

Nutrient Media

When unsealing an ampoule with a culture obtained from the All-Russian Collection of Microorganisms (VKM) and for initial reinoculations, it is recommended to use the medium specified for the strain in the Catalogue.

General comments to the descriptions of the media

1. In all cases when the composition of a medium is given as the description of particular solutions, this implies that the given solutions should be sterilized separately and mixed upon sterilization.
2. In all cases, besides when specified otherwise, a given mode of sterilization assumes autoclaving. If the sterilization conditions of a medium are not specified, they can be set arbitrarily within the limits accepted in the general microbiological practice.
3. The purity of the reagents is not specified in the descriptions. It is assumed that the respective reagents will be used, specifically, peptone, tryptone, yeast extract, etc. marked 'Bacto' or 'For microbiological work'. For some media, descriptions for preparing the components are given instead of using commercial concentrates.
4. The pH values given indicate the magnitudes, which a medium shall have prior to inoculation. In the cases when some solutions are described and errors are possible, it is specified: pH of the medium. An insufficiently alkaline medium shall be alkalized, usually with a sterile solution of NaOH or NaHCO₃; an insufficiently acidic one should be acidified, usually with a sterile solution of HCl.

1. *ALLOMONAS ENTERICA* MEDIUM

Peptone 10.0 g

NaCl 20.0 g

Beef extract 5.0 g*

Distilled water 1000.0 ml,

pH 7.0.

Autoclave at 121°C for 15 min.

*Beef extract may be replaced with 3.0 g yeast extract.

2. YEAST WATER

Pressed yeast 200.0 g

Tap water 1000.0 ml

Preparation of yeast water: suspend 200.0 g of pressed yeast in 1000.0 ml of tap water and boil for 30 min. Twice filter hot through a paper filter or centrifuge.

Autoclave at 121°C for 15 min.

3. POTATO AGAR

Potato 200.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil 200.0 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, fill up distilled water to 1000.0 ml, add agar. Do not use new potatoes.

pH 7.0

Autoclave at 121°C for 15 min.

4. WORT AGAR

Wort extract (malt extract) 20.0 g

Agar 20.0 g

Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.

5. PEPTONE MEAT AGAR

Peptone 10.0 g
NaCl 5.0 g
Beef extract 3.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

6. PEPTONE MEAT BROTH

Peptone 10.0 g
NaCl 5.0 g
Beef extract 3.0 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C for 15 min.

7. OATMEAL AGAR (OA)

Oatmeal 30.0 g
Agar 15.0 g
Tap water 1000.0 ml
Cook 30.0 g of oatmeal in 1000.0 ml tap water for 20 min, filter through 2 layers of gauze, dilute to 1000.0 ml and add 15.0 g agar.
Autoclave at 121°C for 15 min.

8. PEPTONE MAIZE AGAR

Peptone 5.0 g
Maize extract 5.0 g
Starch (soluble) 10.0 g
NaCl 5.0 g
CaCl₂ 0.5 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C for 15 min.

9. MALT AGAR 7 BALLING (MA7B)

Malt extract Balling 7 degrees 1000.0 ml
Agar 20.0 g
Adjust pH to 7.0
Autoclave at 111°C for 30 min.

10. MALT AGAR 2 BALLING (MA2B)

Malt extract Balling 2 degrees 1000.0 ml
Agar 20.0 g
Autoclave at 111°C for 30 min.

11. MALT AGAR 3.5 BALLING (MA3.5B)

Malt extract Balling 3.5 degrees 1000.0 ml

Agar 20.0 g
Autoclave at 111°C for 30 min.

12. CZAPEK MEDIUM (CZ)

NaNO₃ 3.0 g
K₂HPO₄ 1.0 g
KCl 0.5 g
MgSO₄ × 7 H₂O 0.5 g
FeSO₄ × 7 H₂O 0.01 g
Sucrose 30.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 6.0
Autoclave at 121°C for 30 min.

13. POTATO-GLUCOSE AGAR (PDA)

Grated potato 200.0 g
Glucose 20.0 g
Agar 20.0 g
Tap water 1000.0 ml
Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.
pH 6.5-7.0
Autoclave at 121°C for 15 min.

