and vitamins) and adjust pH to 8.1. Dispense medium under air atmosphere into culture vessels suitable for anaerobic cultivation (e.g., 10 ml medium per Balch-type tube), seal and autoclave. Add glucose, peptone and vitamins from sterile stock solutions sterilized by filtration. Adjust pH of complete medium to 7.5, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1526: SALINISPIRA (L21 LS) MEDIUM

NaCl	60.00	g
$MgCl_2$ x 6 H_2O	6.00	g
KCl	1.50	g
Na_2SO_4	1.00	g
NH_4Cl	1.00	g
$CaCl_2$ x 2 H_2O	0.40	g
K_2HPO_4	0.40	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Trypticase peptone (BD BBL)	1.00	g
D-Glucose	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, peptone, glucose, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add peptone, glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.

2. Note: Use at least 5-10% (v/v) inoculum.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g

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Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1527: SEDIMENTISPHAERA (L21 HS) MEDIUM

NaCl	120.00	g
$MgCl_2$ x 6 H_2O	6.00	g
KCl	1.50	g
Na_2SO_4	1.00	g
NH_4Cl	1.00	g
$CaCl_2$ x 2 H_2O	0.40	g
K_2HPO_4	0.40	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
D-Glucose	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, glucose, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.5, if necessary.

2. Note: Use at least 5-10% (v/v) inoculum.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1528. HALOLAMINA MEDIUM (NOM-3)

KCl	5.40	g
K_2HPO_4	0.30	g
$CaCl_2x2H_2O$	0.25	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	26.80	g
$MgCl_2x6H_2O$	23.00	g
NaCl	184.00	g
Yeast extract	1.00	g
Bacto Peptone (BD 211677)	0.50	g
Sodium formate	0.25	g
Sodium lactate	0.25	g
Sodium actate	0.25	g
Sodium pyruvate	0.25	g
Agar	20.00	g

Add distilled water to give a final volume of 1000 ml.
Adjust pH to 7.0

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1529: GEOGLOBUS AHANGARI MEDIUM

Ferric citrate monohydrate	11.20	g
NaCl	19.00	g
$MgCl_2$ x 6 H_2O	9.00	g
$MgSO_4$ x 7 H_2O	0.15	g
$CaCl_2$ x 2 H_2O	0.30	g
KCl	0.10	g
KH_2PO_4	0.60	g
$(NH_4)_2SO_4$	0.10	g
NaBr	0.05	g
SrCl2	0.02	g
Trace element solution SL-10	1.00	ml
$NaHCO_3$	2.50	g
Yeast extract	0.10	g
Na-pyruvate	1.00	g
$FeCl_2$ x 4 H_2O	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.10	g
Distilled water	1000.00	ml

1. First, dissolve ferric citrate in water by heating, adjust to pH 5.8, then cool to room temperature. Add other compounds (except bicarbonate, yeast extract, pyruvate, vitamins, cysteine, and ferrous chloride) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Then add cysteine and ferrous chloride and adjust pH to 6.8 - 7.0 with bicarbonate. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation add yeast extract, pyruvate, and vitamins, from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust the pH of the complete medium to 6.8 - 7.0.

2. Note: Use 5 - 10% (v/v) inoculum. The color of the medium supernatant changes from orange-brown to yellow-greenish during growth.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg

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Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1531. PLANCTOMYCES PYREFORMIS MEDIUM

N-acetylglucosamine	1.00	g
KH_2PO_4	0.10	g
$(NH_4)_2SO_4$	0.20	g
$MgSO_4$ x 7 H_20	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
Hutners basal salts (see below) (see medium 590)	1.00	ml
Staley's vitamin solution (see medium 621)	0.10	g
Yeast extract	0.20	g
Glucose	0.50	g
Agar-agar (Difco)	18.00	g
Distilled water	1000.00	ml

Adjust pH to 5.8 – 6.5.

Hutners basal salts:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$(NH_4)MoO_7O_{24}$ x 4 H_2O	9.25	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.0	mg
$ZnSO_4$ x 7 H_2O	1095.0	mg
$FeSO_4$ x 7 H_2O	500.0	mg
$MnSO_4$ x H_2O	154.0	mg
$CuSO_4$ x 5 H_2O	39.2	mg
$Co(NO_3)_2$ x 6 H_2O	24.8	mg
$Na_2B_4O_7$ x 10 H_2O	17.7	mg
Distilled water	1000.0	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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1532. ANTIBIOTIC MEDIUM 1

Yeast extract	3.0	g
Beef extract	1.5	g
Pancreatic Digest of Casein	4.0	g
Peptone	6.0	g
Glucose	1.0	g
Agar	20.0	g

pH 6.55

or Difco Antibiotic Medium 1 (BD, order number 226340)

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1533. MARINIFILUM MEDIUM (IRD MEDIUM)

KH_2PO_4	0.3	g
K_2HPO_4	0.3	g
NH_4Cl	1.0	g
NaCl	25.0	g
KCl	0.1	g
$CaCl_2$ x2H_2O	0.1	g
Cysteine HCl	0.5	g
Yeast extract Oxoid	1.0	g
Trace mineral element solution (see medium 941)	1	mL
Resazurin (0.1%)	1	mL
Distilled water	1000.00	ml

pH 7.0

Add after autoclave

$MgCl_2$ x 6 H_2O (150.0 g/l)	20 ml/l
$NaHCO_3$ (10%)	20 ml/l
Na_2S x 9 H_2O (2%)	20 ml/l
Glucose (1M)	20 ml/l

Prepare the medium without $NaHCO_3$, Na_2S x 9 H_2O, $MgCl_2$ x 6 H_2O and glucose. Boil the medium in a water bath and cool under a stream of N_2/CO_2 (80/20). Add the $NaHCO_3$ to the cooled medium (the pH should be 7.0) and dispense the medium into Hungate tubes or serum bottles under a stream of N_2/CO_2 (80/20). Seal the tubes under N_2/CO_2 (80/20) and autoclave. To the cooled medium at the appropriate amounts of Na_2S x 9 H_2O, $MgCl_2$ x 6 H_2O and glucose from sterile, anaerobic stock solutions.

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1534. MARINIFILUM MEDIUM

NaCl	13.00	g
$MgCl_2$ x 6H2O	2.80	g
KCl	0.36	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
$CaCl_2$ x2H_2O	0.70	g
Na_2SO_4	1.42	g
Glucose	2.00	g
Resazurin (0.1%)	1.00	ml
Trace mineral element solution (SL10 medium 320)(see below)	1.00	ml
Selenite-tungstate solution (see below)	1.00	ml
$NaHCO_3$	2.52	g
Na_2S x 9 H_2O	0.50	g
Vitamin solution V7 (Pfennig et al.1981) (see below)	1.00	ml
Distilled water	1000.00	ml

pH 7.0- 7.2

Prepare the medium without $NaHCO_3$, Na_2S x 9 H_2O, vitamin solution, Selenite-tungstate solution and glucose. Boil the medium in a water bath and cool under a stream of N_2/CO_2 (80/20). Add the $NaHCO_3$ to the cooled medium (the pH should be 7.0-7.2) and dispense the medium into Hungate tubes or serum bottles under a stream of N_2/CO_2 (80/20). Seal the tubes under N_2/CO_2 (80/20) and autoclave. To the cooled medium at the appropriate amounts of Na_2S x 9 H_2O, vitamin solution, Selenite-tungstate solution and glucose from sterile, anaerobic stock solutions.

Selenite-tungstate solution:

Na_2SeO_3 x5H_2O	3.0	mg
Na_2WO_4 x 2H_2O	4.0	mg
NaOH	0.5	g
Distilled water	1000	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.0	ml
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	6.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	990.0	ml

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First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts.
Finally make up to 1000.0 ml.
Adjust pH to 6.0.

Vitamin solution V7:

Biotin	20.0	mg
p-Aminobenzoic acid	50.0	mg
Vitamin B_{12}	50.0	mg
Thiamine-dichlorid	50.0	mg
Nicotinic acid	100.0	mg
Ca-D-(+)-Panthothenic acid	25.0	mg
Pyridoxymine-Dihydrochloride	250.0	mg
Distilled water	1000.0	ml

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1535. MEDIUM D (20.6%NACL)

NaCL	206.0	g
KCL	0.37	g
MgSO4x7H2O	36.0	g
CaCl2x2H2O	0.5	g
MnCl2	0.013	g
Yeast extract	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml
pH 7.4		

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1536. BAP$^+$ MEDIUM FOR FRANKIA

KH_2PO_4	0.950	g
K_2HPO_4	0.600	g
NH_4Cl	0.270	g
Na-propionate	0.480	g
$MgSO_4$ x 7 H_2O	0.025	g
$CaCl_2$ x 2 H_2O	0.010	g
Vitamine solution (see below)	1.000	ml
Chelated iron solution (see below)	1.000	ml
Trace element solution (see below)	0.100	ml
Distilled water	1000.000	ml

Vitamine solution:

Thiamine HCl	0.010	g
Nicotinic acid	0.050	g
Pyridoxine HCl	0.050	g
Distilled water	100.000	ml

Chelated iron solution:

Citronensäure	1.000	g
Eisencitrat	1.000	g
Distilled water	100.000	ml

Trace element solution:

$CoSO_4$ x 7 H_2O	0.001	g
$CuSO_4$ x 5 H_2O	0.080	g
H_3BO_3	2.860	g
$MnCl_2$ x 4 H_2O	1.810	g
Na_2MoO_4 x 2 H_2O	0.025	g
$ZnSO_4$ x 7 H_2O	0.220	g
Distilled water	100.000	ml

The pH of the final solution is adjusted to 6.3.

Note: Please autoclave the chelated iron solution before you add to the medium, so that solves everything!!!!

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1537. SPIRIBACTER MEDIUM (830 + 12% Marine salts)

R_2A Broth	3.0	g
Marine Salts 12% (see below)	1000.0	ml
Agar	20.0	g
pH 7.0-7.2		

Prepare the medium. Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

NaHCO3	0.71	g

Marine Salts 12%:

NaCl	78.57	g
$MgCl_2$ x 6 H_2O	15.37	g
$MgSO_4$ x 7 H_2O	19.37	g
KCl	2.06	g
$CaCl_2$ x $2H_2O$	4.00	g
Distilled water	1000.00	ml

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1538. ANAEROBACTERIUM MEDIUM

KH_2PO_4	0.2	g
NH_4Cl	0.25	g
NaCl	1	g
$MgCl_2$ x 6 H_2O	0.4	g
KCl	0.5	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	1.0	g
Cellobiose	10.0	g
$NaHCO_3$	2.52	g
Na_2S x 9 H_2O	0.36	g
Trace element solution SL-10	1	ml
Vitamin solution	1	ml
Selenite-tungstate solution	1	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Vitamin solution:

Biotin	20.0	mg/l
p-Aminobenzoic acid	80.0	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Vitamin B_{12}	50.00	mg
Distilled water	1000.00	ml

Selenite-tungstate solution:

NaOH	0.5	g
Na_2SeO_3 x 5 H_2O	3.0	mg
Na_2WO_4 x 2 H_2O	4.0	mg
Distilled water	1000.0	ml

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1539: THERMOTOGA PROFUNDA MEDIUM

K_2HPO_4	0.05	g
KH_2PO_4	0.09	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	1.00	g
Trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.00	g
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, glucose, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.0 - 7.5, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve

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mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1540. BHA MEDIUM

Bacto peptone	5.00	g
Bacto yeast extract	5.00	g
Tris HCl	6.00	g
NaCl	11.70	g
$MgSO_4$ x $7H_2O$	7.84	g
KCl	0.74	g
NH_4Cl	3.00	g
$CaCl_2$	0.15	g
Distilled water	1000.00	ml

Final pH should be 7.8 ± 0.2 at 25°C. The medium may be solidified by adding 15.0g/l agar.

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1542. CY/H LIQUID MEDIUM FOR MYXOBACTERIA

Solution A:

Casiton	1.5	g
Yeast extract	1.5	g
Starch	4.0	g
Soy flour, defatted	1.0	g
D-Glucose	1.0	g
$CaCl_2$ X 2 H_2O	1.0	g
$MgSO_4$ x 7 H_2O	0.5	g
HEPES	11.9	g
Distilled water	1000.0	ml

Solution B:

EDTA-iron	0.80	g
Distilled water	100.0	ml

Solution C:

Vitamin B_{12}	0.05	g
Distilled water	100.0	ml

Adjust pH of solution A to pH 7.4. Autoclave solutions A and B separately. Filter sterilize vitamin solution. After cooling, add 1 ml solution B and 1 ml solution C per liter solution A.

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1543. GY - MEDIUM

Glucose	50.0	g
Yeast extract	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

pH 7.0

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1544 . PIRELLULA MEDIUM (M30)

Solution 1:

Hutner's basal salts (see medium 590)	20.00	ml
0.1M Tris/HCl at pH 7.5	50.00	ml
Gelrite	8.00	g
$MgCl_2$	1.00	g
Biomaris Seawater	700.00	ml
Distilled water	190.00	ml

Adjust pH to 7.5, autoclave at 121°C for 20 min.

Solution 2:

N-acetylglucosamine	2.00	g
$Na_2HPO_4 \times 2H_2O$	0.01	g
Vitamin solution No.6 (see medium 621)	10.00	ml
Distilled water	40.00	ml

Adjust pH to 7.5, filter-sterilize, and add to solution 1.

Vitamin solution No.6:

Vitamin B_{12}	0.2	mg
Biotin	4.0	mg
Thiamine-HCl x 2 H_2O	10.0	mg
Ca-pantothenate	10.0	mg
Folic acid	4.0	mg
Riboflavin	10.0	mg
Nicotinamide	10.0	mg
p-Aminobenzoic acid	10.0	mg
Pyridoxine hydrochloride	20.0	mg
Distilled water	1000.0	ml

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1545. MODIFIED YMA MEDIUM (HAMBI)

K_2HPO_4 x $3H_2O$	0.6	g
$MgSO_4$ x $7H_2O$	0.2	g
Mannitol	10.0	g
Yeast extract	2.5	g
Agar	20.0	g
Distilled water	1000.0	ml

adjust to pH 7.0

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1550. EX-LM MEDIUM

Casitone	5.0	g
Yeast Extract	5.0	g
$MgSO_4 \times 7H_2O$	2.0	g
Distilled water	1000.0	ml

pH needs no adjustment

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1552. AIDINGIMONAS MEDIUM

Yeast extract (BD Difco)	4.0	g
Proteose peptone Nr. 3 (BD Difco)	8.0	g
NaCl	60.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.2

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1553. MICROVIRGA FLOCULATUS MEDIUM

Peptone (BD 211677)	0.25	g
Yeast extract (BD 212750)	0.25	g
K_2HPO_4	0.10	g
NaH_2PO_4	0.05	g
$MgSO_4$ x 7 H_2O	0.02	g
Distilled water	1000.0	ml

Adjust pH to 7.0

Media may be solidified by adding 15.0 g/l agar.

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1555. Medium for tick cells (Rickettsia)

Cultivation of IRE11 cells for infection with *Rickettsia monacensis*

L15C (Modified Leibovitzs' L15) used to culture host cells (IRE11). L15C medium is a modification of L15B medium, previously described in detail (Munderloh UG, Kurtti TJ. 1989. Formulation of medium for tick cell culture. Exp. Appl. Acarol. 7:219–29).

Alterations were made to the following reagents:

L-aspartic acid	0.4485 g/L
L-glutamine	0.5 g/L
L-proline	0.45 g/L
L-glutamic acid	0.25 g/L
α–ketoglutaric acid	0.4485 g/L
D-glucose	18.018 g/L
NaOH	5 mM

Prior to use in supplemented medium, L15C was diluted by the addition of 33 % water by volume.

Supplemented L15C medium is used with

Foetal Bovine Serum	10 %
Tryptose Phosphate Broth	5%
lipoprotein concentrate (LPC; MPBiomedical)	0.1 %
HEPES	25 mM
$NaHCO_3$	0.25 %

The pH is adjusted to pH 7.5 using 1 M NaOH.

This medium and the cultivation of tick cells is described in the "**Basic Tick Cell Culture Methods**" provided as pdf-file (see strain entry for DSM 29017: cultivation conditions) by Tim Kurtti & Uli Munderloh, University of Minnesota.

Infection with *Rickettsia monacensis* or *R. buchneri*:

One 25 cm^2 flask of well-grown cells is infected with 1 ml of the Rickettsia strain (quickly thawed from -80°C) and centrifuged for 1 h onto the cell layer at 1600 rpm at 20°C. Incubate at 30°C with closed lid (without CO_2) and look for bacteria and destroyed cells after 4-6 days.

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1556. BM7 MEDIUM

Cultivation of Liberibacter sp.

ACES-buffer	10.0	g
Na_2 α-ketoglutarate	2.0	g
KOH	3.75	g
destilled water	550	ml

adjust pH to 6.9, autoclave for 15 minutes and allow to cool.
Add aseptically

Fetal Bovine Serum	150	ml
TMN-FH Medium (Sigma-Aldrich, T3285)	300	ml

For preparation of agar plates add 15 g agar per liter of medium.

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1557. PALUDISPHAERA BOREALIS MEDIUM (PX4)

N-acetylglucosamine	1.00	g
KH_2PO_4	0.10	g
$MgSO_4$ x 7 H_20	0.05	g
$CaCl_2$ x 2 H_2O	0.01	g
Hutners basal salts (see below)(see medium 590)	20.00	ml
Na-ampicillin	0.20	g
Peptone	0.10	g
Yeast extract	0.10	g
Glucose	0.10	g
Agar-agar (Difco)	18.00	g
Distilled water	1000.00	ml

Adjust pH to 5.8 – 6.5.