14. POTATO-CARROT AGAR (PCA)

Grated potato 20.0 g
Grated carrot 20.0 g
Agar 20.0 g
Tap water 1000.0 ml
Boil potato and carrot in 1000.0 ml of water for 1 h, filter cold through a cotton-gauze filter, add water to the initial volume and add agar. Do not use new potatoes.
pH 7.0-7.1
Autoclave at 121°C for 15 min.

15. LB MEDIUM

Tryptone 10.0 g
Yeast extract 5.0 g
NaCl 10.0 g
Tap water 1000.0 ml
pH 7.5
Autoclave at 121°C for 15 min.

16. YT MEDIUM

Tryptone 8.0 g
Yeast extract 5.0 g
NaCl 5.0 g
Tap water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

17. GLUCOSE PEPTONE AGAR (GPA)

Glucose 40.0 g

Peptone 10.0 g

Yeast extract 5.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min.

18. GLUCOSE PEPTONE AGAR WITH 5% NaCl

Glucose 40.0 g

Peptone 10.0 g

Yeast extract 5.0 g

NaCl 50.0 g

Agar 20.0 g

Distilled water 1000.0 ml

Autoclave at 111°C for 30 min.

19. MALT AGAR 7 BALLING WITH 5% NaCl (MA7B+5% NaCl)

Malt extract Balling 7 degrees 1000.0 ml

NaCl 50.0 g

Agar 20.0 g

Autoclave at 111°C for 30 min.

20. LIESKE MEDIUM

Mn-acetate 0.1 g

Agar 15.0 g

Distilled water 1000.0 ml

Autoclave at 111°C for 30 min.

21. MANNITOL AGAR WITH YEAST WATER

Mannitol 10.0 g

Agar 15.0 g

Yeast water 100.0 ml

Tap water 900.0 ml

Preparation of yeast water: suspend 200.0 g of pressed yeast in 1000.0 ml of tap water. Twice filter hot through a paper filter or centrifuge.

Autoclave at 121°C for 15 min.

22. MALT AGAR 3.5 BALLING WITH 60% SUCROSE (MA60S)

Malt extract Balling 3.5 degrees 1000.0 ml

Sucrose 600.0 g

Agar 20.0 g

Autoclave at 121°C for 15 min.

23. MALT AGAR 3.5 BALLING WITH 40% SUCROSE (MA40S)

Malt extract Balling 3.5 degrees 1000.0 ml

Sucrose 400.0 g

Agar 20.0 g

Autoclave at 121°C for 15 min.

24. MALT AGAR 3.5 BALLING WITH 20% SUCROSE (MA20S)

Malt extract Balling 3.5 degrees 1000.0 ml
Sucrose 200.0 g
Agar 20.0 g
Autoclave at 121°C for 15 min.

25. MALT AGAR 3.5 BALLING WITH FILTER PAPER

Malt extract Balling 3.5 degrees 1000.0 ml
Agar 20.0 g
Autoclave at 121°C for 15 min.
Sterilize filter paper strips with dry heat and soak it with sterile medium.

26. MANURE AGAR

Horse manure 100-125 g
Agar 25.0 g
Distilled water 1000.0 ml
pH 6.5-7.0
Boil manure in 1000.0 ml of water for 10 min, then keep for 16-20 hours, filter through 1-2 layers of filter paper, adjust to the initial volume, add agar.
Autoclave at 121°C for 15 min.

27. PEPTONE LACTOSE AGAR

Peptone 10.0 g
Lactose 10.0 g
Agar 15.0 g
Tap water 1000.0 ml
Autoclave at 121°C for 15 min.

28. TRYPTOSE AGAR

Tryptose 20.0 g
Glucose 1.0 g
NaCl 5.0 g
Agar 15.0 g
Thiamine-HCl 0.05 g
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.

29. PEA AGAR

Yellow peas 100.0 g
 K_2HPO_4 0.5 g
Sucrose 10.0 g
Agar 20.0 g
Tap water 1000.0 ml
Boil peas in 1000.0 ml of water, filter through gauze, add water to the initial volume; add phosphate, sucrose and agar.
Autoclave at 121°C for 15 min.

30. AZOTOBACTER MEDIUM 1

Glucose 5.0 g
Mannitol 5.0 g
 $CaCl_2 \times 2 H_2O$ 0.1 g
 $MgSO_4 \times 7 H_2O$ 0.1 g
 $Na_2MoO_4 \times 2 H_2O$ 5.0 mg

K₂HPO₄ 0.9 g
KH₂PO₄ 0.1 g
FeSO₄ × 7 H₂O 0.01 g
CaCO₃ 5.0 g
Agar 15.0 g
Distilled water 950.0 ml
pH 7.3
Autoclave at 121°C for 15 min.
Sterilize glucose and mannitol separately (in 50.0 ml H₂O) and add to the medium after autoclaving.

31. CABBAGE AGAR

Cabbage 50.0 g
Glucose 20.0 g
Peptone 10.0 g
Agar 20.0 g
Tap water 1000.0 ml
Boil 50.0 g of cabbage in 1000.0 ml of water, filter cabbage and adjust the volume of broth to the initial value.
pH 7.0
Autoclave at 111°C for 30 min.

32. CURD DECOCTION

Pour 9000.0 ml of distilled water into 3.0 kg of curd and add 150.0 of dry *Aspergillus terricola* mycelium, shake, pour chloroform, seal with a stopper. Decoct at 37°C for 10 days, adjust pH to 7.0 with 1 N NaOH. The decoction shall contain 400 mg% amine nitrogen and 300 mg% tryptophan.

33. PEPTONE MEAT AGAR WITH 0.2% UREA

Peptone 10.0 g
NaCl 5.0 g
Beef extract 3.0 g
Agar 20.0 g
Distilled water 1000.0 ml
Do not adjust pH; pH raises to about 8 due to heat degraded urea.
Autoclave at 121°C for 15 min.
10.0 ml filter-sterilized 20% urea solution is added aseptically after autoclaving to 1000.0 ml cooled, molten, agar. The medium is then immediately dispensed aseptically.

34. SOIL EXTRACT

Dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121°C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121°C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

35. NITROBACTER MEDIUM 1

Solution 1 (see below) 0.5 ml
Solution 2 (see below) 0.5 ml
Solution 3 (see below) 1.0 ml

Solution 4 (see below) 0.5 ml
Solution 5 (see below) 0.5 ml
Solution 6 (see below) 0.1 ml
Distilled water to 1000.0 ml

Solution 1:

CaCl₂ 2.0 g
Distilled water 100.0 ml

Solution 2:

MgSO₄ × 7 H₂O 20.0 g
Distilled water 100.0 ml

Solution 3:

Chelated iron 0.1 g
Distilled water 100.0 ml

Solution 4:

Na₂MoO₄ 0.1 g
MnCl₂ × 4 H₂O 0.2 g
CoCl₂ × 6 H₂O 0.02 g
ZnSO₄ × 7 H₂O 0.1 g
CuSO₄ × 5 H₂O 0.02 g
Distilled water 1000.0 ml

Solution 5:

NaNO₃ 41.4 g
Distilled water 100.0 ml

Solution 6:

K₂HPO₄ 1.74 g
Distilled water 100.0 ml

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

36. PROPIONIBACTERIUM MEDIUM

Yeast extract 10.0 g
KH₂PO₄ 1.0 g
Na₂HPO₄ × 2 H₂O 3.0 g
Na-lactate (70%) 40.0 ml
Distilled water 1000.0 ml
Dissolve all ingredients and add Na-lactate.
pH 7.0
Autoclave at 121°C for 15 min.

37. KNOP MEDIUM WITH FILTER PAPER

Ca(NO₃)₂ 1.0 g
KH₂PO₄ 0.25 g
MgSO₄ 0.25 g
FeCl₃ Traces
Agar 20.0 g
Distilled water 1000.0 ml
pH 6.5-7.0
Autoclave at 121°C for 15 min.
Sterilize filter paper strips by dry heat and soak with sterile medium.