Hutners basal salts:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$(NH_4)MoO_7O_{24}$ x 4 H_2O	9.25	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.0	mg
$ZnSO_4$ x 7 H_2O	1095.0	mg
$FeSO_4$ x 7 H_2O	500.0	mg
$MnSO_4$ x H_2O	154.0	mg
$CuSO_4$ x 5 H_2O	39.2	mg
$Co(NO_3)_2$ x 6 H_2O	24.8	mg
$Na_2B_4O_7$ x 10 H_2O	17.7	mg
Distilled water	1000.0	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

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1560. Planctomyces Medium AG Jogler

Peptone (Bacto)	0.25	g
Yeast extract (Bacto)	0.25	g
HEPES	2.38	g
Mineral salt solution (see below)	20.00	ml
Trace element solution (see below)	1.00	ml
Artifical sea water (see below)	250.00	ml
Agar (Bacto)	15.00	g
Double distilled water	730.00	ml

Sterilize 20 min./121°C. After cooling to 60°C add:

N-Acetylglucosamine (10% stock solution)	8.0	ml
Glucose sol. (2.5%, sterile-filtered)	10.0	ml
Vitamin sol. (double conc.)(see below, medium 621)	5.0	ml

Adjust pH to 7.5 (the medium is only weakly buffered; one needs approx. 10 drops/l medium of 6 N KOH).

Artifical sea water:

NaCl	46.94	g
Na_2SO_4	7.84	g
$MgCl_2$ x 6 H_2O	21.28	g
$CaCl_2$	2.86	g
$NaHCO_3$	0.38	g
KCl	1.38	g
KBr	0.192	g
H_3BO_3	0.052	g
$SrCl_2$	0.08	g
NaF	0.006	g
Double distilled water	1000.000	ml

Mineral salts solution:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$Na_2MoO_7O_4$ x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

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"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Trace element solution:

Na-Nitrilotriacetat	1.50	g
$MnSO_4$ x H_2O	0.50	g
Fe SO_4 x 7 H_2O	0.10	g
$Co(NO_3)_2$ x 6 H_2O	0.10	g
$ZnCl_2$	0.10	g
$NiCl_2$ x 6 H_2O	0.05	g
H_2SeO_3	0.05	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.01	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
Na_2WO_4 x 2 H_2O	0.01	g
Double distilled water	1000.00	ml

Vitamin solution (double conc.):

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Double distilled water	1000.00	ml

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1561. Medium for broad range phototrophic bacteria

Yeast extract	0.30	g
NH_4Cl	0.50	g
KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.30	g
KCl	0.50	g
$Na_2S_20_3$	0.10	g
NaCl	0.50	g
$CaCl_2$ x 2 H_2O	0.05	g
sodium acetate	0.50	g
sodium malate	0.50	g
HEPES	3.00	g
Trace element solution	1.00	ml
Distilled water	1000.00	ml

Adjust pH to 7.5.
Autoclave at 121°C for 15 min. Before inoculation add 1.0 ml/ l 5% sulfide solution pH 7.5 and 1.0 ml/l vitamin solution.

Trace element solution:

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

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Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1564. HAHA100MOD-MEDIUM

NaCl	26.40	g
$MgSO_4$ x 7 H_2O	6.80	g
$MgCl_2$ x $6H_2O$	5.70	g
$CaCl_2$ x 2 H_2O	1.50	g
KCl	0.70	g
KBr	0.10	g
NH_4Cl	0.80	mg
KH_2PO_4	0.02	g
Casamino acids (Oxoid)	0.20	g
Tryptone Peptone	0.20	g
Trace elements SL-8 (see below)	1.00	ml
Distilled water	1000.00	ml

Autoclave at 121 °C for 20 min.

Before inoculation add from sterile stock solutions:

Glucose	0.40	g/l
Mannose	0.40	g/l
$NaHCO_3$	0.17	g/l

Adjust pH to 7.5 with 1 M HCl or 1 M $NaHCO_3$

Trace elements SL 8:

EDTA-Na_2	5.2	g
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	62.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	17.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
$NaMoO_4$ x 2 H_2O	36.0	mg
Distilled water	1000.0	ml

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1565: ALKALIPHILUS TRANSVAALENSIS MEDIUM

Final pH: 9.8 - 10.0
Final volume: 1000 ml

MJ(-N) synthetic seawater	100.00	ml
Yeast extract (BD Bacto)	2.00	g
Tryptone (BD Bacto)	2.00	g
Na_2-tartrate x 2 H_2O	1.00	g
Na_3-citrate	1.00	g
$NaNO_3$	0.25	g
$(NH_4)_2SO_4$	0.25	g
Ferric citrate monohydrate	0.20	g
Na_2SO_4	1.00	g
Na_2CO_3	1.25	g
$NaHCO_3$	2.50	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
Distilled water	900.00	ml

Dissolve ingredients except carbonate, bicarbonate, and thiosulfate, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve carbonate and bicarbonate, adjust pH to 10 with KOH, then dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate to the autoclaved medium from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration. Adjust the pH of the complete medium to 9.8 - 10.0.

MJ(-N) synthetic seawater

NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
$NiCl_2$ x 6 H_2O	0.50	mg
Na_2SeO_3 x 5 H_2O	0.50	mg
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.01	g
Modified Wolin's mineral solution	10.00	ml
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g

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1565: ALKALIPHILUS TRANSVAALENSIS MEDIUM

$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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1566. HAHA100 mod V2– MEDIUM

NaCl	26.40	g
$MgSO_4$ x 7 H_2O	6.80	g
$MgCl_2$ x $6H_2O$	5.70	g
$CaCl_2$ x 2 H_2O	1.50	g
KCl	0.70	g
KBr	0.10	g
NH_4Cl	0.80	mg
KH_2PO_4	0.02	g
Casamino acids (Oxoid)	0.20	g
Tryptone Peptone	0.20	g
Trace elements SL-8 (see below, medium 1162)	1.00	ml
Distilled water	1000.00	ml

Autoclave at 121 °C for 20 min.

Before inoculation add from sterile stock solutions:

Glucose	0.40	g/l
Mannose	0.40	g/l
Vitamin solution (see below, medium 428)	10.00	ml/l
$NaHCO_3$	0.17	g/l

Adjust pH to 7.5 with 1 M HCl or 1 M $NaHCO_3$

Trace elements SL 8:

EDTA-Na_2	5.2	g
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	62.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	17.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
$NaMoO_4$ x 2 H_2O	36.0	mg
Distilled water	1000.0	ml

continued on next page

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Vitamin solution:

Riboflavin	10.0	mg
Thiamine-HCl x 2 H_2O	50.0	mg
Nicotinic acid	50.0	mg
Pyridoxine-HCl	50.0	mg
Ca-pantothenate	50.0	mg
Biotin	0.1	mg
Folic acid	0.2	mg
Vitamin B_{12}	1.0	mg
Distilled water	100.0	ml

Filter sterilise

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1567. METHYLOPROFUNDUS SEDIMENTI MEDIUM

Solution 1 (10x NMS salts):

KNO_3	10.00	g
$MgSO_4$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	2.00	g
Distilled water	1000.00	ml

Dissolve the ingredients listed above (in that order) in about 700 ml of distilled water, and then make up to 1 litre.

Solution 2 *Phosphate buffer (10x)*

Na_2HPO_4 x 12 H_2O	71.60	g
KH_2PO_4	26.00	g
Distilled water	1000.00	ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Solution 3 *Trace element solution (medium 141)*

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Solution 4 *Vitamin solution (medium 141)*

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Continued on next page(Fe EDTA):

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Solution 5 *Sodium chloride (15x)*

NaCl	300.0	g
Distilled water	1000.0	ml

Solution 6 (Fe EDTA):

Fe EDTA	3.80	g
Distilled water	1000.00	ml

To make liquid 1x NMS

Solution 1 (NMS)	100.0	ml
Solution 2 (Phosphate buffer)	10.0	ml
Solution 3 (Trace elements)	1.0	ml
Solution 4 (vitamine solution)	1.0	ml
Solution 5 (NaCl)	66.0	ml
Solution 6 (Fe EDTA))	0.4	ml

Bring to 1 liter with distilled water.

Prepare the growth medium as follows:
Dilute 100 ml solution 1 to 1 litre with distilled water and then add 1 ml of solution 3, 66 ml solution 5 and 0.4 ml of solution 6. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 50% methane to the gas phase and autoclave at 121°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. Filter sterilized the vitamin solution. When the growth medium is cool 1.0 ml/l vitamin solution and 10 ml/l of the phosphate buffer are added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.

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1568. MEDIUM FOR METHYLOCALDUM (RS11D-PR)

$NaNO_3$	1.00	g
$MgSO_4$ x 7 H_2O	0.10	g
Na_2HPO_4	0.50	g
KH_2PO_4	0.22	g
$CaCl_2$ x 2 H_2O	0.03	g
$FeSO_4$ x 7 H_2O	2.00	mg
Trace elements (see below)	1.00	ml
Distilled water	1000.00	ml

Medium was adjusted pH 7.0 with NaOH

Trace elements:

$ZnSO_4$ x 7 H_2O	0.44	g
$CuSO_4$ x 5 H_2O	0.20	g
$MnSO_4$ x 2 H_2O	0.17	g
Na_2MoO_4	0.06	g
H_3BO_3	0.10	g
$CoCl_2$ x 6 H_2O	0.08	g
Distilled water	1000.00	ml

Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 20% methane to the gas phase and autoclave at 121°C for 15 minutes.

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1569. ORENIA CHITINITROPHA

Mineral Base:

NaCl	120.0	g
K_2HPO_4	2.5	g
$(NH_4)_2SO_4$	0.5	g

Adjust pH to 7.0 with KH_2PO_4 or K_2HPO_4. Make anoxic under nitrogen stream and autoclave at 121°C.

After autoclaving add sterile, anoxic solutions of:

Chitin (amorphous)	1 – 2g/l (from 5% solution)
$MgCl_2$ x $6H_2O$	5 mM (final concentration)
Yeast extract	20 mg/l
$NaHCO_3$	20 mM (final concentration)
Na_2S	1 mM (final concentration)
Acidic trace metals (Pfennig & Lippert, 1965)	1 ml/l
Vitamine mixture (Pfennig & Lippert, 1965)	1 ml/l

Acidic trace metals (Pfennig & Lippert, 1965)

1. EDTA	5000 mg/l
2. FeSO4 x 7H2O	2000 mg/l
3. ZnSO4 x 7H2O	100 mg/l
4. MnCl2	30 mg/l
5. H3BO3	300 mg/l
6. CoCl2 x 6H2O	200 mg/l
7. CuCl2	10 mg/l
8. NiCl2 x 2H2O	20 mg/l
9. Na2MoO4 x 2H2O or NH4MoO4	20 mg/l

adjust pH to 3-4 with HCl; autoclave in closed bottles, 120°C, 20 min.

Vitamine mixture (Pfennig & Lippert, 1965)

1: acidic solution (HCl, pH 3; 100 ml)

Thiamin	5 mg
Ca pantotenate	5 mg
B12	0.5 mg

2: basic solution (100 ml 0.1 N NaOH)

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Biotin	2 mg
PABA	5 mg
Nicotinic acid	5 mg
Pyridoxine	10 mg

3: Neutral solution (100 ml Aqua dem.)

Folic acid	2 mg
Riboflavin	5 mg

Sterilize by filtration and keep at 4°C. Before addition to the medium mix 1:1:1 (vol:vol:vol).

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1570: THIOGRANUM MEDIUM

NaCl	30.00	g
$CaCl_2$ x 2 H_2O	0.15	g
$MgCl_2$ x 6 H_2O	3.05	g
KCl	0.33	g
$(NH_4)_2SO_4$	0.66	g
Modified Wolin's mineral solution	10.00	ml
Phosphate buffer	5.00	ml
$Na_2S_2O_3$ x 5 H_2O	1.50	g
Na_2CO_3	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except phosphate buffer, thiosulfate, carbonate and vitamins) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add phosphate buffer, thiosulfate, carbonate, and vitamin solution to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N_2 gas. Phosphate buffer, stock solutions of vitamins and thiosulfate are sterilized by filtration and stored under N_2, whereas the solution of carbonate is prepared under 80% N_2 and 20% CO_2 gas mixture and autoclaved. Adjust pH of the complete medium to 6.7, if necessary.

2. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.

Phosphate buffer

KH_2PO_4	119.00	g
K_2HPO_4	21.00	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g

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$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1571. MICROBACTER MEDIUM

KH_2PO_4	0.41	g
Na_2HPO_4 x2 H_2O	0.53	g
NH_4Cl	0.3	g
NaCl	0.3	g
$MgCl_2$ x 6 H_2O	0.1	g
$CaCl_2$ x 2 H_2O	0.01	g
Yeast extract	2.0	g
Na_2S x 9 H_2O	0.36	g
Resazurin	0.5	g
Trace element solution SL-10	1	ml

Dissolve in 1 liter, boil under N2, add 0.5 g of L-cysteine and adjust pH to 6.4 (should be pH 5.5 after autoclaving). Disperse in tubes and bottles (leave 50% of container volume as gas phase). Change to gas mixture N2/CO2; 80/20%) and autoclave for 20 minutes at 121°C.

To 100 ml sterile medium add aseptically

Vitamin solution	0.5	ml
Selenite-tungstate solution	0.5	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg

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p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Selenite-tungstate solution:

NaOH	0.5	g
Na_2SeO_3 x 5 H_2O	3.0	mg
Na_2WO_4 x 2 H_2O	4.0	mg
Distilled water	1000.0	ml

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1572. HMD-MEDIUM

$MgCl_2$ X $6H_2O$	20.0	g
K_2SO_4	5.0	g
$CaCl_2$ x $2H_2O$	0.1	g
Yeast extract	0.1	g
Casamino acids	0.5	g
NaCl	20-25	%
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0 – 7.5

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1573. T 5 MEDIUM

Peptone	1.0	g
Tryptone	0.5	g
D(+)-Glucose	1.0	g
Yeast extrakt	2.0	g
$CaCO_3$	1.0	g
Trace elements solution(see below)	1.0	ml
Distilled water	1000.0	ml

Adjust pH to 7.0.

Trace element solution:

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$KAl(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

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1577: SPOROHALOBACTER SALINUS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.80	g
Na-acetate	0.50	g
NaCl	200.00	g
Yeast extract (OXOID)	0.50	g
Tryptone (BD Bacto)	5.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
$MgSO_4$	5.00	g
Na_2CO_3	1.00	g
D-Glucose	4.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, magnesium sulfate, carbonate, glucose and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve cysteine, adjust pH to 6.0, dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add magnesium sulfate, glucose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Prior to inoculation check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. Note: This medium cannot be stored over a longer period and should be prepared freshly before use!

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

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$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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1578. 1/10 R2A MEDIUM

Yeast extract	0.05	g
Proteose Peptone (Difco no. 3)	0.05	g
Casamino acids	0.05	g
Glucose	0.05	g
Soluble starch	0.05	g
Na-pyruvate	0.03	g
K_2HPO_4	0.03	g
$MgSO_4$ x 7 H_2O	0.005	g
Agar	15.00	g
Distilled water	1000.00	ml

Final pH 7.2; adjust with crystalline K_2HPO_4 or KH_2PO_4 before adding agar. Add agar, heat medium to boiling to dissolve agar, and autoclave for 15 min at 121°C.

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1579: PYROBACULUM FERRIREDUCENS MEDIUM

NH_4Cl	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
KNO_3	1.00	g
$NaHCO_3$	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except yeast extract, nitrate, bicarbonate and vitamins) and sparge medium with 100% CO_2 gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add yeast extract, nitrate and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 6.5 - 6.8, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg

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Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1580. MODIFIED EGGERTH-GAGNON MEDIUM

Peptone	10.0	g
Na_2HPO_4 x $2H_2O$	4.0	g
Mucin from porcine stomach Type III	2.0	g
BactoAgar	15.0	g
distilled water	950	ml

Autoclave at 121°C, let agar cool to 50°C and aseptically add 50 ml sheep blood.

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1582. MELISSOCOCCUS PLUTONIUS MEDIUM

KH_2PO_4	13.5	g
Yeast extract	10.0	g
Glucose	10.0	g
Soluble starch	10.0	g
Cysteine hydrochloride	1.0	g
add deionized water to	1000.0	ml

Adjust pH to 6.6 with NaOH. Boil medium and cool under N_2, distribute under N_2 and sterilize by autoclaving at 121°C for 20 minutes.

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1583. MEDIUM FOR AMMONIA OXIDIZING BACTERIA (STRAINS FROM SOIL)

Solution A:

NH_4Cl	535	mg
KH_2PO_4	54	mg
KCl	74	mg
$MgSO_4$ x 7 H_2O	49	mg
$CaCl_2$ x 2 H_2O	147	mg
NaCl	584	mg
Trace element solution	1	ml
Cresol red solution	2	ml
Distilled water	ad 1000	ml

Autoclave, subsequently adjust pH of the medium to about 7.8 by addition of sterile 10% $NaHCO_3$ (medium is turning from yellow to pink).
Important: During growth, add $NaHCO_3$ to readjust pH as soon as pH indicator changes to yellow. For stock cultures, 5 g l^{-1} $CaCO_3$ (will stay as a precipitate) or 4.8 g l^{-1} HEPES may be added to keep pH neutral for a longer time.
Aeration must be adjusted according to the density and activity of the culture. At low cell concentrations (which is the case most of the time) cells are sensitive to strong aeration (don't shake the cultures).

Trace element solution

Distilled water	975	ml
HCl, 1M	25	ml
$MnSO_4$ x 4 H_2O	45	mg
H_3BO_3	49	mg
$ZnSO_4$ x 7 H_2O	43	mg
$(NH_4)_6$ Mo_7O_{24} x 4 H_2O	37	mg
$FeSO_4$ x 7 H_2O	973	mg
$CuSO_4$ x 5 H_2O	25	mg

Cresol red solution

Cresol red	50	mg
Distilled water	100	ml

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1584: DESULFUROMONAS CARBONIS MEDIUM

NaCl	8.80	g
KCl	0.34	g
NH_4Cl	0.25	g
$MgCl_2$ x 6 H_2O	4.84	g
$CaCl_2$ x 2 H_2O	0.15	g
K_2HPO_4	0.15	g
$Fe(NH_4)_2(SO_4)_2$ (0.1% w/v)	3.00	ml
$NiCl_2$ x 6 H_2O (0.1% w/v)	9.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na_2CO_3	1.50	g
Na_2-fumarate	8.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, fumarate and vitamins) and sparge medium with 80% N_2 and 20% CO_2 gas mixture to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding fumarate and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of fumarate and vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1585: SULFURIFERULA MULTIVORANS MEDIUM

Na_2HPO_4 x 12 H_2O	3.00	g
KH_2PO_4	1.80	g
$MgSO_4$ x 7 H_2O	0.10	g
$(NH_4)_2SO_4$	0.10	g
$CaCl_2$ x 2 H_2O	0.04	g
$FeCl_3$ x 6 H_2O	0.03	g
$MnSO_4$ x 5 H_2O	0.03	g
$Na_2S_2O_3$ x 5 H_2O	10.00	g
Distilled water	1000.00	ml

Dissolve ingredients except thiosulfate and adjust pH of medium to 6.5 -7.0. Dispense medium under air atmosphere into serum vials suitable for anaerobic cultivation (e.g., 20 ml medium in 100 ml bottles) and close with butyl rubber septa. After autoclaving complete medium with thiosulfate added from a stock solution sterilized by filtration and add 3% (v/v) sterile CO_2 to the gas phase.

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1587. MSV MEDIUM FOR TETRACOCCUS CECHII

(NH4)2SO4	0.500	g
MgSO4 x 7 H2O	0.100	g
CaCl2 x 2 H2O	0.050	g
K2HPO4	0.110	g
KH2PO4	0.085	g
FeCl3 x 6 H2O	0.002	g
Na2-EDTA	0.003	g
Distilled water	1000.000	ml

After autoclaving the mineral base add the glucose at final concentration of 0.5g/l.

If solid media is required add agar to a final concentration of 15 g/l.

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1588. BRADYMONAS SEDIMINIS MEDIUM

Solution A:

Peptone	10.0	g
Meat extract	3.0	g
NaCl	5.0	g
D(+)-Trehalose	1.0	g
Agar if appropriate	19.0	g
Tap water	250.0	ml
Sea water*	750.0	ml

Dissolve beef extract and peptone by heating in tap water, adjust pH to 7.8 and boil for 10 min. Readjust pH to 7.3. Add agar and autoclave at 121°C for 20 min. Directly after autoclaving, add warm (55°C) sterile sea water. Liquid medium without agar should be combined when cooled to room temperature.

*Natural sea water is stored in the dark for at least three weeks to "age". If natural sea water is not available use artificial sea water:

Artificial sea water

NaCl	28.13	g
KCl	0.77	g
$CaCl_2$ x 2 H_2O	1.60	g
$MgCl_2$ x 6 H_2O	4.80	g
$NaHCO_3$	0.11	g
$MgSO_4$ x 7 H_2O	3.50	g
Distilled water	1000.0	ml

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1590. modified BAP$^+$ (PAGFS) MEDIUM FOR FRANKIA

KH_2PO_4	0.950	g
K_2HPO_4	0.600	g
NH_4Cl	0.270	g
Na-propionate	0.480	g
Glucose	0.480	g
Fructose	0.480	g
Sodium succinate dibasic	0.480	g
Pyruvic acid sodium salt	0.480	g
Sodium acetate	0.480	g
$MgSO_4$ x 7 H_2O	0.025	g
$CaCl_2$ x 2 H_2O	0.010	g
Vitamine solution (see below)	1.000	ml
Chelated iron solution (see below)	1.000	ml
Trace element solution (see below)	0.100	ml
Distilled water	1000.000	ml

Vitamine solution:

Thiamine HCl	0.010	g
Nicotinic acid	0.050	g
Pyridoxine HCl	0.050	g
Distilled water	100.000	ml

Chelated iron solution:

Citronensäure	1.000	g
Eisencitrat	1.000	g
Distilled water	100.000	ml

Trace element solution:

$CoSO_4$ x 7 H_2O	0.001	g
$CuSO_4$ x 5 H_2O	0.080	g
H_3BO_3	2.860	g
$MnCl_2$ x 4 H_2O	1.810	g
Na_2MoO_4 x 2 H_2O	0.025	g
$ZnSO_4$ x 7 H_2O	0.220	g
Distilled water	100.000	ml

The pH of the final solution is adjusted to 6.3.

Note: Please autoclave the chelated iron solution before you add to the medium, so that

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solves everything!!!!

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1591. L-15B Tick Cell Medium

For medium and tick cell cultivation please see 'Tick Cell Culture Methods'

https://www.dsmz.de/fileadmin/Bereiche/Microbiology/Dateien/Kultivierungshinweise/TickCellCultureMethods-4.pdf

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1592. Cyanobacteria Medium BG11-

	Volume [ml/l]	Stock Solutions [g/l]
$Mg(SO_4)$ x $7H_2O$	10	7.5
$CaCl_2$ x $2H_2O$	10	3.6
Na_3-citrate x $2H_2O$	10	0.6
Na_2-EDTA x $2H_2O$	10	0.1
Trace metal mix	1.0	-

Adjust to 1000ml with H_2O dist. and autoclave.

After cooling, add the following filter components

	Volume [ml/l]	Stock Solutions [g/l]
BG11-Mix	1.0	-
Vitamine B12	1.0	0.02

BG11 - Stock Solutions

		[g/l]
BG11-Mix	K_2HPO_4 x $3H_2O$	20
-	Na_2CO_3	10
-	Fe-NH_4-citrate	3.0

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.9	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8g/100ml Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

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1593. Cyanobacteria Medium BG11+

Minerals	Volume [ml/l]	Stock Solutions [g/l]
$Mg(SO_4)$ x $7H_2O$	10	7.5
$CaCl_2$ x $2H_2O$	10	3.6
Na_3-citrate x $2H_2O$	10	0.6
Na_2-EDTA x $2H_2O$	10	0.1
Trace metal mix	1.0	-

Adjust to 1000 ml with H_2O dist. and autoclave.

After cooling, add the following filter sterilized components

Minerals	Volume [ml/l]	Stock Solutions [g/l]
$NaNO_3$	5.0	300
BG11-Mix	2.0	-
Vitamine B12	1.0	0.02

		[g/l]
BG11-Mix	K_2HPO_4 x $3H_2O$	20
	Na_2CO_3	10
	Fe-NH_4-citrate	3.0

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.9	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8 g/100 ml Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5 g/100ml Stock Sol.

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1594. MEDIUM S FOR RHIZORHABDUS

Enzymatic casein hydrolysate	5.0	g
Glucose	2.5	g
.K_2HPO_4 x 3 H_2O	1.3	g
.KNO_3	0.5	g
.$MgSO_4$ x 7 H_2O	0.5	g
Agar, if necessary	13.0	g
Distilled water	1000.0	ml

Autoclave phosphate component separately and add after cooling. Adjust pH to 7.2.

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1595. CM MEDIUM

Beef extract	0.50	g
Yeast extract	0.50	g
Peptone	1.00	g
NaCl	0.50	g
D-Glucose	1.00	g
Distilled water	1000.0	ml

Adjust pH to 7.0-7.2.

Media may be solidified by adding 14.0 g/l agar.

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1596: METHANOCALCULUS ALKALIPHILUS MEDIUM

NaCl	3.00	g
K_2HPO_4	1.00	g
$NaHCO_3$	20.00	g
Na_2CO_3	15.00	g
NH_4Cl	0.20	g
$MgSO_4$ x 7 H_2O	0.50	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Na-formate	3.40	g
Na-acetate	0.16	g
Yeast extract (OXOID)	0.02	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

1. Dissolve sodium chloride and hydrogenphosphate, then sparge solution with 100% N_2 gas for at least 30 - 45 min to make it anoxic. Add and dissolve hydrogencarbonate and carbonate while gassing the head space only, then dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add ammonium chloride, magnesium sulfate, trace elements, formate, acetate, yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. The pH of the complete medium should be 9.5.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

For DSM 27087: Supplement the medium with 15 ml/l methanol solution (15% v/v). Omit Na-acetate and Na-formate.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g

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Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1597: METHANOSALSUM NATRONOPHILUM MEDIUM

Solution A	750.00	ml
Solution B	250.00	ml
Solution C	1.00	ml
Solution D	1.00	ml
Solution E	5.00	ml
Solution F	3.00	ml
Solution G	5.00	ml
Solution H	10.00	ml
Solution I	1.00	ml
Solution J	10.00	ml

1. Sterilize solutions A and B in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of solution A after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 - 4 days. It is best to remove precipitates by decantation before using solution A for medium preparation.

2. Combine solution A with solution B and sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions C to J are sterilized separately under 100% N_2 gas. Vitamins and coenzyme M should be sterilized by filtration. To complete the medium appropriate amounts of solutions C to J are added to the combined sterile solutions A and B in the sequence as indicated. Final pH of the medium should be 9.5.

3. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

Solution A

Na_2CO_3	71.25	g
$NaHCO_3$	11.25	g
NaCl	4.00	g
K_2HPO_4	0.75	g
Distilled water	750.00	ml

Solution B

NaCl	30.00	g
K_2HPO_4	0.25	g
KCl	0.25	g
Distilled water	250.00	ml

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Solution C

Trace elements solution (Pfennig & Lippert,1966)	1.00	ml

Solution D

Selenite-tungstate solution	1.00	ml

Solution E

NH_4Cl	0.20	g
$MgSO_4$ x 7 H_2O	0.25	g
Distilled water	5.00	ml

Solution F

Methanol	3.00	ml

Solution G

Yeast extract	0.02	g
Distilled water	5.00	ml

Solution H

2-Mercaptoethanesulfonic acid (coenzyme M)	0.15	g
Distilled water	10.00	ml

Solution I

Wolin's vitamin solution (10x)	1.00	ml

Solution J

Na_2S x 9 H_2O	0.25	g
Distilled water	10.00	ml

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g

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Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1599: CONTUBERNALIS MEDIUM

NH_4Cl	0.50	g
KH_2PO_4	0.20	g
$MgCl_2 \times 6\ H_2O$	0.10	g
KCl	0.20	g
NaCl	4.20	g
Na_2SO_4	3.00	g
Yeast extract	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	3.30	g
$NaCO_3$	6.50	g
Na-acetate	3.00	g
$Na_2S \times 9\ H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonates, acetate and sulfide) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Then, add and dissolve bicarbonate and carbonate, adjust pH to 9.5, dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add acetate and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas atmosphere. Adjust pH of complete medium to 9.5 - 9.7, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g

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Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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1601. MODIFIED M13 VERRUCOMICROBIUM MEDIUM

Peptone	0.25	g
Yeast extract	0.25	g
Glucose	0.25	g
Vitamin solution (see medium 603)	10.00	ml
Hutner's basal salts (see medium 590)	20.00	ml
0.1M Tris/HCl at pH 7.5	50.00	ml
Biomaris MeeresTIEFwasser	900.00	ml
Distilled water	30.00	ml

Autoclave at 121°C for 15 min.

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1603. ACETOBACTER MUSTI MEDIUM

Yeast extract	10.00	g
Glucose	10.00	g
Distilled water	1000.00	ml

For solid media 20 g/l agar may be added.

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1604: ATHALASSOTOGA MEDIUM

Ferric citrate monohydrate	2.40	g
$(NH_4)_2SO_4$	0.20	g
KH_2PO_4	3.00	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
Yeast extract (OXOID)	0.50	g
Allen's trace element solution	10.00	ml
$FeCl_2$ x 4 H_2O	0.26	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve ferric citrate in water by heating and adjust to pH 6.0, then add other medium ingredients except ferrous chloride, vitamins and cysteine. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferrous chloride (dissolved in 0.1 N HCl), vitamins (sterilized by filtration) and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to inoculation check pH and adjust to 6.0, if necessary.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg

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1604: ATHALASSOTOGA MEDIUM

Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1606. RUBROBACTER CARBOXYDOVORANS MEDIUM

Na_2HPO_4 x 2 H_2O	4.50	g
KH_2PO_4	1.50	g
$MnSO_4$ x H_2O	0.01	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.01	g
Ferric ammonium citrate	5.00	mg
Trace element solution SL-6 (see medium 27)	3.00	ml
Yeast extract	1.00	g
Tryptone	1.00	g
Distilled water	1000.00	ml

Adjust pH to 8.0. Agar if necessary 15.0 g/l. Incubate without agitation at 50°C.

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1609. HEART INFUSION BLOOD AGAR

Bacto Heart Infusion Broth (BD 238400)	25.0	g
Agar	15.0	g
add deionized water to	1000.0	ml

Allow to soak for 10 minutes, swirl to mix then sterilize by autoclaving at 121°C for 15 minutes. Cool to 47°C then aseptically add 5-10% of sterile defibrinated blood (horse or sheep), mix well and pour plates. pH: 7.2 ± 0.2

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1611. YCFA MEDIUM (modified)

Casitone	10.00	g
Yeast extract	2.50	g
Glucose	5.00	g
$MgSO_4$ x 7 H_2O	45.00	mg
$CaCl_2$ x 2 H_2O	90.00	mg
K_2HPO_4	0.45	g
KH_2PO_4	0.45	g
NaCl	0.9	g
Resazurin	1.0	mg
Distilled water	1000.00	ml
$NaHCO_3$	4.00	g
L-Cysteine-HCl	1.00	g
Hemin	10.00	mg
Volatile fatty acids:		
Acetic acid	1.90	ml
Propionic acid	0.70	ml
iso-Butyric acid	90.00	µl
n-Valeric acid	100.00	µl
iso-Valeric acid	100.00	µl
Vitamin solution:		
Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Dissolve ingredients except $NaHCO_3$, hemin, cysteine in water and boil for 10 min, then cool to room temperature while gassing with 100% CO_2.Add the $NaHCO_3$, hemin, cysteine and adjust pH to 6.7 -6.8. Distribute under N_2 into Hungate tubes (10 ml) and autoclave. Filter sterilize vitamin solution and aseptically add 100 µl per Hungate tube.

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1613. FLOUR MEDIUM

Plain flour	4.00	g
$CaCO_3$	0.40	g
Distilled water	1000.00	ml

pH 7.0

For solid media add 16.0 g/l agar.

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1614. AMB MEDIUM FOR RACEMICYSTIS SPP.

Soluble starch	5.00	g
Casitone	2.50	g
K_2HPO_4	0.25	g
$MgSO_4$ x 7 H_2O	0.50	g
Agar	14.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2. HEPES (10 mM) may be added to keep pH constant.

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1616: SEDIMENTIBACTER ACIDAMINIVORANS MEDIUM

NaCl	20.00	g
NH_4Cl	0.54	g
KH_2PO_4	0.10	g
$MgCl_2$ x 7 H_2O	4.00	g
$CaCl_2$ x 2 H_2O	1.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-pyruvate	2.75	g
Wolin's vitamin solution (10x)	2.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, pyruvate, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add pyruvate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pyruvate and vitamin solutions should be sterilized by filtration. Check pH of complete medium and adjust to 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1617. TRYPTIC SOY BROTH MEDIUM (SIGMA T8907-1KG)

Casein (pancreatic digest)	17.0	g
Soya pepton (papaic digest)	3.0	g
D(+)-Glucose	2.5	g
NaCl	5.0	g
K_2HPO_4	2.5	g
Distilled water	1000.0	ml

Adjust pH to 7.3.

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1618. TRYPTONE YEAST EXTRACT GLUCOSE MEDIUM (TYG)

Tryptone	1.00	g
Yeast extract	1.00	g
D-glucose	0.50	g
KCl	6.34	g
NaCl	1.20	g
$MgSO_4$ x 7 H_2O	0.25	g
K_2HPO_4	0.13	g
$CaCl_2$ x 2 H_2O	0.22	g
K_2SO_4	0.17	g
Na_2SO_4	2.40	g
$NaHCO_3$	0.50	g
Na_2CO_3	0.09	g
Fe EDTA	0.07	g
Agar	20.00	g
Distilled water	1000.00	ml

Adjust pH to 7.0

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1619. ISP 7 MEDIUM

ISP 7 Medium (Himedia)	23.74	g
Glycerol	15.00	ml
Distilled water	1000.00	ml

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1623. Half-concentrated LB (Luria-Bertani) MEDIUM

Tryptone	5.0	g
Yeast extract	2.5	g
NaCl	5.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

DSM 103618 for liquid cultures use half-concentrated LB (Luria Bertani) Medium with the addition of 5% Sea sand.

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1624. METHYLOPHAGA MURALIS MEDIUM

KH_2PO_4	1.00	g
KNO_3	1.00	g
$MgSO_4$ x 7 H_2O	0.22	g
NaCl	30.00	g
Distilled water	890.00	ml

pH 5.0

pH is adjusted to 9.0 by adding 100 ml/l 1M sterilized $NaHCO_3$ and 10 ml/l 1M sterilized Na_2CO_3. 5 ml/l sterile methanol is added to the cooled medium. When preparing liquid media cool the mineral salts solution, Na_2CO_3 solution and $NaHCO_3$ solution to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salts solution and autoclave. Cool the Na_2CO_3 solution, $NaHCO_3$ solution and agar to 50-55°C before mixing.

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1625. TAP WATER YEAST EXTRACT MEDIUM (TWYE)

Yeast extract	0.25	g
K_2HPO_4	0.50	g
Agar	18.00	g
Tap water	1000.00	ml

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1626. YEAST EXTRACT MANITOL MEDIUM (YEM)

Yeast extract	0.5	g
Mannitol	5.0	g
K_2HPO_4	0.5	g
$MgSO_4 \times 7H_2O$	0.2	g
NaCl	0.1	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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1628. M408 MEDIUM

Yeast extract	1.0	g
Mannitol	10.0	g
K_2HPO_4	0.5	g
$MgSO_4 \times 7H_2O$	0.2	g
NaCl	0.1	g
Agar	20.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0.