38. MALT AGAR 7 BALLING WITH 12% NaCl (MA12NaCl)

Malt extract Balling 7 degrees 1000.0 ml
NaCl 120.0 g

Agar 20.0 g
Autoclave at 121°C for 15 min.

39. MALT 7 BALLING AGAR WITH 1% NaCl

Malt extract Balling 7 degrees 1000.0 ml
NaCl 10.0 g
Agar 20.0 g
Autoclave at 121°C for 15 min.

40. AZOTOBACTER MEDIUM 2

KH₂PO₄ 0.2 g
K₂HPO₄ 0.8 g
MgSO₄ × 7 H₂O 0.2 g
CaSO₄ × 2 H₂O 0.1 g
FeCl₃ Traces
Na₂MoO₄ Traces
Yeast extract 0.5 g
Sucrose 20.0 g
Agar 15.0-20.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 111°C for 30 min.

41. FLAVOBACTERIUM MEDIUM

Na-caseinate 2.0 g
Yeast extract 0.5 g
Peptone 1.0 g
K₂HPO₄ 0.5 g
Agar 12.0 g
Distilled water 1000.0 ml
pH 7.4
Autoclave at 121°C for 15 min.

42. PSEUDOMONAS SACCHAROPHILA MEDIUM

Solution 1:

KH₂PO₄ 4.4 g
Na₂HPO₄ 4.8 g
NH₄Cl 1.0 g
MgSO₄ × 7 H₂O 0.5 g
Agar (if needed) 20.0 g
Distilled water 985.0 ml

Solution 2:

Ferric ammonium citrate 50.0 mg
CaCl₂ 5.0 mg
Distilled water 5.0 ml

Solution 3:

Sucrose 1.0 g
Distilled water 10.0 ml

Autoclave solutions 1 and 2 separately at 121°C for 15 min, solution 3 at 111°C for 30 min and mix aseptically.

43. SEA WATER MEDIUM WITH YEAST EXTRACT

Sea salt 37.9 g
Yeast extract 3.0 g
Peptone 10.0 g
Agar 20.0 g
Distilled water to 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C for 15 min.

44. HALOBACTERIUM MEDIUM 1

Solution 1:

NaCl 250.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 10.0 g
KCl 5.0 g
 $\text{CaCl}_2 \times 6 \text{H}_2\text{O}$ 0.2 g
Tap water 800.0 ml

Solution 2:

Yeast extract 10.0 g
Tryptone 2.5 g
Agar 20.0 g
Tap water 200.0 ml
Autoclave solutions separately at 121°C for 15 min and mix aseptically.

45. STARVED AGAR

Agar 20.0 g
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.

46. SP MEDIUM FOR STIGMATELLA AURANTIACA

Raffinose 1.0 g
Sucrose 1.0 g
Galactose 1.0 g
Starch (soluble) 5.0 g
Casitone 2.5 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.5 g
 K_2HPO_4 0.25 g
Agar 15.0 g
Distilled water 1000.0 ml
Autoclave at 111°C for 30 min.

47. CM + YE MEDIUM

Casamino acids 7.5 g
Yeast extract 10.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 20.0 g
Na-citrate 3.0 g
KCl 2.0 g
NaCl 200.0 g
Agar 15.0 g
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ in 0.01N HCl (see below) 1.0 ml
Distilled water 1000.0 ml
Solution of $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$:
HCl (0.01 N) 100.0 ml
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 4.98 g

pH 7.4 (adjust with 1N NaOH)

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

48. CASEIN MEDIUM

NaCl 250.0 g

Casein hydrolysate 5.0 g

Yeast extract 5.0 g

MgCl₂ × 6 H₂O 20.0 g

KCl 2.0 g

CaCl₂ × 2 H₂O 0.2 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.4 (adjust with 1N NaOH)

Autoclave at 121°C for 15 min.