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1629. MINIMAL MEDIA CONTAINING METHANOL

NH_4Cl	1.62	g
$MgSO_4$ x 7 H_2O	0.20	g
K_2HPO_4	2.40	g
NaH_2PO_4 x $2H_2O$	1.10	g
Na_2EDTA	15.00	mg
$FeSO_4$ x 6 H_2O	3.00	mg
$ZnSO_4$ x $7H_2O$	4.50	mg
$CoCl_2$ x 6 H_2O	3.00	m g
$MnCl_2$ x 4 H_2O	0.64	mg
H_3BO3	1.00	mg
Na_2MoO_4 x 2 H_2O	0.40	mg
$CuSO_4$ x 2 H_2O	0.30	mg
$CaCl_2x6H_2O$	3.00	mg
Methanol	5.00	ml
Agar	15.00	g
Distilled water	1000.000	ml

Prepare the medium without methanol. Adjust pH to 7.1.

Autoclave and after cooling to 50°C add the methanol from a filter-sterilized stock solution.

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1630. NITROSOSPHAERA MEDIUM

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
KH_2HPO_4	0.20	g
KCl	0.50	g
Na_2SO_4	0.05	g
HEPES buffer solution (see below)	10.00	ml
NH_4Cl	0.05	g
Trace elements solution SL-11 (see medium 722)	1.00	ml
Na-pyruvate	0.05	g
$NaHCO_3$	0.25	g
Wolin's vitamin solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except trace elements, HEPES buffer, ammonium chloride, pyruvate, bicarbonate and vitamins) and dispense medium under air atmosphere into culture vessels suitable for anaerobic cultivation (e.g., 20 ml medium per 100 ml serum bottle), seal with grey butyl rubber stoppers and autoclave. Add HEPES buffer and ammonium chloride from stock solutions sterilized by autoclaving. Trace elements, pyruvate, bicarbonate, and vitamins are added from sterile stock solutions sterilized by filtration. Adjust pH of the complete medium to 7.5 using a sterile solution of bicarbonate (5% w/v) or 1 N HCl.

HEPES buffer solution:

NaOH (pellets)	24.00	g
HEPES (free acid)	238.40	g
Distilled water	1000.00	ml

Fist dissolve NaOH in ca 600 ml water, then add HEPES, adjust pH to 7.5 and fill up to 1000 ml volume.

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1631. VERRUCOMICROBIUM MEDIUM (IG)

Mineral salt solution (see below, medium 621)	20.00	ml
Peptone (Bacto)	0.25	g
Yeast extract (Bacto)	0.25	g
HEPES	2.38	g
Agar (Bacto)	15.00	g
Distilled water	965.00	ml

Sterilize 20 min./121°C. After cooling to 60°C add to the medium:

Glucose sol. (2.5%, sterile-filtered)	10.00	ml
Vitamin sol. (double conc.)	5.00	ml

Adjust pH to 7.5 (the medium is only weakly buffered; one needs approx. 10 drops/l medium of 6 N KOH).

Mineral salt solution:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_2O	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
Na_2MoO_4 x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
Metall salt sol. "44" (see medium 590)	50.00	ml
Distilled water	900.00	ml

Dissolve NTA first by neutralizing with KOH, then add other salts. Adjust pH to 7.2 with KOH or H_2SO_4. Adjust volume to 1000.0 ml with distilled water.

Vitamin solution (double conc.):

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Distilled water	1000.00	ml

Store in the dark and cold (5°C).

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1633. V2 MEDIUM

Peptone (Bacto)	0.250	g
Yeast extract (Bacto)	0.250	g
HEPES	2.380	g
Mineral salt solution (see below)	20.000	ml
Trace element solution (see below)	1.000	ml
$NaHCO_3$	0.025	g
$CaC0_3$	0.025	g
$CaCl_2$ x 2 H_2O	0.015	g
$MgSO_4$ x 7 H_20	0.049	g
KNO_3	0.010	g
KH_2PO_4	1.400	mg
NH_4Cl	0.530	mg
Agar (Bacto)	15.00	g
Double distilled water	730.00	ml

Sterilize 20 min./121°C. After cooling to 60°C add:

N-Acetylglucosamine (10% stock solution)	8.0	ml
Glucose sol. (2.5%, sterile-filtered)	10.0	ml
Vitamin sol. (double conc.)(see below, medium 621)	5.0	ml

Adjust pH to 7.0 with 40% KOH.

Mineral salts solution:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$Na_2MoO_7O_4$ x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

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"Metals 44":		
Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Trace element solution:		
Na-Nitrilotriacetat	1.50	g
$MnSO_4$ x H_2O	0.50	g
Fe SO_4 x 7 H_2O	0.10	g
$Co(NO_3)_2$ x 6 H_2O	0.10	g
$ZnCl_2$	0.10	g
$NiCl_2$ x 6 H_2O	0.05	g
H_2SeO_3	0.05	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.01	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
Na_2WO_4 x 2 H_2O	0.01	g
Double distilled water	1000.00	ml

Vitamin solution (double conc.):		
Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Double distilled water	1000.00	ml

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1634. SERPENTINOMONAS MINIMAL MEDIUM

Na_2SO_4	0.0284	g
$MgCl_2$ x 6 H_2O	0.0404	g
NH_4Cl	0.0802	g
CAPS buffer	3.32	g
dDistilled water	1000.00	ml

Adjust the pH to 11 with NaOH. Fill into to screw-cap tubes, leaving 60% headspace and flush headspace with N_2. Add 30% air and for DSM 103920 additionally add 50% H_2.

Autoclave medium for 20 min at 121°C, cool and add from sterile stock solutions while stirring:
0.3 mM K_2HPO_4 (1ml of 300 mM stock solution per liter)
5 mM $CaCl_2$x2 H_2O (5ml of 1 M stock solution per liter)
4 mM Na-acetate (2ml of 2 M stock solution per liter)
Vitamin solution 10 ml per liter
Trace element solution 10 ml per liter
Some precipitate will form.

Vitamin solution :

Folic acid	2.0	mg
Biotin	2.0	mg
Riboflavin	5.0	mg
Nicotinic acid	5.0	mg
Thiamine	5.0	mg
p-Aminobenzoic acid	5.0	mg
Pyridoxine hydrochloride	10.0	mg
Ca-pantothenate	5.0	mg
Vitamin B_{12}	0.1	mg
Thioctic acid	5.0	mg
Monopotassium phosphate	900.0	mg
Distilled water	1000.0	ml

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Trace element solution:

Na-EDTA x 2 H_2O	500	mg
$MgSO_4$ x 7 H_2O	3000	mg
$MnSO_4$ x H_2O	500	mg
NaCl	1000	mg
$FeSO_4$ x 7 H_2O	100	mg
$Co(NO_3)_2$ x 6 H_2O	100	mg
$CaCL_2$ (anhydrous)	100	mg
$ZnSO_4$ x 7 H_2O	100	mg
$CuSO_4$ x 5 H_2O	10	mg
$AlK(SO_4)_2$(anhydrous)	10	mg
H_3BO_3	10	mg
Na_2MoO_4 x 2 H_2O	10	mg
Na_2SeO_3 (anhydrous)	1	mg
Na_2WO_4 x 2 H_2O	10	mg
$NiCl_2$ x 6 H_2O	20	mg
Distilled water	1000.0	ml

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1635: SULFURIFLEXUS MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
$MgSO_4$ x 7 H_2O	0.30	g
$CaCl_2$	0.10	g
NH_4Cl	0.10	g
KH_2PO_4	0.10	g
KCl	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$Na_2S_2O_3$ x 5 H_2O	5.00	g
$NaHCO_3$	0.40	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except thiosulfate, bicarbonate and vitamins), adjust pH to 6.0 and dispense medium under air atmosphere into serum vials up to a volume of 20%, close with rubber septa and autoclave. Add thiosulfate, vitamins and bicarbonate to the autoclaved medium from sterile stock solutions sterilized by filtration. Adjust pH of the complete medium to 7.2.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1636: TEPIDIBACILLUS MEDIUM

NaCl	10.00	g
KH_2PO_4	0.33	g
$MgCl_2 \times 6\ H_2O$	0.33	g
KCl	0.33	g
NH_4Cl	0.33	g
Modified Wolin's mineral solution	1.00	ml
Yeast extract	0.10	g
$CaCl_2 \times 2\ H_2O$	0.33	g
Na_2CO_3	1.50	g
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add calcium chloride, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.4, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)_2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.03	g
$Na_2SeO_3 \times 5\ H_2O$	0.30	mg
$Na_2WO_4 \times 2\ H_2O$	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1637: METHANOMETHYLOPHILUS MEDIUM

Final pH: 7.6 - 7.8
Final volume: 1020 ml

Trypticase peptone (BD BACTO)	16.00	g
Yeast extract (OXOID)	7.00	g
NaCl	5.00	g
L-Cysteine HCl x H_2O	0.50	g
$NaHCO_3$	0.50	g
Haemin solution	5.00	ml
Vitamin K_1 solution	5.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Methanol (15% v/v)	10.00	ml
DL-Dithiothreitol	1.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients except for methanol and dithiothreitol. Adjust pH to 7.5 with 1 N NaOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Distribute medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add methanol from a sterile anoxic stock solution (15% v/v) autoclaved under 100% N_2 gas atmosphere and dithiothreitol from an anoxic stock solution sterilized by filtration. Before use check the pH of the complete medium and adjust it to 7.6 - 7.8, if necessary.

2. After inoculation, add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 bar overpressure. After growth becomes visible overpressure of 80% H_2 and 20% CO_2 gas mixture can be increased to 1 bar.

3. Note: Use 10% (v/v) as inoculum.

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml
Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K_1 solution (from medium 78)

Vitamin K_1	0.10	ml
Ethanol (95 %)	20.00	ml

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Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

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1639: AZOARCUS TAIWANENSIS MEDIUM

NaCl	10.00	g
KNO_3	0.76	g
NH_4Cl	0.10	g
K_2HPO_4	0.25	g
Trace element solution SL-11	1.00	ml
$NaHCO_3$	1.50	g
Na-acetate	0.44	g
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate and sulfide) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Then, add and dissolve bicarbonate, adjust pH to 8.5 - 9.0, dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add acetate and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas atmosphere. Adjust pH of complete medium to 8.5 - 9.0, if necessary.

Trace element solution SL-11 (from medium 722)

Na_2-EDTA x 2 H_2O	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

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1646. GEMMATIMONAS MEDIUM

Yeast extract	0.25	g
Peptone (Difco)	0.50	g
Glucose	0.10	g
HEPES	1.95	g
$MgSO_4$ x 7 H_2O	0.37	g
$CaSO_4$ x 2 H_2O	0.43	g
Agar	14.00	g
Distilled water	1000.00	ml

Adjust pH to 7.0 prior to the addition of agar.
After autoclaving and cooling to about 55°C, add 5 ml of vitamin solution.

Vitamin solution: (see also medium 461).

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

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1647. ACETOBACTEROIDES MEDIUM

Yeast extract	0.20	g
Glucose	0.18	g
KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	ml
Cysteine-HCl x H_2O	0.30	g
$NaHCO_3$	2.50	g
Na_2S x 9 H_2O	0.30	g
Resazurin	1.00	mg
Vitamin solution (see below)	1	ml
Trace element solution (see below)	1.00	ml
Se/W solution (see below)	1.00	ml
Distilled water	1000.00	ml

The vitamin solution, trace element solution, Se/W solution, cysteine, glucose, Na_2S and $NaHCO_3$ are added after the medium has been autoclaved.

Trace element solution (modified; for preparation see Medium 318):

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
Distilled water	1000.00	ml

Se/W solution:

Na_2SeO_3	4.0	mg
Na_2WO_4	4.0	mg
Distilled water	1000.00	ml

Vitamin solution (for preparation see Medium 141):

Biotin	2.00	mg
Folic acid	2.00	mg

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Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H_2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1654. GILLIAMELLA MEDIUM

Proteose peptone	20.00	g
Beef extract	3.00	g
Yeast extract	3.00	g
Malt extract	3.00	g
Dextrose	5.00	g
ACES	3.64	g
Agar if appropriate	14.00	g
Charcoal for medical application (in agar only)	2.00	g
Distilled water	990.00	ml
Na-Ascorbate solution	10.00	ml

Add ascorbate solution (20 g per litre) after autoclaving and cooling. Final pH of the medium is 7.2. Charcoal should be omitted in liquid medium.

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1655. MODIFIED THERMUS MEDIUM 878

Yeast extract	2.50	g
Tryptone	2.50	g
Agar	28.00	g
Nitrilotriacetic acid	100.00	mg
$CaSO_4$ x 2 H_2O	40.00	mg
$MgCl_2$ x 6 H_2O	200.00	mg
0.01 M Fe citrate	0.50	ml
Trace element solution (see below)	0.50	ml
Phosphate buffer (see below)	100.00	ml
Distilled water	900.00	ml

Adjust pH to 7.2 with NaOH. Autoclave at 121°C for 15 min. Autoclave the phosphate buffer separately and then add to the medium.

Phosphate buffer:

KH_2PO_4	5.44	g
Na_2HPO_4 x 12 H_2O	43.00	g
Distilled water	1000.00	ml

Adjust pH to 7.2

Trace element solution:

H_2SO_4	0.50	ml
$MnSO_4$ x H_2O	2.28	g
$ZnSO_4$ x 7 H_2O	0.50	g
H_3BO_3	0.50	g
$CuSO_4$ x 5 H_2O	25.00	mg
Na_2MoO_4 x 2 H_2O	25.00	mg
$CoCl_2$ x 6 H_2O	45.00	mg
Distilled water	1000.00	ml

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1660. modified BAP$^+$ (PSA) MEDIUM FOR FRANKIA

KH_2PO_4	0.950	g
K_2HPO_4	0.600	g
NH_4Cl	0.270	g
Na-propionate	0.480	g
Sodium succinate dibasic	0.480	g
Pyruvic acid sodium salt	0.480	g
Sodium acetate	0.480	g
$MgSO_4$ x 7 H_2O	0.025	g
$CaCl_2$ x 2 H_2O	0.010	g
Vitamine solution (see below)	1.000	ml
Chelated iron solution (see below)	1.000	ml
Trace element solution (see below)	0.100	ml
Distilled water	1000.000	ml

Vitamine solution:

Thiamine HCl	0.010	g
Nicotinic acid	0.050	g
Pyridoxine HCl	0.050	g
Distilled water	100.000	ml

Chelated iron solution:

Citronensäure	1.000	g
Eisencitrat	1.000	g
Distilled water	100.000	ml

Trace element solution:

$CoSO_4$ x 7 H_2O	0.001	g
$CuSO_4$ x 5 H_2O	0.080	g
H_3BO_3	2.860	g
$MnCl_2$ x 4 H_2O	1.810	g
Na_2MoO_4 x 2 H_2O	0.025	g
$ZnSO_4$ x 7 H_2O	0.220	g
Distilled water	100.000	ml

The pH of the final solution is adjusted to 6.3.

Note: Please autoclave the chelated iron solution before you add to the medium, so that solves everything!!!!

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1661: ALKALIPHILUS NAMSARAEVII MEDIUM

KH_2PO_4	0.50	g
NaCl	25.00	g
Yeast extract	2.50	g
$NaHCO_3$	1.85	g
Na_2CO_3	1.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Fe(III) citrate (19% Fe)	7.30	g
$MgCl_2$ x 6 H_2O	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, carbonate, trace elements, ferric citrate, magnesium chloride and vitamins) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and carbonate, then adjust pH to 9.5 and dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferric citrate (10% w/v stock solution; dissolve by boiling and adjust pH to 7.5-8.0), trace elements, selenite-tungstate solution, magnesium chloride and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas. Sterilize vitamins by filtration. Adjust pH of the complete medium to 9.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg

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Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1662: ALKALIPHILUS HYDROTHERMALIS MEDIUM

KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
NH_4Cl	0.50	g
KCl	0.10	g
NaCl	5.00	g
$MgCl_2$ x 6 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
Yeast extract (OXOID)	5.00	g
Trypticase peptone (BD BBL)	5.00	g
Modified Wolin's mineral solution	10.00	ml
Na_2CO_3	3.00	g
Na-crotonate solution (1 M)	10.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, crotonate and sulfide), adjust pH to 8.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add crotonate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The crotonate solution should be sterilized by filtration. Adjust pH of the complete medium to 9.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Na-crotonate solution (1 M) (from medium 870)

Crotonic acid (ALDRICH 113018)	86.00	g
NaOH (10 N)	100.00	ml
Distilled water	900.00	ml

Dissolve crotonic acid in 800 ml distilled water, add around 100 ml of 10 N NaOH and adjust pH to around 7. Then add water to reach a volume of 1000 ml. Sterilize by filtration under 100% N_2 gas atmosphere.

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1663. BSM medium

NaCl	3.62	g
K_2HPO_4	0.26	g
(NH4)Cl	0.08	g
$NaNO_3$	0.85	g
Na_2SO_4	0.05	g
Trace element solution (see below)	1.00	ml
Vitamin solution (see medium 141)	10.00	ml
Na-DL-lactate	1.12	g
Yeast extract	0.20	g
Distilled water	936.50	ml
$NaHCO_3$ (8%)	52.50	ml

Dissolve ingredients (except vitamins and trace elements) in distilled water; boil for 1- 2 minutes and cool down to room temperature under 100% N_2 gas, dispense in culture vessels and autoclave. After sterilization add $NaHCO_3$, vitamins, and trace elements from sterile anoxic stock solutions prepared under 100% N_2. Adjust pH to 8.2 and add sodium sulfide (Na_2S) from a sterile anoxic solution to achieve a final concentration of 100 µM prior inoculation.

Trace element solution

HCl (25%; 7.7 M)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$MgCl_2$ x 6 H_2O	3.00	g
CaCl2 x 2 H_2O	0.10	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

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1664 Sphaerotilus natans subspec. sulfidivorans medium

(NH4)Cl	0.3	g
NaCl	3.62	g
KH_2PO_4	0.09	g
K_2HPO_4	0.22	g
Na_2HPO_4 x $7H_20$	0.35	g
$MgSO_4$ x $7H_20$	0.23	g
$CaCl_2$	0.28	g
$FeCl_3$ x $6H_2$	0.25	mg
Na_2SO_4	0.05	g
$Na_2S_2SO_3$	1.0	g
Distilled water	1000	ml

Dissolve ingredients (except vitamins) in distilled water. After sterilization add the 25ml SLM.

Trace element solution

Trace element solution:

EDTA	0.2	g
$FeSO_4$ x 7 H_2O	0.7	g
$ZnSO_4$ x 7 H_2O	0.01	g
$MnSO_4$ x 4 H_2O	2.0	mg
$CuSO_4$ x 5 H_2O	5.0	mg
H_3BO_3	0.01	g
$Co(NO_3)_2$	1.0	g
Na_2MoO_4 x 2 H_2O	1.0	g
Distilled water	1000	ml

Standard vitamin solution:

Riboflavin	10.00	mg
Thiamine-HCl x 2 H_2O	50.0	mg
Nicotinic acid	50.0	mg
Pyridoxine-HCl	50.0	mg
Ca-pantothenate	50.0	mg
Biotin	0.1	mg
Folic acid	0.2	mg
Vitamin B_{12}	1.0	mg
Distilled water	100	ml

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1665: DESULFAMPLUS MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	6.00	g
Na_2SO_4	3.00	g
$CaCl_2$ x 2 H_2O	0.20	g
KCl	0.50	g
NH_4Cl	0.30	g
Wolfe's mineral elixir	2.00	ml
HEPES (SIGMA)	2.40	g
Na_2-fumarate	1.00	g
Yeast extract (OXOID)	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
K-phosphate buffer (0.5 M, pH 7.0)	2.00	ml
$NaHCO_3$	1.00	g
$FeCl_2$ x 4 H_2O (0.2% w/v in 0.02 N HCl)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphate buffer, bicarbonate, ferrous chloride, vitamins and cysteine), adjust pH to 7.2 with NaOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add phosphate buffer, ferrous chloride, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.2 - 7.5 , if necessary.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g

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Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1666. MAG Modified arabinose gluconate medium

KH_2PO_4	0.22	g
Hepes	1.30	g
NH_4Cl	0.32	g
$MgSO_4$ x $7H_2O$	0.18	g
Na_2SO_4	0.25	g
Arabinose	1.00	g
Yeast extract	1.00	g
Gluconic acid	1.00	g
MES	1.10	g
$FeCL_3$ 0.67%	1.00	ml
$CaCl_2$ 1.5%	1.00	ml
$NaMoO_4$ x $2H_2O$ 1%	1.00	ml
$NiCl_2$ 1.2%	0.10	ml
Distilled water	1000.00	ml
Adjust pH to pH 7.2		

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1667: HYPNOCYCLICUS MEDIUM

Na_2SO_4	0.02	g
KH_2PO_4	1.00	g
NH_4Cl	0.10	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
$NaNO_3$	0.70	g
Yeast extract	1.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Seven vitamins solution	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, vitamins and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1667: HYPNOCYCLICUS MEDIUM

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1668. MDM (modified dehority medium)

Yeast Extract	0.50	g
Tryptone	0.50	g
Stock A	250	ml
Stock B	2.5	ml
Distilled water	250	ml

To make medium anoxic boil it, cool under N_2CO_2 gas atmosphere, add 1 g/l L-cysteine hydrochloride and adjust pH to 6.8. Finally gas over with N_2 . Dispense under N_2CO_2 into suitable vessels for anaerobic growth (serum bottles or Hungate tubes sealed with rubber stoppers) and sterilize by autoclaving.

For **DSM 104696, DSM 104698, DSM 104699**

For 10 ml in hungate tube, add:		
Vitamin solution	5-10	µl
Rumen fluid	500	µl
Cellobiose (10%)	200	µl

For **DSM 104697**

For 10 ml in hungate tube, add:		
Vitamin solution	5-10	µl
Rumen fluid	500	µl
Cellobiose (10%)	200	µl
CM-Mix	500	µl
Vitamin K1 solution	5-10	µl

Stock A: Salt solution 2x (50 ml stock per 100 ml medium)

K_2HPO_4	6804	mg
$NaHCO_3$	6721	mg
NaCl	4675	mg
NH_4Cl	1605	mg
$CaCl_2$	89	mg
$MgCl_2$	95	mg
$FeSO_4$ x 7 H_2O	28	mg
Distilled water	1000	ml

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Stock B: Salt solution 200x (0,5 ml stock per 100 ml medium)

$MgCl_2$ x 2 H_2O	971	mg
$ZnCl_2$	273	mg
$CoCl_2$	104	mg
Na_2 MoO_4 x 2 H_2O	48	mg
Na_2SeO_3	35	mg
$NiCl_2$ x 6 H_2O	237	mg
Na_2WO_4 x 2 H_2O	66	mg
Distilled water	1000.00	ml

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H2O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B12	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Filter sterilize and make anoxic with N_2.

Cellobiose (10%)

Mix 10 g cellobiose with 100 ml distilled water and filter sterilize, make anoxic with N_2.

CM-Mix (only for DSM 104697)

D-Glucose	4.0	g
Cellobiose	1.0	g
Maltose	1.0	g
Starch (soluble)	1.0	g
Distilled water	1000.00	ml

Make anoxic with N_2.

Vitamin K_1 solution (only for DSM 104697)

Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Make anoxic with N_2. Store refrigerated in a brown bottle.

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1669. SCHAEDLER BROTH (Roth; 5772)

Casein peptone	5.66	g
Soy peptone	1.00	g
Yeast extract	5.00	g
Peptone mixture	5.00	g
Glucose	5.83	g
K_2HPO_4	0.83	g
NaCl	1.66	g
Tris (hydroxymethyl aminomethane)	3.00	g
Resazurin	1.00	mg
Hemin	0.01	g
Cysteine-HCl x H_2O	0.40	g
Distilled water	1000.00	ml

Boil medium under CO_2 until the resazurin indicator changes from pink to colourless. Cool down under CO_2 and adjust pH to 7.6. Switch to N_2 to prevent further lowering of the pH. Fill up into Hungate tubes under N_2 and autoclave.

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1672. Cyanobacteria Medium BA-

	Volume [ml/l]	Stock Solutions [g/l]
NaCl	33.3	250
MgCl2 x 6H2O	3.3	200
KCl	3.3	50
$Mg(SO_4)$ x $7H_2O$	3.3	350
$CaCl_2$ x $2H_2O$	3.3	50
Na_3-citrate x $2H_2O$	8.3	0.6
Na_2-EDTA x $2H_2O$	8.3	0.1
Trace Metal Mix	1.0	-

Adjust to 1000ml with H_2O dist. and autoclave.

After cooling, add the following filter sterilized components

	Volume [ml/l]	Stock Solutions [g/l]
K_2HPO_4 x$3H_2O$	4.15	8.0
Na_2CO_3	2.3	20
Fe-NH_4-citrate	0.8	6.0
Vitamin B12	1.0	0.02

BA- Stock Solutions

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.86	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8g/100ml Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

1673. Cyanobacteria Medium ASN3+

	Volume [ml/l]	Stock Solutions [g/l]
NaCl	100	250
MgCl2 x 6H2O	10	200
KCl	10	50
$Mg(SO_4)$ x $7H_2O$	10	350
$CaCl_2$ x $2H_2O$	10	50
Na_3-citrate x $2H_2O$	5.0	0.6
Na_2-EDTA x $2H_2O$	5.0	0.1
Trace Metal Mix	1.0	-

Adjust to 1000ml with H_2O dist. and autoclave.

After cooling, add the following filter sterilized components to complete the medium:

	Volume [ml/l]	Stock Solutions [g/l]
$NaNO_3$	2.5	300
K_2HPO_4 x $3H_2O$	2.5	8.0
Na_2CO_3	1.0	20
Fe-NH_4-citrate	0.5	6.0
Vitamin B12	1.0	0.02

ASN3+ Stock Solutions

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.9	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8g/100ml Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

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1674. Cyanobacteria Medium BG11- Cu

	Volume [ml/l]	Stock Solutions [g/l]
$Mg(SO_4)$ x $7H_2O$	10	7.5
$CaCl_2$ x $2H_2O$	10	3.6
Na_3-citrate x $2H_2O$	10	0.6
Na_2-EDTA x $2H_2O$	10	0.1
Trace metal mix **-Cu**	1.0	-

Adjust to 1000ml with H_2O dist. and autoclave.

After cooling, add the following filter components

	Volume [ml/l]	Stock Solutions [g/l]
BG11-Mix	2.0	-
Vitamine B12	1.0	0.02

BG11-Cu Stock Solutions

		[g/l]
BG11-Mix	K_2HPO_4 x $3H_2O$	20
-	Na_2CO_3	10
-	Fe-NH_4-citrate	3.0

		[g/l]	[ml/l]	
Trace metal Mix -Cu	H_3BO_3	2.9	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	-	-	-
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

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1675: DESULFOTHERMOBACTER MEDIUM

NH_4Cl	0.33	g
KCl	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
KH_2PO_4	0.33	g
Na_2SO_4	2.80	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve all ingredients except vitamins and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 4.5 with HCl, dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 4.5 - 4.8, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1676. Cyanobacteria Medium ASN3 -

	Volume [ml/l]	Stock Solutions [g/l]
NaCl	100	250
MgCl2 x 6H2O	10	200
KCl	10	50
$Mg(SO_4)$ x $7H_2O$	10	350
$CaCl_2$ x $2H_2O$	10	50
Na_3-citrate x $2H_2O$	5	0.6
Na_2-EDTA x $2H_2O$	5	0.1
Trace Metal Mix	1	-

Adjust to 1000ml with H_2O dist. and autoclave.

After cooling, add the following filter sterilized components to complete the medium:

	Volume [ml/l]	Stock Solutions [g/l]
K_2HPO_4 x $3H_2O$	2.5	8.0
Na_2CO_3	1.0	20
Fe-NH_4-citrate	0.5	6.0
Vitamine B12	1.0	0.02

ASN3 -

Stock Solutions

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.9	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8g/100ml Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

1677: Cyanobacteria Medium BA+

Final volume: 1000 ml

NaCl (250 g/l stock solution)	33.30	g
$MgCl_2$ x 6 H_2O (200 g/l stock solution)	3.30	g
KCl (50 g/l stock solution)	3.30	g
$Mg(SO_4)$ x 7 H_2O (350 g/l stock solution)	3.30	g
$CaCl_2$ x 2 H_2O (50 g/l stock solution)	3.30	g
Na_3-citrate x 2 H_2O (0.6 g/l stock solution)	8.30	ml
Na_2-EDTA x 2 H_2O (0.1 g/l stock solution)	8.30	ml
T-Trace Metal Mix *	1.00	ml

1. Make up to 1000 ml with MilliQ water.
2. Autoclave at 121 °C for 20 min.
3. After cooling, add the following filter sterilized components:

$NaNO_3$ (300 g/l stock solution)	4.15	ml
K_2HPO_4 x 3 H_2O (8.0 g/l stock solution)	4.15	ml
Na_2CO_3 (20 g/l stock solution)	2.30	ml
$Fe(NH_4)$citrate (6.0 g/l stock solution)	0.80	ml
Vitamin B_{12} (0.02 g/l stock solution)	1.00	ml

T-Trace Metal Mix *

$ZnSO_4$ x 7 H_2O	0.22	g/l
$MnCl_2$ x 4 H_2O	1.81	g/l
H_3BO_3	2.86	g/l
Na_2MoO_4 x 2 H_2O	0.39	g/l
$CuSO_4$ x 5 H_2O (0.08 g/l stock solution)	10.00	ml/l
$Co(NO_3)_2$ x 6 H_2O (0.05 g/l stock solution)	10.00	ml/l

Make up to 1000 ml with MilliQ water.

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1678. Cyanobacteria Medium BA+50+B+N/2

	Volume [ml/l]	Stock Solutions [g/l]
NaCl	33.3	250
MgCl2 x 6H2O	3.3	200
KCl	3.3	50
$Mg(SO_4)$ x $7H_2O$	3.3	350
$CaCl_2$ x $2H_2O$	3.3	50
Na_3-citrate x $2H_2O$	8.3	0.6
Na_2-EDTA x $2H_2O$	8.3	0.1
Trace Metal Mix	1.0	-

Adjust to 1000ml with H_2O dist. and autoclave.

After cooling, add the following filter sterilized components

	Volume [ml/l]	Stock Solutions [g/l]
$NaNO_3$	2.075	300
K_2HPO_4 x $3H_2O$	4.15	8.0
$NaHCO_3$	3.43	52.5
Fe-NH_4-citrate	0.8	6.0
NH_4Cl	0.5	53.5
Vitamine B12	1.0	0.02

BA + 50 +B +N/2 Stock Solutions

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.86	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO_4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8g/100ml Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

1679. Cyanobacteria Medium 2xASN3

	Volume [ml/l]	Stock Solutions [g/l]
NaCl	200	250
$MgCl_2 \times 6H_2O$	20	200
KCl	20	50
$Mg(SO_4) \times 7H_2O$	20	350
$CaCl_2 \times 2H_2O$	20	50
Na_3-citrate x $2H_2O$	10	0.6
Na_2-EDTA x $2H_2O$	10	0.1
Trace Metal Mix	1.0	-

Adjust to 1000ml with H_2O dist. water and autoclave.

After cooling, add the following filter sterilized components

	Volume [ml/l]	Stock Solutions [g/l]
$NaNO_3$	2.5	300
$K_2HPO_4 \times 3H_2O$	2.5	8.0
Na_2CO_3	1.0	20
Fe-NH_4-citrate	0.5	6.0
Vitamine B12	1.0	0.02

2xASN3 Stock Solutions

		[g/l]	[ml/l]	
Trace metal Mix	H_3BO_3	2.9	<-	
	$MnCl_2 \times 4H_2O$	1.81	<-	
	$ZnSO_4 \times 7H_2O$	0.22	<-	
	$Na_2MoO_4 \times 2H_2O$	0.39	<-	
	$CuSO_4 \times 5H_2O$	->	10 ml	0.8g/100ml Stock Sol.
	$Co(NO_3)_2 \times 6H_2O$	->	10 ml	0.5g/100ml Stock Sol.

1680: Cyanobacteria Medium MCL

Final volume: 1000 ml

Artificial Sea Salt (hw Marinemix 21010; Wiegandt GmbH – Products for aquaristics, Sterkenhofweg 13, 47807 Krefeld, Germany)	33.00	g
MCL Stock Solution A *	5.00	ml
MCL Stock Solution B **	5.00	ml

1. Adjust to 1000 ml with MilliQ water and autoclave (121°C, 20 min).

2. After cooling add the following filter sterilized component under sterile conditions:

MCL Vitamin Stock Solution ***	1.00	ml

MCL Stock Solution A * (from medium 1680)

$CuSO_4$ x 7 H_2O (1.92 g/l stock solution)	5.00	ml
$MnSO_4$ x H_2O (2.4 g/l stock solution)	100.00	ml
$ZnSO_4$ x 7 H_2O (0.44 g/l stock solution)	100.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (5.6 g/l stock solution)	100.00	ml

Adjust to 500 ml with MilliQ water.

MCL Stock Solution B ** (from medium 1680)

$FeCl_3$ (0.96 g/l stock solution)	100.00	ml
H_3BO_3 (22.4 g/l stock solution)	100.00	ml
$NaNO_3$ (84.0 g/l stock solution)	100.00	ml
Na_2-EDTA x 2 H_2O (25.3 g/l stock solution)	100.00	ml
Sodium beta-glycerophosphate (8.0 g/l stock solution)	100.00	ml

Mix to obtain 500 ml of MCL Stock Solution B.

MCL Vitamin Stock Solution *** (from medium 1680)

Vitamin B_{12} (1.0 mg/ml stock solution)	160.00	µl
Biotin (1.0 mg/ml stock solution)	160.00	µl
Thiamine (10.0 mg/ml stock solution)	800.00	µl

Adjust to 100 ml with MilliQ water.

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1681. Mineral salt medium with colloidal chitin

Solution A	50.00	ml
Solution B	50.00	ml
Trace element solution (see medium 141)	10.00	ml
Vitamin solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	1.00	ml
Yeast extract	1.00	g
Polypeptone	10.00	g
$NaHCO_3$	3.52	g
Colloidal chitin	5.00	g
Distilled water	890.00	ml

Solution A

KH_2PO_4	8.00	g
NH_4Cl	5.00	g
$MgCl_2$ x 6 H_2O	4.00	g
$CaCl_2$ x $2H_2O$	1.00	g
Distilled water	1000	ml

Solution B

K_2HPO_4	8.00	g
Distilled water	1000	ml

Reducing agents

Flush each solution under N_2 for 30 minutes, stopper and autoclave.
L-Cysteine-HCL x H_2O solution (10%): add 12.5 µl per 10 ml medium
Na_2S x 9 H_2O solution (3%): add 40 µl per 10 ml medium.

Colloidal chitin preparation

Cut 5 gram commercial crab (shrimp) shell flakes into small pieces, and dissolve in 100 ml of 12M HCL while stirring in a fume hood at room temperature. Add chitin-HCL mixture slowly into pre-cooled water to obtain the colloidal chitin, centrifuge and wash in pre-cooled water till the colloidal chitin reaches a pH of 6 to 6.5. Mix 0.5% with MS medium as the sole carbon source. As an alternative commercial available chitin powder can be used.

Prepare MS medium with chitin

Dissolve the ingredients of MS medium (except $NaHCO_3$ and vitamins), boil medium under CO_2 until the resazurin indicator changes from pink to colourless. Cool down under CO_2, add $NaHCO_3$ and adjust pH to 7.0. Switch to N_2 to prevent further lowering of the pH. Fill up into Hungate tubes under N_2 and autoclave. Before use add vitamins (sterilized by filtration) and reducing agents.

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1684. Alicyclobacillus cellulosilyticus Medium

Carboxymethylcellulose (CMC), sodium salt	2.50	g
$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.37	g
Glucose	1.00	g
Yeast extract	0.25	g
Tryptone	0.90	g
$MgSO_4$ x 7 H_2O solution (25%)	1.00	ml
$CaCl_2$ x 2 H_2O solution (7%)	1.00	ml
$FeCl_3$ x 6 H_2O solution (2%)	1.00	ml
Distilled water	1000.00	ml

Adjust to pH 4.8-5.0 (liquid medium) and pH 5.5 - 6.0 agar medium. Autoclave MgSO4 x 7 H_2O solution, CaCl2 x 2 H_2O solution and FeCl3 x 6 H_2O solution separately. Aseptically add all solutions to the basal medium.