49. HALOBACTERIUM MEDIUM 2

Solution 1:

NaCl 120.0 g

MgCl₂ × 6 H₂O 50.0 g

K₂SO₄ 5.0 g

CaCl₂ × 6 H₂O 0.2 g

Distilled water 500.0 ml

Solution 2:

Tryptone 5.0 g

Yeast extract 5.0 g

Agar 20.0 g

Distilled water 500.0 ml

pH 6.8

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

50. YEAST GLUCOSE AGAR

Yeast extract 5.0 g

Peptone 5.0 g

Glucose 10.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 111°C for 30 min.

51. CASITONE AGAR

Casitone 3.0 g

CaCl₂ × 2 H₂O 1.36 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 121°C for 15 min.

52. CASITONE YEAST MEDIUM

Casitone 3.0 g

CaCl₂ × 2 H₂O 1.36 g

Yeast extract 1.0 g

Agar 15.0 g

Distilled water 1000.0 ml
pH 7.2
Autoclave at 121°C for 15 min.

53. *THERMUS THERMOPHILUS* MEDIUM

Yeast extract 4.0 g
Polypeptone 8.0 g
NaCl 2.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

54. *DESULFOVIBRIO* MEDIUM WITH 1% NaCl

K₂HPO₄ 0.01 g
NaCl 10.0 g
MgSO₄ × 7 H₂O 0.2 g
Na-lactate (40%) 4.0 ml
Solution of *More* salt (see below) 1.0 ml
Yeast extract 1.0 g
Ascorbic acid 0.1 g
Agar 6.0 g
Distilled water 1000.0 ml
Solution of More salt:
Fe(NH₄)₂(SO₄)₂ × 6 H₂O 1.0 g
Distilled water 5.0 ml
Autoclave solutions separately at 121°C for 15 min and mix aseptically.

55. *CAULOBACTER* MEDIUM

Peptone 2.0 g
Yeast extract 1.0 g
MgSO₄ × 7 H₂O 0.2 g
Agar 10.0 g
Tap water 1000.0 ml
Autoclave at 121°C for 15 min.

56. *CAULOBACTER* MEDIUM WITH GLUCOSE

Peptone 2.0 g
Yeast extract 1.0 g
Glucose 2.0 g
Riboflavin 1.0 mg
MgSO₄ × 7 H₂O 0.2 g
Agar 10.0 g
Tap water 1000.0 ml
pH 7.0
Autoclave at 111°C for 30 min.

57. PEPTONE MEAT AGAR WITH 1% UREA

Peptone 10.0 g
Beef extract 3.0 g
NaCl 5.0 g
Urea 10.0 g

Agar 20.0 g
Distilled water 1000.0 ml
Do not adjust pH; pH raises to about 8 due to heat degraded urea.
Autoclave at 121°C for 15 min.

58. HALOBACTERIUM MEDIUM 3

Solution 1:

Casamino acids 7.5 g
Yeast extract 10.0 g
Na-citrate 3.0 g
KCl 2.0 g
MgSO₄ × 7 H₂O 20.0 g
FeCl₃ traces
NaCl 250.0 g
Distilled water 750.0 ml

Solution 2:

Agar 25.0 g
Distilled water 250.0 ml
pH 7.4
Autoclave solutions separately at 121°C for 15 min. and mix aseptically.

59. PSEUDONOCARDIA THERMOPHILA MEDIUM

Peptone 2.5 g
Meat extract 2.5 g
NaCl 2.5 g
Yeast extract 0.1 g
Glucose 2.5 g
Sucrose 5.0 g
Casein acidic hydrolysate 0.1 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 111°C for 30 min.

60. ALFALFA AGAR

K₂HPO₄ 0.5 g
MgSO₄ × 7 H₂O 0.2 g
KH₂PO₄ 0.5 g
NaCl 0.2 g
CaSO₄ 0.1 g
(NH₄)₂MoO₄ Traces
Mannitol 20.0 g
Alfalfa meal 10.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0-7.2.
Autoclave at 111°C for 20 min.

61. MEDIUM FOR NITROGEN-FIXING SPIRILLUM

K₂HPO₄ 0.1 g
KH₂PO₄ 0.4 g
MgSO₄ × 7 H₂O 0.2 g

NaCl 0.1 g
CaCl₂ 0.02 g
FeCl₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.02 g
Na-malate 5.0 g
Yeast extract 50.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C for 15 min.