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1687. THERMOANAEROBACULUM MEDIUM

Solution A:

KH_2PO_4	0.020	g
NH_4Cl	0.025	g
NaCl	0.100	g
$MgCl_2$ x 6 H_2O	0.040	g
KCl	0.050	g
$CaCl_2$ x 2 H_2O	0.015	g
$NaNO_3$	0.85	g
yeast extract	0.10	g
Na-resazurin solution (0.1% w/v)	0.050	ml
Distilled water	994.00	ml

Solution B:

Trace element solution SL-10 (see medium 320)	0.100	ml

Solution C:

Selenite-tungstate solution (see medium 385)	0.100	ml

Sparge *solution A* with 100% N_2 gas for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. S*olutions B* and *C* are autoclaved separately under 100% N_2 gas. Adjust pH of complete medium to 7.2 - 7.4, if necessary.

Use 1.10 g/l Na-pyruvate as substrate. Na-pyruvate is added to the autoclaved medium from a sterile anoxic stock solution sterilized by filtration.

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1688. ACTINOBACTERIA MEDIUM

KH_2PO_4	0.050	g
Na_2SO_4	0.150	g
$(NH_4)_2SO_4$	0.450	g
$MgSO_4$ x 7 H_2O	0.500	g
$Ca(NO_3)_2$ x 4 H_2O	0.015	g
KCl	0.050	g
yeast extract	0.100	g
glucose	0.900	g
Distilled water	add 1000.000	ml

Adjust the pH to 3.0 with H_2SO_4 or HCl.

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1690: NITROSPIRA INOPINATA MEDIUM

KH_2PO_4	1.00	g
KCl	1.50	g
$MgSO_4$ x 7 H_2O	1.00	g
NaCl	11.68	g
Distilled water (MilliQ)	1000.00	ml
$CaCO_3$	4.00	g

1. Dilute stock solution with MilliQ water (1+19 v/v), add 4 g $CaCO_3$ per litre and autoclave. After autoclaving add sterile solutions:

Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Borate-copper solution	1.00	ml
Ammonium chloride solution	10.00	ml

2. Complete medium can be stored at 4°C.

3. Check pH 7.6-8.0

For DSM 105286: Do not shake cultures. Growth takes place without visible turbidity and may be best detected by measuring ammonium and/or nitrite or nitrate concentrations in the culture broth

Borate-copper solution

H_3BO_3	54.00	mg
$CuCl_2$ x 2 H_2O	18.00	mg
Distilled water	1000.00	ml

Incubate at 42°C without shaking. Activity and growth can be followed by measuring the depletion of ammonium or the production of nitrite and/or nitrate. Higher ammonium concentrations are inhibitive. For that reason our recommendation is follow nitrite content. As soon as nitrite has been produced and is depleting again (to form nitrate) another dose of ammonium can be added. Incubation time may be up to several weeks. We checked the nitrite content (absence/presence) every 5-7 days and added ammonium chloride about every 2-3 weeks. Don't expose cultures to intense light.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg

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H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Ammonium chloride solution

Ammonium chloride	0.80	g
Distilled water	100.00	ml

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1693. Cyanobacteria Medium ES

Minerals	Volume [ml/l]	Stock Solutions [g/l]
Soil Extract	30	see recipe below
KNO_3	10.5	19
K_2HPO_4	4.6	4.35
$MgSO_4 \times 7H_2O$	2.7	7.5
Micronutrient solution	5.0	see recipe below

Adjust to 1000mL with H_2O dist. and autoclave.

Micronutrients Component 1	Volume [g/50ml]	
$FeSO_4 \times 7H_2O$	0.7	(solutions remains slightly turbid)
Na-EDTA x $2H_2O$	2.0	(solution remains slightly turbid)

Combine both solutions (yields a clear solution)

Micronutrients Component 2	Volume [ml/l]	[g/l] stock solution
$ZnSO_4 \times 7H_2O$	1.0	1.0
$MnSO_4 \times H_2O$	2.0	0.76
H_3BO_3	5.0	2.0
$Co(NO_3)_2 \times 6H_2O$	0.2	5.0
$Na_2MoO_4 \times 2H2O$	5.0	0.2
$CuSO_4 \times 5H_2O$	0.02	0.250
	[g/L]	
Na-EDTA x $2H_2O$	0.4	

For Micronutrient solution combine Component 1 and Component 2 and adjust to 1 l

Freeze aliquots for storage

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Recipe for the preparation of soil extract:

- Fill 300ml garden soil in a 1 l laboratory flask and fill up with H_2O_{dist} to 700 ml

  (The soil should not contain too much humus and not too much clay – avoid soil contaminated with fertilizers, herbicides or fungicides)

- Shake vigorously
- Autoclave for 1 h at 121 °C and let cool down
- After 24 h autoclave for a second time (for 1 h at 121 °C)
- Remove bigger particles and debris by centrifugation
- Filter through Whatmanfilter 595 (1/2, 320 mm) to remove residual particles
- Filter sterilize (PES membrane, pore size 0,22 µm)
- Store at 4°C – 10°C

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1695. MARINES PLANCTOMYCES MEDIUM MODIFIED I

Peptone (Bacto)	1.00	g
Yeast extract (Bacto)	1.00	g
HEPES	2.38	g
Mineral salt solution (see below)	20.00	ml
Trace element solution (see below)	1.00	ml
Artifical sea water (see below)	250.00	ml
Agar (Bacto)	15.00	g
Double distilled water	730.00	ml

Sterilize 20 min./121°C. After cooling to 60°C add:

N-Acetylglucosamine (10% stock solution)	10.0	ml
Glucose sol. (25%, sterile-filtered)	4.0	ml
Vitamin sol. (double conc.)(see below, medium 621)	5.0	ml

Adjust pH to 7.5 (the medium is only weakly buffered; one needs approx. 10 drops/l medium of 6 N KOH).

Artifical sea water:

NaCl	46.94	g
Na_2SO_4	7.84	g
$MgCl_2$ x 6 H_2O	21.28	g
$CaCl_2$	2.86	g
$NaHCO_3$	0.38	g
KCl	1.38	g
KBr	0.192	g
H_3BO_3	0.052	g
$SrCl_2$	0.08	g
NaF	0.006	g
Double distilled water	1000.000	ml

Mineral salts solution:

Nitrilotriacetic acid (NTA)	10.00	g
$MgSO_4$ x 7 H_20	29.70	g
$CaCl_2$ x 2 H_2O	3.34	g
$Na_2MoO_7O_4$ x 2 H_2O	12.67	mg
$FeSO_4$ x 7 H_2O	99.00	mg
"Metals 44" (see below)	50.00	ml
Distilled water	950.00	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

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"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Trace element solution:

Na-Nitrilotriacetat	1.50	g
$MnSO_4$ x H_2O	0.50	g
Fe SO_4 x 7 H_2O	0.10	g
$Co(NO_3)_2$ x 6 H_2O	0.10	g
$ZnCl_2$	0.10	g
$NiCl_2$ x 6 H_2O	0.05	g
H_2SeO_3	0.05	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.01	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
Na_2WO_4 x 2 H_2O	0.01	g
Double distilled water	1000.00	ml

Vitamin solution (double conc.):

Biotin	4.00	mg
Folic acid	4.00	mg
Pyridoxine-HCl	20.00	mg
Riboflavine	10.00	mg
Thiamine-HCl x 2 H_2O	10.00	mg
Nicotinamide	10.00	mg
D-Ca-pantothenate	10.00	mg
Vitamin B_{12}	0.20	mg
p-Aminobenzoic acid	10.00	mg
Double distilled water	1000.00	ml

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1697: THIOSOCIUS MEDIUM

NaCl	16.00	g
Na_2SO_4	2.50	g
$CaCl_2$ x 2 H_2O	1.00	g
KCl	0.45	g
NH_4Cl	0.25	g
K_2HPO_4	0.14	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
HEPES buffer (1 M, pH 8.0)	20.00	ml
$MgCl_2$ x 6 H_2O	6.00	g
$NaHCO_3$	1.00	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	980.00	ml

Dissolve ingredients (except HEPES buffer, magnesium chloride, bicarbonate, thiosulfate and vitamins), dispense medium in test tubes or Erlenmeyrer flasks to 20% of volume, close with screw caps and autoclave. Add HEPES buffer, magnesium chloride, bicarbonate, thiosulfate and vitamins to the autoclaved medium from sterile stock solutions sterilized by filtration. Adjust pH of complete medium to 8.0.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg

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1697: THIOSOCIUS MEDIUM

Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1699: SULFURIMONAS CRATERIS MEDIUM

Solution A	981.00	ml
Solution B	25.00	ml
Solution C	1.00	ml

Dissolve compounds of solution A except sulfur and sparge it with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials that contain the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Solution B is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is prepared under 100% N_2 gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.5 - 8.0, if necessary.

Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	10.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
$NaNO_3$	0.85	g
Modified Wolin's mineral solution	1.00	ml
Sulfur (powdered)	5.00	g
Distilled water	980.00	ml

Solution B

Na_2CO_3	1.25	g
Distilled water	25.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g

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$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1700: GEOBACTER AMMONIFICANS MEDIUM

KH_2PO_4	0.80	g
K_2HPO_4	0.80	g
KNO_3	0.50	g
Na-acetate	0.80	g
L-Methionine (1% w/v)	0.80	ml
Trace element solution SL-10	1.00	ml
Na-thioglycolate	0.10	g
Ascorbic acid	0.10	g
$CaCl_2$ x 2 H_2O	0.02	g
$MgSO_4$ x 7 H_2O	0.15	g
Na_2CO_3	1.00	g
Distilled water	1000.00	ml

Dissolve all ingredients except thioglycolate, ascorbic acid, calcium chloride, magnesium sulfate and carbonate, adjust pH to 6.7 and then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve thioglycolate and ascorbic acid, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride and magnesium sulfate from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2. Adjust pH of complete medium to pH 7.0 - 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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1703. CMKA MEDIUM (HALOACTINOMYCES MEDIUM)

Mannitol	1.50	g
Casein acids hydrolysate	0.50	g
$MgCl_2$	98.00	g
NaCl	64.00	g
$CaCl_2$	28.00	g
KCl	10.00	g
$(NH_4)_2SO_4$	2.00	g
KNO_3	1.00	g
K_2HPO_4	0.50	g
$CaCO_3$	0.50	g
Agar	20.00	g
add Distilled water	1000.00	ml

Adjust the pH to 7.5.

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1704. YMA

Yeast extract	0.30	g
Na_2-succinate	1.00	g
(NH_4)-acetate	0.50	g
Fe(III) citrate solution (0.1% in H_2O)	5.00	ml
KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Vitamin B_{12} solution (10 mg in 100 ml H_2O)	0.40	ml
Trace element solution SL-6 (see below)	1.00	ml
L-Cysteiniumchloride	0.30	g
Resazurin(0,1%)	0.50	ml
Distilled water	1000.00	ml

Adjust pH to 6.8.
Boil the medium for a few minute. Bubble the medium with nitrogen gas and fill 10 ml in 15 ml tubes with a rubber septum under a stream of nitrogen gas. Autoclave at 121°C for 15 min. Sterile syringes are used to inoculate and remove samples.
Incubate in the light using a tungsten lamp.

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

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1708. THERMOANAEROMYCES MEDIUM

K_2HPO_4	0.350	g
KH_2PO_4	0.230	g
NH_4Cl	0.500	g
$MgSO_4$ x 7 H_2O	0.500	g
$CaCl_2$ x 2 H_2O	0.050	g
NaCl	2.250	g
$FeSO_4$ x 7 H_2O	0.002	g
Yeast extract	2.000	g
Lactose	1.000	g
Na-acetate	0.500	g
$NaHCO_3$	0.500	g
L-cysteine-HCl x H_2O	0.500	g
Trace element solution SL-10 (see medium 1426)	1.000	ml
Na-resazurin solution (0.1% w/v)	1.000	ml
Distilled water	1000.00	ml

Add after autoclaving 0.25/50 ml of sterile Na_2S x 9 H_2O (stock solution 12 g/l)

Dissolve ingredients (except bicarbonate, trace elements, resazurin, cysteine and sulfide) and sparge medium with N_2 for 30 – 45 min to make it anoxic. Then add and dissolve L-cysteine and bicarbonate, adjust pH to 6.8 and dispense medium under N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave.
The reducing agent is autoclaved separately under 100% N_2 gas atmosphere as concentrated solutions in tightly closed tubes. Trace elements are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.8 – 7.0, if necessary.

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1712. AROMATOLEUM MEDIUM

Based on the medium in *Rabus and Widdel, 1995, Arch. Microbiol., 163, 96-103*.

1. Anoxic medium

KH_2PO_4	0.5	g
NH_4CL	0.3	g
$MgSO_4$ x $7H_2O$	0.5	g
$CaCl_2$ x $2H_2O$	0.1	g
$NaNO_3$	0.6	g
distilled water	950	mL

The medium was autoclaved and removed from the autoclave at 85°C. Oxygen dissolution was prevented by exchanging the gas-phase with N_2. The medium was complemented by addition of the following stock solutions:

Trace element solution	1.0	mL/L
Vitamin mixture	1.0	mL/L
Vitamin B_{12} solution	1.0	mL/L
1 M $NaHCO_3$	40.0	mL/L

After adding bicarbonate the gas was changed from N_2 to N_2/CO_2 (90/10 %, v/v) and the pH was adjusted to 7.2 – 7.4 with sterile 2 M HCL.

1 M Ascorbate	4.0	mL/L
1 M Benzoate	4.0	mL/L

2. Preparation of stock solutions

Trace element solution

Na_2-EDTA	5.2	g
$FeSO_4$ x $7H_2O$	2.1	g
H_3BO_3	0.03	g
$MnCl_2$ x $4H_2O$	0.1	g
$CoCl_2$ x $6H_2O$	0.19	g
$NiCl_2$ x $6H_2O$	0.024	g
$CuSO_4$ x $5H_2O$	0.029	g
$ZnSO_4$ x $7H_2O$	0.144	g
Na_2MoO_4 x $2H_2O$	0.036	g

The trace element solution was prepared by dissolving the salts in 800 mL warm H_2O_{bidest}
NaOH was used to adjust the pH to 6.5. The solution was added up to 1000 mL with H_2O_{bidest} and autoclaved.

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Vitamin mixture

Na_2HPO_4/NaH_2PO_4, 25 mM, pH 7.1	100	mL
4-Aminobenzoic acid	4.0	mg
D-(+)-Biotin	1.0	mg
Nicotinic acid	10.0	mg
Calcium D-(+)-pantothenate	5.0	mg
Pyridoxolhydrochloride	15.0	mg
DL-α-lipoic acid	1.5	mg
Folic acid	4.0	mg
2- Mercaptoethanesulfonic acid, sodium salt	25.0	mg

The solution was filter-sterilized and stored at 4°C in the dark.

Vitamin B_{12} solution

Cyanocobalamine	2.5	mg
H_2O_{bidest}	ad 50.0	mL

The solution was filter-sterilized and stored at 4°C in the dark.

Bicarbonate solution (1M)

$NaHCO_3$	84.0	g
H_2O_{bidest}	ad 1000.0	mL

The solution was dispensed in the required portions (40 mL per 1 L medium). The head space of the bottles was flushed with CO_2. Saturation of CO_2 in the solution was achieved by repeated flushing and shaking of the bottles. Finally, the bottles were sealed with rubber stoppers and aluminum crimps and autoclaved.

Ascorbate solution (1M)

Ascorbic acid	17.6	g
H_2O_{bidest}	ad 100.0	mL

In an ice bath ascorbic acid was dissolved in 80 mL anoxic $H_2O_{bidest\ (autoclaved,}$ cooled under N_2 atmosphere) while flushing with N_2. The pH was adjusted to 7.0 with NaOH and the solution added up to 100 ml with anoxic $H_2O_{bidest.}$ After filter-sterilization the solution was stored under N_2 atmosphere in a special flask (which can be flushed with gas) at 4°C in the dark.

Benzoate solution (1M)

Sodiumbenzoate	14.4	g
H_2O_{bidest}	ad 100.0	mL

The solution was filter-sterilized and stored at room temperature.

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1713. GAM Broth (Gifu Anaerobic Medium Broth)

GAM Broth powder (HyServe)	59.0	g
add deionized water to	1000.0	ml

Suspend the dehydrated medium in deionized water, and heat do dissolve the medium, sterilize by autoclaving at 115 °C for 15 minutes.

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1714: CONEXIVISPHAERA MEDIUM

$(NH_4)_2SO_4$	0.20	g
KH_2PO_4	3.00	g
Allen's trace element solution	10.00	ml
$Na_2S_2O_3$ x 5 H_2O	2.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
Yeast extract (OXOID)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.05	g
$FeCl_2$ x 4 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ammonium sulfate, potassium phosphate and trace elements, adjust pH to 5.2 using 10 N H_2SO_4 and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add magnesium sulfate, calcium chloride, yeast extract, vitamins (sterilized by filtration), cysteine and ferrous chloride from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 5.0 - 5.2, if necessary.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg

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1714: CONEXIVISPHAERA MEDIUM

Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1715. GAM Broth, mod. (Gifu Anaerobic Medium Broth, modified)

GAM Broth mod. powder (HyServe)	41.7	g
add deionized water to	1000.0	ml

Suspend the dehydrated medium in deionized water, and heat do dissolve the medium, sterilize by autoclaving at 115 °C for 15 minutes.

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1716. DILUTED TRYPTONE SOYA BROTH (TSB) 1:10

Peptone from casein	1.70	g
Peptone from soymeal	0.30	g
D(+)-Glucose	0.25	g
NaCl	0.50	g
K_2HPO_4	0.25	g
Distilled water	1000.0	ml

Adjust pH to 5.5.
The same as Caso Bouillon (Merck or Oxoid, Germany).

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1717.PELLABORIMONAS MEDIUM

KH_2PO_4	0.33	g
NH_4Cl	0.33	g
KCl	0.33	g
NaCl	10.00	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 6 H_2O	0.033	g
$NaHCO_3$	2.00	g
Yeast extract	0.05	g
Tryptone	2.00	g
Fructose	2.00	g
Resazurin	1.00	mg
Trace element solution (see medium 141)	10.0	ml
Vitamin solution (see medium 141)	10.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.0	ml

Dissolve ingredients (except carbonate, and sulfide), adjust pH to 8.0, boil for 10 min. and sparge medium with 100% N_2 gas for 30 min to make it anoxic. Distribute under N2 into Hungate tubes (10 ml) and autoclave. Add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Filter sterilize vitamin solution and aseptically add 100 µl per Hungate tube. Adjust pH of the complete medium to 8.0, if necessary.

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1721. MMJHS MEDIUM

$NaNO_3$	1.00	g
$NaHCO_3$	1.00	g
$Na_2S_2O_3$ x 5 H_20	1.00	g
Sulfur, powdered	10.00	g
MJ synthetic seawater (see below)	1000.00	ml

Synthetic seawater:

NaCl	25.00	g
K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.70	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.20	g
KCl	0.50	g
Trace mineral solution (see below)	10.00	ml
Distilled water	1000.00	ml

Trace mineral solution:

Nitrilotriacetic acid	1.500	g
$MgSO_4$ x 7 H_2O	3.000	g
$MnSO_4$ x X H_2O	0.500	g
NaCl	1.000	g
$FeSO_4$ x 7 H_2O	0.100	g
$CoSO_4$ x 7 H_2O	0.180	g
$CaCl_2$ x 2 H_2O	0.100	g
$ZnSO_4$ x 7 H_2O	0.180	g
$CuSO_4$ x 5 H_2O	0.010	g
$AlK(SO_4)_2$	0.002	g
H_3BO_3	0.010	g
Na_2MoO_4 x 2 H_2O	0.010	g
Na_2WO_4 x 2 H_2O	0.100	g
$NiCl_2$ x 6 H_2O	0.025	g
Na_2SeO_3 x 5 H_2O	0.050	g
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

For **DSM113300** add 0,1% O2

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Prepare the medium under an atmosphere of H_2/CO_2 (80:20) without adding $NaNO_3$, $Na_2S_2O_3$ x 5 H_20 and $NaHCO_3$ in serum bottles and seal the serum tubes with butyl rubber stoppers. Steam medium for 3 hours on each of 3 successive days. To the sterile medium add, from filter sterilised stock solutions, $NaNO_3$, $Na_2S_2O_3$ x 5 H_20 and $NaHCO_3$. Increase the 80% H_2 + 20% CO_2 gas phase pressure to 0.3 MPa. The final pH is 7.0.

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1722. T5 MEDIUM modified

Tryptone	0.5	g
Yeast extract	2.0	g
Glucose	1.0	g
Soluble starch	1.0	g
Distilled water	1000.0	ml

Adjust to pH 7.0 and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

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1725. TRYPTONE-YEAST EXTRACT MEDIUM modified

Tryptone	10.0	g
Yeast extract	5.0	g
NaCl	5.0	g
Distilled water	1000.0	ml

pH 7.5

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1727. Z45/4 Medium for Cyanobacteria

Composition:

KH_2PO_4	41.0 mg
K_2HPO_4	17.0 mg
$CaCl_2 \times 2\ H_2O$	37.0 mg
$MgSO_4 \times 7\ H_2O$	25.0 mg
Fe-EDTA complex *	10.0 ml
Micronutrient solution **	0.06 ml
Deionized or distilled water	1000.0 ml

*** Fe-EDTA complex**

Combine in deionized or distilled water and make up to 500 ml:

$FeCl_3 \times 6\ H_2O$	135.0 mg
NaEDTA	186.0 mg

**** Micronutrient solution** (after H. Gaffron)

Add to 100 ml of deionized or distilled water:

H_3BO_3	310.0 mg
$MnSO_4 \times H_2O$	223.0 mg
$Na_2WO_4 \times 2\ H_2O$	3.3 mg
$(NH_4)_6Mo_7O_{24} \times 4\ H_2O$	8.8 mg
KBr	11.9 mg
KJ	8.3 mg
$ZnSO_4 \times 7\ H_2O$	28.7 mg
$Cd(NO_3)_2 \times 4\ H_2O$	15.4 mg
$Co(NO_3)_2 \times 6\ H_2O$	14.6 mg
$CuSO_4 \times 5\ H_2O$	12.5 mg
$NiSO_4(NH_4)_2SO_4 \times 6\ H_2O$	19.8 mg
$Cr(NO_3)_3 \times 7\ H_2O$	3.7 mg
$VOSO_4 \times 2\ H_2O$	2.0 mg
$Al_2(SO_4)_3K_2SO_4 \times 24\ H_2O$	47.4 mg

Reference: A. Zehnder, EAWAG, ETH Zuerich, Switzerland

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1728. MMB MEDIUM (ATCC 2254)

Peptone	0.10	g
Yeast extract	0.10	g
Glucose	1.00	g
$(NH_4)_2SO_4$	0.20	g
Hutner's salts (see below)	20.00	ml
Vitamin solution (see below)	10.00	ml
Distilled water	970.00	ml

pH 7.0- 7.5

For solid medium, add 15g of agar per liter.
Glucose and vitamin solution should be filter-sterilized separately.

Hutner's salts:

Nitrilotriacetic acid (NTA)	10.000	g
$MgSO_4$ x 7 H_2O	29.700	g
$CaCl_2$ x 2 H_2O	3.335	g
$(NH_4)_6MoO_7O_{24}$ x 4 H_2O	9.250	mg
$FeSO_4$ x 7 H_2O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4.

"Metals 44":

Na-EDTA	250.000	mg
$ZnSO_4$ x 7 H_2O	1095.000	mg
$FeSO_4$ x 7 H_2O	500.000	mg
$MnSO_4$ x H_2O	154.000	mg
$CuSO_4$ x 5 H_2O	39.200	mg
$Co(NO_3)_2$ x 6 H_2O	24.800	mg
$Na_2B_4O_7$ x 10 H_2O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of the heavy metal ions.

Vitamin solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl	5.00	mg
Riboflavin	5.00	mg

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Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1729. MEDIUM FOR THIORHODOVIBRIO FRISIUS

KH_2PO_4	0.25	g
NH_4Cl	0.34	g
KCl	0.34	g
$MgSO_4x7H_2O$	0.50	g
$CaCl_2x2H_2O$	0.25	g
NaCl	20.0	g
$MgCl_2x6H_2O$	2.80	g
$NaCH_3COO$	0.25	g
SL-12B	1.00	ml
double distilled water	1000.00	ml

Bubble with nitrogen gas and fill in tubes with a rubber septum under a stream of nitrogen gas. Autoclave at 121°C for 15 min.

After sterilization add 50 ml/l 3% $NaHCO_3$, 1 ml/l filter-sterilized Vitamin B12 solution (2mg/100ml), 1 ml/l 10-Vitamins-solution (1000x), 1.25 ml/l Na_2Sx9H_2O (1M stock solution). Adjust pH to 7.2.
Add 1 ml/100ml of culture Sulfide feeding solution (Siefert and Pfennig 1984) every 3 days or when the sulfide is consumed.

3% NaHCO3

$NaHCO_3$	1.50	g
double distilled water	50.00	ml

Fill 50 ml in 100 ml serum bottles, bubble for 15 min with CO2 and autoclave bottle capped tightly at 121°C for 15 min

Trace element solution SL-12 B:

Na_2-EDTA	3.00	g
$FeSO_4$ x 7 H_2O	1.10	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 2 H_2O	50.00	mg
$ZnCl_2$	42.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	18.00	mg
H_3BO_3	300.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
Distilled water	1000.00	ml

Adjust pH to 6.0.

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10-Vitamins-solution (1000x)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiaminehydrochloride	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
DL-Calciumpanthetonate	50.00	mg
Vitamin B_{12} (Cyanocobalamin)	1.00	mg
p-Aminobenzoic acid	50.00	mg
Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Vitamin B12 solution

Vitamin B12 (Cyanocobalamin)	2.00	mg
Distilled water	100.00	ml

Sulfide feeding solution (Siefert and Pfennig 1984)

Na_2S x9 H_2O	7.00	g
Na_2CO_3	2.65	g
Distilled water	250.00	ml

Autoclave under CO2 pressure in a 500 ml bottle. Sterile solution is saturated with CO2 (~0.8 bar) until pH is approx.7.3. 1 ml is added to 100 ml of culture every 3 days or when the sulfide is consumed.

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1730. FarD MEDIUM

NaCl	100.00	g
Trisodium citrate	3.00	g
$MgSO_4$ x 7 H_2O	1.00	g
KCl	3.00	g
Na_2CO_3	5.00	g
Casamino acids	7.00	g
Yeast extract (Difco)	10.00	g
Distilled water	1000.00	ml

Final pH 7.5

Sterilize NaCl solution separately and combine autoclaved solutions after cooling.

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1731: PONTIELLA MEDIUM

Final pH: 7.3 - 7.5
Final volume: 1011 ml

NaCl	18.00	g
$MgCl_2$ x 6 H_2O	6.00	g
KCl	1.50	g
Na_2SO_4	1.00	g
NH_4Cl	1.00	g
$CaCl_2$ x 2 H_2O	0.40	g
K_2HPO_4	0.40	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
L-Fucose	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, fucose, vitamins, cysteine, and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fucose, vitamins, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Before use adjust the pH of the complete medium to 7.3 - 7.5, if necessary.

For DSM 113125: Supplement medium with 1 g/l D-Galactose. Omit L-Fucose. Adjust the pH of the complete medium to 7.5 - 8.0.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g

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H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1732. Z Medium for Cyanobacteria

Composition:

$NaNO_3$	480.0 mg
$Ca(NO_3)_2 \times 4\ H_2O$	59.0 mg
K_2HPO_4	31.0 mg
$MgSO_4 \times 7\ H_20$	25.0 mg
Na_2CO_3	21.0 mg
Fe-EDTA complex *	10.0 ml
Micronutrient solution **	0.08 ml
Deionized or distilled water	1000.0 ml

*** Fe-EDTA complex**

Combine in deionized or distilled water and make up to 500 ml:

$FeCl_3 \times 6\ H_2O$	135.0 mg
NaEDTA	186.0 mg

**** Micronutrient solution** (after H. Gaffron)

Add to 100 ml of deionized or distilled water:

H_3BO_3	310.0 mg
$MnSO_4 \times H_2O$	223.0 mg
$Na_2WO_4 \times 2\ H_2O$	3.3 mg
$(NH_4)_6Mo_7O_{24} \times 4\ H_2O$	8.8 mg
KBr	11.9 mg
KJ	8.3 mg
$ZnSO_4 \times 7\ H_2O$	28.7 mg
$Cd(NO_3)_2 \times 4\ H_2O$	15.4 mg
$Co(NO_3)_2 \times 6\ H_2O$	14.6 mg
$CuSO_4 \times 5\ H_2O$	12.5 mg
$NiSO_4(NH_4)_2SO_4 \times 6\ H_2O$	19.8 mg
$Cr(NO_3)_3 \times 7\ H_2O$	3.7 mg
$VOSO_4 \times 2\ H_2O$	2.0 mg
$Al_2(SO_4)_3K_2SO_4 \times 24\ H_2O$	47.4 mg

Reference: A. Zehnder, EAWAG, ETH Zuerich, Switzerland

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1734: THIOMICROSPIRA SP. MEDIUM

NaCl	17.50	g
NH_4Cl	0.40	g
$MgSO_4$ x 7 H_2O	0.10	g
Trace element solution	10.00	ml
$Na_2S_2O_3$ x 5 H_2O	10.00	g
KH_2PO_4	0.14	g
$NaHCO_3$	0.50	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except hydrogenphosphate, hydrogencarbonate and vitamins), adjust pH to 7.5 and autoclave. K_2HPO_4 is autoclaved separately. Filter sterilize the vitamins and $NaHCO_3$ stock solutions. Adjust pH of the complete medium to 8.0 with sterile 0.4% (w/v) Na_2CO_3 solution, if necessary.

2. Note: Incubate in Erlenmeyer flasks with shaking.

Trace element solution (from medium 333)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	11.00	g
$CaCl_2$ x 2 H_2O	7.34	g
$MnCl_2$ x 4 H_2O	2.50	g
$CoCl_2$ x 6 H_2O	0.50	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	0.50	g
$FeSO_4$ x 7 H_2O	5.00	g
$CuSO_4$ x 5 H_2O	0.20	g
NaOH	11.00	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds. Adjust pH of final solution to 6.0 with NaOH.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

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1735. MEDIUM FOR MITSUARIA

Peptone	2.00	g
Magnesium sulfate heptahydrate	0.20	g
Calcium chloride dihydrate	0.15	g
Agar, if necessary	15.0	g
Distilled water	1000.0	ml

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1736. BEIJERINCKIA MEDIUM (LMG 18)

Glucose	10.00	g
K_2HPO_4	0.80	g
KH_2PO_4	0.20	g
$MgSO_4$ x 7 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	0.02	g
$MnSO_4$ x H_2O	1.30	mg
$ZnSO_4$ x 7 H_2O	5.00	mg
$CuSO_4$ x 5 H_2O	4.00	mg
Na_2MoO_4 x 2 H_2O	5.00	mg
Agar	15.00	g
Distilled water	1000.00	ml

Final pH is 6.5.

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1743: GHIORSEA MEDIUM

NaCl	27.50	g
$MgCl_2$ x 6 H_2O	5.38	g
$MgSO_4$ x 7 H_2O	6.78	g
KCl	0.72	g
NH_4Cl	0.50	g
$CaCl_2$ x 2 H_2O	1.40	g
KH_2PO_4	0.05	g
Trace element solution	10.00	ml
$NaHCO_3$	1.70	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except vitamins), then sparge medium with 80% N_2 and 20% CO_2 gas mixture to make it anoxic and until a pH of 6.5 is reached. Dispense medium under same gas atmosphere into anoxic serum vials to 20% of their volume and autoclave. After autoclaving add vitamins from a sterile stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Prior to inoculation add 5% (v/v) sterile H_2 and 5% (v/v) sterile air to the headspace by using syringes. Check pH of the complete medium and adjust pH to 6.5, if necessary. Incubate without shaking.

2. Note: After growth has started cell density of the culture can be increased by replenishing H_2 and air every two days.

Trace element solution (from medium 705)

Na_2-EDTA x 2 H_2O	0.50	g
$CoCl_2$ x 6 H_2O	0.15	g
$MnCl_2$ x 4 H_2O	0.10	g
$FeSO_4$ x 7 H_2O	0.10	g
$ZnCl_2$	0.10	g
AlCl3 x 6 H_2O	40.00	mg
Na_2WO_4 x 2 H_2O	40.00	mg
Na_2SeO_3 x 5 H_2O	30.00	mg
$NiCl_2$ x 6 H_2O	20.00	mg
$CuCl_2$ x 2 H_2O	20.00	mg
H_3BO_3	10.00	mg
Na_2MoO_4 x 2 H_2O	10.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7 using 2 N NaOH, then dissolve remaining compounds.

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Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1748. HALOFERAX GIBBONSII MEDIUM

Mineral Salts Solution for liquid 23% MGM

Salt Water Solution	786.00	ml
Peptone (Oxoid)	5.00	g
Yeast extract (Fisher)	1.00	g
Make up Tap water to	1000.00	ml

Adjust to pH 7.2 with 1 M Tris-Base and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

Mineral Salts Solution for solid 20%MGM

Salt Water Solution	666.00	ml
Peptone (Oxoid)	5.00	g
Yeast extract (Fisher)	1.00	g
Make up tap water to	1000.00	ml

Adjust to pH 7.2 with 1 M Tris-Base and sterilize by autoclaving. The medium may be solidified by adding 20.0 g/l agar.

Salt Water Solution (30% Stock Solution):

NaCl	240.0	g
KCl	7.0	g
$MgCl_2$ x 6 H_20	30.0	g
$MgSO_2$ x 7 H_2O	35.0	g
CaCl 1M	5.0	ml
Distilled water	1000.0	ml

Adjust to pH 7.2 with 1 M Tris-HCL and sterilize by autoclaving.

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1749. Sucrose-Asparagine Medium

Mineral Salts Solution with Asparagin (L)

$MgSO_2$ x 7 H_2O	0.5	g
K_2HPO_4	1.0	g
L-Asparagin	2.0	g
Make up tap water to	990.0	ml

Adjust to pH 7.0 sterilize by autoclaving. The medium may be solidified by adding 15.0 g/l agar.

Sucrose Solution (10 mL)

Sucrose	20	g

Sterilize by autoclaving.

After autoclaving add 10 mL of the Sucrose solution to the Mineral Salts Solution with Asparagine (final volume 1 L).

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1761. PFENNIG'S MEDIUM III

Solution A:

$CaCl_2$ x 2 H_2O	0.25	g
Distilled water	460.00	ml

Fill 10x 46ml in 100 ml screw-cap bottles. Bubble with N_2/CO_2 and autoclave at 121°C for 15 min.

Solution B:

Na_2S x 9 H_2O	2.00	g
Distilled water	135.00	ml

Prepare in a screw-cap bottle, bubble with N_2 to replace air, close tightly and autoclave.

Solution C:

$NaHCO_3$	1.50	g
H_2O	50.00	ml

Bubble with CO_2 and filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution D

Resazurin (0,1%)	0.5	ml
Distilled water	450.00	ml

Autoclave in a cotton-stoppered Erlenmeyer flask with an outlet tube for medium, connected to a glass outlet at the bottom of the vessel and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. Cool to room temperature under an atmosphere of N_2/CO_2 in an ice bath (10-15 min).

Solution E:

Ammonium chloride	0.40	g
$MgSO_4x7H_2O$	0.50	g
KCL	0.34	g
KH_2PO_4	0.34	g
Trace element solution SL-12 B	1.00	ml
Distilled water	25	ml

Filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution F:

Vitamin B_{12}	0.002	g
Distilled water	100.00	ml

Filter sterilized

Solution G:

Ammonium acetate	2.5	g
Magnesium acetate	2.5	g
Distilled water	100.00	ml

Autoclave at 121°C for 15 min.