62. MANURE TINCTURE

Cow manure (fresh) 1.0 kg
Distilled water 3000.0 ml
Boil, squeeze through gauze into a bottle and dilute to 3000.0 ml.

63. MEDIUM WITH CURD DECOCTION

Curd decoction 61.0 ml
Manure tincture 184.0 ml
Na-acetate 1.0 g
Agar 20.0 g
Tap water 735.0 ml
pH 7.8

Preparation of curd decoction: pour 9000.0 ml of distilled water into 3.0 kg of curd and add 150.0 of dry *Aspergillus terricola* mycelium, shake, pour chloroform, seal with a stopper. Decoct at 37°C for 10 days, adjust pH to 7.0 with 1 N NaOH. The decoction shall contain 400 mg% amine nitrogen and 300 mg% tryptophan.

Preparation of manure tincture: mix cow manure (fresh) 1.0 kg and distilled water 3000.0 ml. Boil, squeeze through gauze into a bottle and dilute to 3000.0 ml.
Autoclave at 121°C for 15 min.

64. PEPTONE YEAST AGAR

Peptone 5.0 g
Yeast extract 3.0 g
KH₂PO₄ 0.2 g
Glucose 5.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.0-7.2
Autoclave at 111°C for 30 min.

65. OATMEAL AGAR FOR BACTERIA

Oatmeal 2.0 g
Peptone 0.5 g
NaCl 1.0 g
Galactose 0.5 g
Agar 12.0 g
Distilled water 1000.0 ml
pH 7.5
Autoclave at 111°C for 30 min.

66. MEDIUM WITH SOIL EXTRACT

Peptone 5.0 g

Beef extract 3.0 g
Agar 15.0 g
Soil extract 250.0 ml
Tap water 750.0 ml
pH 7.0

Autoclave at 121°C for 15 min.

Preparation of soil extract: dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121°C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121°C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

67. PEPTONE MEAT AGAR WITH TRACE ELEMENTS

Peptone 10.0 g
NaCl 5.0 g
Beef extract 3.0 g
Yeast autolysate 2.0 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml
Agar 20.0 g

Trace element solution:

H₃BO₃ 5.0 g
(NH₄)₂MoO₄ 5.0 g
KI 0.5 g
NaBr 0.5 g
ZnSO₄ 0.2 g
Al₂(SO₄)₃ 0.3 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C for 15 min.

68. PEPTONE MEAT AGAR WITH 3% SEA SALT

Peptone 10.0 g
NaCl 5.0 g
Sea salt 30.0 g
Beef extract 3.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C for 15 min.

69. DAVIS SUPPLEMENTED MINIMAL MEDIUM

Solution 1:

Yeast extract 2.0 g
Casein hydrolysate 2.0 g
K₂HPO₄ 7.0 g
KH₂PO₄ 3.0 g
Na-citrate × 3 H₂O 0.5 g
MgSO₄ × 7 H₂O 0.1 g
(NH₄)₂SO₄ 1.0 g
Agar 15.0 g

Distilled water 980.0 ml

Solution 2:

Glucose 2.0 g

Distilled water 20.0 ml

pH 7.0

Autoclave at 121°C for 15 min.

70. MEDIUM VY/2 FOR *STIGMATELLA AURANTIACA*

Baker's yeast 5.0 g

CaCl₂ × 2 H₂O 1.36 g

Vitamin B₁₂ 0.5 mg

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2 (adjust with KOH before adding agar)

Autoclave at 111°C for 30 min. Vitamin B₁₂ sterilize separately with filtration.