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Trace element solution SL-12 B:

Distilled water	1000.00	ml
Na_2-EDTA	3.00	g
$FeSO_4$ x 7 H_2O	1.10	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 2 H_2O	50.00	mg
$ZnCl_2$	42.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	18.00	mg
H_3BO_3	300.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg

Adjust pH to 6.0.

Mix solution D, C and E in the cotton-stoppered Erlenmeyer flask. Bubble with CO_2 in an ice bath under sterile conditions (5-10 min).

Fill 50 ml of the mixed solution (C,D,E) into each 100 ml bottle of **solution A** (for a total volume of approx. 96 ml).

Before using, add **2,6 ml solution B** (Na_2S x 9 H_2O), **0.1 ml solution F** (Vitamin B_{12}) and **1 ml** of **solution G** (2,5% Ammonium-acetate and Magnesium-acetate) to each 100ml bottle.

Adjust the pH with filter-sterilised 1M Na_2CO_3 to 7.1-7.3.
Fill in sterile, N_2 gassed screw-cap tubes under N_2 gas.

For the growth of DSM 11081 feed the culture with neutralized sulfide feeding solution regularly (approx. every 3 days). *0,5-1 ml/ 100 mL*

Neutralized sulfide solution:

Distilled water	100.00	ml
Na_2S x 9 H_2O	3.00	g

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.
Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

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1774: THIOTHRIX MEDIUM II

$(NH_4)_2SO_4$	0.300	g
Ammonium acetate	0.200	g
KH_2PO_4	0.100	g
$MgSO_4$ x 7 H_2O	0.100	g
$CaCl_2$ x 2 H_2O	0.050	g
$NaHCO_3$	0.125	g
Trace elements solution (Pfennig & Lippert,1966)	10.000	ml
Agar	15.000	g
Distilled water (ad)	1000.000	ml

Adjust pH to 7.4-7.5 before autoclaving.

After autoclaving, add:

Vitamin B_{12} solution	4.000	ml
Na_2S x 9 H_2O solution	25.000	ml

Vitamin B12 solution

Vitamin B_{12}	5	mg
Distilled water	1000	ml

Na2S x 9 H2O solution

Na_2S x 9 H_2O	3	%(w/v)
Distilled water	1000	ml

Adjust pH to 7.0.

For DSM 21228: For growth of DSM 21228 use agar medium (e.g., agar slants) overlayed with some autoclaved tap water.

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1784: modified MGM medium, 23% saltwater

1. The medium is a modified MGM which contains 5 g peptone (Oxoid) and 1 g Bacto yeast extract (Difco Laboratories) per liter, and 23% Saltwater (SW)

Salt Water Solution (30% Stock Solution)	767.00	ml
Peptone (Oxoid)	5.00	g
Yeast extract (Difco)	1.00	g

2. Make up to 1000 ml with distilled water.

3. Adjust pH to 7.2 with Tris-Base (2M).

4. Autoclave at 121 °C for 20 min.

Salt Water Solution (30% Stock Solution) (from medium 1339)

NaCl	240.00	g
KCl	7.00	g
$MgCl_2$ x 6 H_2O	30.00	g
$MgSO_4$ x 7 H_2O	35.00	g
$CaCl_2$ (1 M)	5.00	ml
Distilled water	995.00	ml

Adjust to pH 7.5 with 2 M Tris-Base and sterilize by autoclaving.

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1785: IM1

$CaCl_2$	0.80	g
$MgSO_4$ x 7 H_2O	81.80	g
NaCl	40.00	g
K_2SO_4	3.00	g
Na_2SO_4	3.00	g
Casamino acids	2.50	g
Agar, if required (optional)	15.00	g
Distilled water	1000.00	ml

1. Adjust pH to 7.5.

2. Autoclave at 121 °C for 20 min.

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1790: Mucispirillum Spezial

Brain heart infusion	9.260	g
Trypticase soy broth	7.500	g
Yeast extract	2.500	g
$NaNO_3$	0.426	g
K_2HPO_4	1.250	g

1. Adjust pH to 7.0.

2. Bring to boil, cool down under N_2CO_2.

Cysteine HCl	0.250	g

3. Fill under N_2CO_2 in hungate tubes (10 ml)

4. Autoclave at 121 °C for 15 min.

Haemin solution	0.050	ml
Vitamin K_3 solution	0.050	ml
Fetal bovine serum	0.500	ml

5. pressurize with 1 bar H_2

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml
Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K3 solution

Vitamin K_3	5.00	mg
Ethanol (95%)	1.00	ml
Water	100.00	ml

Dissolve 5 mg vitamin K_3 in 1 ml 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.

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1791: Bacto(TM) Tryptic Soy Broth without Dextrose (BD 286220)

tryptic soy broth without dextrose (BD 286220)	27.50	g
Agar (optional)	15.00	g
Distilled water	1000.00	ml

Autoclave at 121 °C for 20 min.

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1793: Chlamydia serpentis & poikilotherma Medium

MEM-Medium (+ Glutamine)	500.00	ml
Fetal bovine serum	100.00	ml
D-glucose solution	6.00	ml

1. Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

2. Prepare a 25 cm2 flask and seed cells according to standard protocols. Incubate at 37°C plus 5% CO_2. When a confluent layer has formed, infection can be carried out.

Cycloheximid solution	0.70	ml

3. Exchange medium with Infection Medium (as above with the addition of cycloheximide) and add 500 - 1000 µl of EB stock solution (thawed quickly to 37°C).

4. Centrifuge for 1 h onto the cell layer at 1000 x g at >20°C.

5. Incubate at 37°C + 5% CO_2. Control cells daily and look for inclusions. Not all Chlamydiae form well-visible inclusions, ultimately, immunofluorescence or in situ-hybridization techniques are necessary to visualize inclusions.

D-glucose solution

D-Glucose	3.00	g
Demineralized water	50.00	ml

Filtrate.

Cycloheximid solution

Cycloheximid	10.00	mg
Demineralized water	10.00	ml

Filtrate.

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1796: PAS for Acanthamoeba hatchetti

Trisodium citrate x 2 H_2O	1.00	g
PAS Ah Stock Solution 1	5.00	ml
PAS Ah Stock Solution 2	5.00	ml
PAS Ah Stock Solution 3	5.00	ml

1. Make up to 1000 ml with MilliQ water.
2. Adjust pH to 7.0 with 1 N NaOH.
3. Autoclave at 121 °C for 20 min.
4. Store at room temperature.

PAS Ah Stock Solution 1

$CaCl_2$ x 2 H_2O	5.90	g

Make up to 500 ml with MilliQ water.

PAS Ah Stock Solution 2

Na_2HPO_4 (anhydrous)	35.50	g
KH_2PO_4 (anhydrous)	34.10	g

Make up to 500 ml with MilliQ water.

PAS Ah Stock Solution 3

$MgSO_4$ x 7 H_2O	98.60	g

Make up to 500 ml with MilliQ water.

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1797: RE-103 Medium

NaCl, for Agar medium autoclave separately	206.00	g/l
$MgSO_4$ x 7 H_2O	36.00	g/l
KCl	0.37	g/l
$CaCl_2$ x 2 H_2O	0.50	g/l
Yeast extract (ThermoFischer BP1422)	5.00	g/l
$MnCl_2$ x 2 H_2O	7.50	mg/l
Agar (optional)	15.00	g/l
Distilled water	1000.00	ml

1. Adjust pH to 7.0.

2. Note: When preparing a solid medium, autoclave the NaCl separately.

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1800: Vulcanimicrobium alpinus medium

KH_2PO_4	0.44	g
$(NH_4)_2SO_4$	0.10	g
$MgSO_4$ x 7 H_2O	0.10	g
Yeast extract (Bacto™ Yeast Extract recommended), can be increased to 0.3% = 3g/L	0.30	g
Peptone	0.50	g
$CaCl_2$ x 2 H_2O	0.05	g
Glucose	1.00	g/l
Distilled water	1000.00	ml

1. Adjust pH to 6.0.

2. aliquot 5 mL medium into 50 mL serum bottles

3. add 10% CO_2 to gasphase (air)

4. Autoclave at 121 °C for 15 min.

5. add trace element solution, and vitamins solutions from 1000 x stocks

Trace element solution SL-8	1.00	ml
Vitamin solution A (1000 x stock)	0.25	ml
Vitamin solution B (1000 x stock)	0.25	ml

Vitamin solution A (1000 x stock) (from medium 1783)

Biotin	10.00	mg
Riboflavin	10.00	mg
Thiamine HCl	100.00	mg
Thiamine pyrophosphate	100.00	mg
L-Ascorbic acid	100.00	mg
Calcium pantothenate	100.00	mg
Folic acid	100.00	mg
Nicotinamide	100.00	mg
Nicotinic acid	100.00	mg
p-Aminobenzoic acid	100.00	mg
Pyridoxine hydrochloride	100.00	mg
Lipoic acid	100.00	mg
beta-NAD	100.00	mg
Potassium phosphate buffer (pH 7.2, 10 mM)	100.00	ml

1. Dissolve the vitamins in 10mM phosphate buffer, pH7.2.

2. Titrate with NaOH until vitamins are dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage.

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Vitamin solution B (1000 x stock) (from medium 1783)

Vitamin B_{12}	100.00	mg
Distilled water	100.00	ml

1. Dissolve the vitamin B_{12} in water.

2. Titrate with HCl until vitamin B_{12} is dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage.

Trace element solution SL-8 (from medium 745)

Na_2-EDTA	5.20	g
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	62.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	17.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoSO_4 x 2 H_2O	36.00	mg
Distilled water	1000.00	ml

pH 6.8

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1801: SULFURIMONAS MARISNIGRI MEDIUM

Solution A	1001.00	ml
Solution B	15.00	ml
Solution C	2.00	ml
Solution D	2.00	ml
Solution E	2.00	ml
Solution F	3.00	ml
Solution G	1.00	ml

Dissolve ingredients of solution A (except MnO_2), then sparge with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute solution A under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of MnO_2 and autoclave at 121°C for 15 min. Solution B is autoclaved separately under an 80% N_2 and 20% CO_2 gas atmosphere, and solutions C, D, and E are autoclaved under a 100% N_2 gas atmosphere. Solutions F and G are prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to G are added to the sterile solution A in the sequence as indicated. The final pH of the complete medium should be 7.5.

Solution A

NaCl	17.70	g
$MgCl_2 \times 6\ H_2O$	3.20	g
$CaCl_2 \times 2\ H_2O$	0.40	g
KCl	0.70	g
$MgSO_4 \times H_2O$	2.70	g
Trace element solution SL-10	1.00	ml
MnO_2	0.50	g
Distilled water	1000.00	ml

Solution B

Na_2CO_3	0.75	g
Distilled water	15.00	ml

Solution C

NH_4Cl	0.01	g
Distilled water	2.00	ml

Solution D

K_2HPO_4	0.01	g

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Distilled water	2.00	ml

Solution E

Na-acetate	0.16	g
Distilled water	2.00	ml

Solution F

$Na_2S_2O_3$ x 5 H_2O	0.75	g
Distilled water	3.00	ml

Solution G

Wolin's vitamin solution (10x)	1.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1805: YSG Medium

Yeast extract	2.00	g
Glucose	1.00	g
Starch	1.00	g
$MnSO_4$ x 1 H_2O	10.00	mg
Agar (if needed)	20.00	g
Distilled water (for liquid medium)	1000.00	ml

1. Adjust pH to 3.7 with H_2SO_4.

2. For preparation of agar plates dissolve agar in 500 ml distilled water and autoclave separately. The basis medium should then be dissolved in 500 ml distilled water only. Cool down after sterilization and mix.

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1809: DC-8A Medium

K_2HPO_4	2.90	g
Urea	4.20	g
Yeast extract	2.00	g
$CaCl_2$ x 2 H_2O	0.01	g
Na_2CO_3	1.00	g
L-Cysteine HCl x H_2O	0.50	g
Resazurin	0.50	mg
Mineral solution	0.20	ml
Distilled water	900.00	ml
Xylose (w/v, 10%)	100.00	ml

Mix components thoroughly and adjust pH to 9.0. Distribute the medium into culture vessels (e.g., 9.0 ml in Balch tubes), replace the gas phase with N_2, seal with butyl rubber stoppers, and autoclave. After cooling, aseptically and anaerobically add per 900 ml the following solution (autoclaved under a N_2 atmosphere):

Mineral solution

$MgCl_2$ x 6 H_2O	25.000	g
$CaCl_2$ x 2 H_2O	37.500	g
$FeSO_4$ x 7 H_2O	0.312	g
Distilled water	1000.000	ml

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1810: PSEUDOALTEROMONAS ESPEJIANA MEDIUM

Nutrient broth (Difco 234000)	8.00	g
KCl	0.70	g
$CaCl_2$ x 2 H_2O	1.50	g
$MgSO_4$ x 7 H_2O	12.00	g
NaCl	26.00	g
Agar (optional)	15.00	g
Distilled water	1000.00	ml

1. Adjust pH to 7.0.
2. Autoclave at 121 °C for 15 min.

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1819: Ectothiorhodospira lacustris medium

NH_4Cl	0.50	g/l
KH_2PO_4	0.50	g/l
$MgCl_2$ x 6 H_2O	0.20	g/l
$CaCl_2$ x 2 H_2O	0.10	g/l
KCl	0.30	g/l
Yeast extract	0.10	g/l
Na-acetate	0.50	g/l
$Na_2S_2O_3$ x 5 H_2O	0.50	g/l
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve components (without bicarbonate, sulfide and vitamin solutions).
2. Sparge medium with 100% N_2 gas mixture.
3. Aliquot into vials (e.g, Hungate tubes, 9 mL each).
4. Autoclave at 121 °C for 15 min.
5. After autoclaving, add the following sterile solutions.

$NaHCO_3$ (3% in water, autoclaved)	100.00	ml/l
Na_2S x 9 H_2O (5% solution in water, autoclaved)	10.00	ml/l
Wolin's vitamin solution	1.00	ml/l

6. Adjust pH to 8.5-9.0.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Wolin's vitamin solution (from medium 141)

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1819: Ectothiorhodospira lacustris medium

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B_{12}	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

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1824: HYDROGENOVIBRIO MEDIUM

NaCl	27.50	g
$MgSO_4$ x 7 H_2O	6.78	g
$MgCl_2$ x 6 H_2O	5.38	g
$CaCl_2$ x 2 H_2O	1.40	g
NH_4Cl	1.00	g
KCl	0.72	g
K_2HPO_4	0.05	g
Modified Wolin's mineral solution	1.00	ml
Na_2CO_3	0.50	g
$Na_2S_2O_3$ x 5 H_2O	1.25	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, thiosulfate, and vitamins, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into serum vials or Hungate tubes to 30% of their volume and autoclave. Add vitamins and thiosulfate from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins and thiosulfate should be sterilized by filtration. Adjust the pH of the complete medium to 6.2-6.5, if necessary.

2. After inoculation add sterile air (with a hypodermic syringe through the rubber closure) to a concentration of ca. 1 - 3% (v/v) O_2 in the vial (e.g., add 2 ml air to a Hungate-type tube of 16 ml total volume).

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

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Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

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1829: BSKII medium (Borrelia)

Final pH: 7.3
Final volume: 100 ml

M 1829

HEPES	0.51	g
Trisodium citrate x 2 H_2O	0.05	g
Glucose	0.42	g
Pyruvate	0.06	g
N-Acetylglucosamine	0.03	g
Sodium bicarbonate	0.18	g
Yeast extract	0.17	g
Bacto peptone (Becton Dickinson)	0.42	g
Bovine serum albumin (Fraction V)	0.85	g
Rabbit serum	8.56	ml
MEM alpha Modification (BioWest Cat No. P0440)	0.89	g
Deionized water	fill up to 100	ml

Combine all ingredients, adjust pH to 7.3 with 1 M NaOH and filter-sterilize

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1831: SWES (Seawater - Soil Extract - Salts)

Final volume: 1000 ml

KNO_3 (19.0 g/l stock solution)	10.50	ml
K_2HPO_4 (4.35 g/l stock solution)	4.60	ml
$MgSO_4$ x 7 H_2O (7.5 g/l stock solution)	2.70	ml
Micronutrient Solution *	5.00	ml
Soil Extract **	30.00	ml

1. Make up to 1000 ml with filtered natural seawater.
2. Autoclave at 121 °C for 20 min.
3. Add aseptically after autoclaving:

Vitamin B_{12} (0.02 g/l stock solution, filter sterilised)	250.00	µl

Micronutrient Solution * (from medium 1831)

Milli Q Water	700.00	ml
Fe SO_4 x 7 H_2O (10.0 g/l stock solution)	70.00	ml
Na-EDTA x 2 H_2O (80.0 g/l stock solution)	10.00	ml
$ZnSO_4$ x 7 H_2O (1.0 g/l stock solution)	1.00	ml
$MnSO_4$ x 1 H_2O (0.76 g/l stock solution)	2.00	ml
H_3BO_3 (2.0 g/l stock solution)	5.00	ml
$Co(NO_3)_2$ x 6 H_2O (5.0 g/l stock solution)	0.20	ml
Na_2 MoO_4 x 2 H_2O (0.2 g/l stock solution)	5.00	ml
$CuSO_4$ x 5 H_2O (0.25 g/l stock solution)	20.00	µl

1. Make up to 1000 ml with Milli Q water.
2. Prepare aliquots and store them at -20 °C.

Soil Extract ** (from medium 1831)

Preparation of soil extract: Fill a 1 litre Schott bottle one third with garden or leaf soil of medium, but not too high humus content. The soil must not contain fertilizers or plant protective agents. Success of soil extract depends on selection of suitable soils. Those with high clay content are usually less satisfactory. Make up with Milli Q water to 700 ml and sterilize by autoclaving for one hour twice in a 24 h interval. Separate the decanted extract from particles by centrifugation. Filter the supernatant through Whatman filter paper into a 1 litre Schott bottle, autoclave for 20 min at 121°C and store in the refrigerator. To prepare the medium, aseptically remove 30 ml of the soil extract.

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