71. *NITROSOCOCCUS* MEDIUM 1

(NH₄)₂SO₄ 1.32 g

MgSO₄ × 7 H₂O 380.0 mg

CaCl₂ × 2 H₂O 20.0 mg

Chelated iron (13% iron) 1.0 mg

Na₂MoO₄ × 2 H₂O 100.0 µg

MnCl₂ × 4 H₂O 200.0 µg

CoCl₂ × 6 H₂O 2.0 µg

ZnSO₄ × 7 H₂O 100.0 µg

K₂HPO₄ 8.7 mg

Phenol red (0.04%) 3.25 ml

Sea water 1000.0 ml

pH 7.5-7.8 (adjust with 1 N HCl)

Autoclave at 121°C for 15 min.

72. BEAN AGAR

Beans (peas or pulse) 100.0 g

K₂HPO₄ 0.5 g

Sucrose 10.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil beans in 1000.0 ml of water, filter through gauze, add water to the initial volume; add phosphate, sucrose and agar

pH 7.2-7.4

Autoclave at 111°C for 30 min.

73. GYT-AGAR

Glucose 10.0 g

Yeast extract 1.0 g

Tryptose 2.0 g

FeSO₄ × 7 H₂O 1.0 mg

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 111°C for 30 min.

74. HOTTINGER BROTH

Hottinger broth (HiMedia M1425) 23.0 g
Autoclave at 121°C for 15 min.

75. MODIFICATION OF TWEEN-80 MEDIUM FOR MILK-ACID BACTERIA

Yeast extract 5.0 g
Glucose 2.5 g
Beef extract 1.2 g
Tween-80 1.0 ml
 K_2HPO_4 2.0 g
Na-acetate 5.0 g
 NH_4 -citrate 2.0 g
 $MgSO_4 \times 7 H_2O$ 0.2 g
 $MnSO_4 \times 4 H_2O$ 0.05 g
Agar 5.0 g
Distilled water 1000.0 ml
pH 6.0-6.5
Autoclave at 111°C for 30 min.

76. GLUCOSE MINERAL MEDIUM

KNO_3 0.2 g
 $MgSO_4 \times 7 H_2O$ 0.04 g
 $CaCl_2 \times 2 H_2O$ 0.04 g
 Na_2HPO_4 0.3 g
 KH_2PO_4 0.14 g
Glucose 5.0 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml
Trace element solution:
Nitrilotriacetic acid 1.5 g
 $MgSO_4 \times 7 H_2O$ 3.0 g
 $MnSO_4 \times H_2O$ 0.5 g
NaCl 1.0 g
 $FeSO_4 \times 7 H_2O$ 0.1 g
 $CoSO_4 \times 7 H_2O$ 0.18 g
 $CaCl_2 \times 2 H_2O$ 0.1 g
 $ZnSO_4 \times 7 H_2O$ 0.18 g
 $CuSO_4 \times 5 H_2O$ 0.01 g
 $KAl(SO_4)_2 \times 12 H_2O$ 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
 $Na_2SeO_3 \times 5 H_2O$ 0.3 mg
 $Na_2WO_4 \times 2 H_2O$ 0.4 mg
Distilled water 1000.0 ml
First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).
Autoclave base medium and trace element solution at 121°C for 15 min.

77. PEPTONE MEAT AGAR WITH VITAMINS

Peptone 10.0 g
NaCl 5.0 g

Beef extract 3.0 g
Yeast extract 1.0 g
Glucose 1.0 g
Vitamin B₁₂ 2.0 mg
Thiamin 2.0 mg
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 111°C for 30 min.

78. PEPTONE MEAT AGAR WITH 2% SOLUBLE STARCH

Peptone 10.0 g
Beef extract 3.0 g
NaCl 5.0 g
Soluble starch 20.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

79. PEPTONE MEAT AGAR WITH 1% SOLUBLE STARCH

Peptone 10.0 g
Beef extract 3.0 g
NaCl 5.0 g
Soluble starch 10.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 121°C for 15 min.

80. PEPTONE MEAT AGAR WITH 6% NaCl

Peptone 10.0 g
Beef extract 3.0 g
NaCl 60.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

81. PEPTONE MEAT AGAR WITH 1.8% SEA SALT

Peptone 10.0 g
NaCl 5.0 g
Beef extract 3.0 g
Sea salt 18.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

82. POTATO AGAR WITH 2% GLUCOSE

Potato 200.0 g
Glucose 20.0 g

Agar 20.0 g

Tap water 1000.0 ml

pH 7.0

Boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.

Autoclave at 111°C for 30 min.

83. LOPATINA MEDIUM

Glucose 10.0 g

KH₂PO₄ 0.2 g

MgSO₄ × 7 H₂O 0.2 g

Tyrosine 1.0 g

NaCl 0.2 g

CaSO₄ 0.1 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min

84. MEDIUM WITH CASEIN HYDROLYSATE

Casein hydrolysate 10.0 g

Glucose 5.0 g

p-Aminobenzoic acid 5.0 mg

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min.

85. MEDIUM WITH HOTTINGER BROTH

Peptone 10.0 g

Yeast autolysate 10.0 g

Hottinger broth (HiMedia M1425) 0.23 g

Phosphate solution (see below) 25.0 ml

Salt solution (see below) 25.0 ml

Glucose 5.0 g

Distilled water 950.0 ml

Phosphate solution:

KH₂PO₄ 100.0 mg

K₂HPO₄ 100.0 mg

Distilled water 25.0 ml

Salt solution:

MgSO₄ × 7 H₂O 40.0 mg

NaCl 2.0 mg

FeSO₄ 2.0 mg

Distilled water 25.0 ml

pH 7.0

Autoclave phosphate and salt solutions at 121°C for 15 min, base medium at 111°C for 30 min.

86. NITROSOLOBUS MEDIUM 1

CaCl₂ × 2 H₂O 0.02 g

MgSO₄ × 7 H₂O 0.2 g

Chelated iron 1.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 0.1 mg
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 0.2 mg
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 2.0 mg
 $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 0.02 mg
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 mg
 $(\text{NH}_4)_2\text{SO}_4$ 1.65 g
 K_2HPO_4 0.087 g
Phenol red 5.0 mg
Distilled water 1000.0 ml
pH 7.5 (adjust with 0.1 M NaHCO_3)
Autoclave at 121°C for 15 min.

87. NITROSOCOCCUS MEDIUM 2

NH_4Cl 0.5 g
 KH_2PO_4 0.05 g
 CaCO_3 5.0 g
Chelated iron 1.0 mg
Phenol red (0.04%) 3.25 ml
Sea water 1000.0 ml
pH 7.5-7.8 (adjust with 1 N HCl)
Autoclave at 121°C for 15 min.

88. SPIRILLUM MEDIUM

Peptone 10.0 g
Succinic acid 1.0 g
 $(\text{NH}_4)_2\text{SO}_4$ 1.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 1.0 g
 $\text{FeCl}_3 \times 6 \text{H}_2\text{O}$ 2.0 mg
 $\text{MnSO}_4 \times \text{H}_2\text{O}$ 2.0 mg
Distilled water 1000.0 ml
pH 6.8
Autoclave at 121°C for 15 min.

89. MILK MEDIUM FOR HALOPHILS

Solution 1:

Milk 500.0 ml

Solution 2:

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 10.0 g

KNO_3 2.0 g

NaCl 200.0 g

Distilled water 100.0 ml

Solution 3:

Peptone 5.0 g

Glycerol 10.0 g

Agar 25.0 g

Distilled water 400.0 ml

pH 8.4

Autoclave at 121°C for 15 min.

Sequence of mixing: add warm skim milk to a hot mixture of solutions 1 and 2.

90. DESULFOVIBRIO GIGAS MEDIUM

Solution 1:

KH₂PO₄ 1.0 g
NH₄Cl 0.5 g
MgSO₄ × 7 H₂O 0.4 g
Na₂SO₄ 2.0 g
CaCl₂ × 2 H₂O 0.1 g
Trace element solution SL-6: (see below) 1.0 ml
2 M H₂SO₄ 1.0 ml
Na L-lactate 2.0 g
Distilled water 950.0 ml

Solution 2:

NaHCO₃ 2.0 g
Distilled water 40.0 ml

Solution 3:

Na₂S × 9 H₂O 300.0 mg
Distilled water 6.0 ml

Vitamin solution:

Biotin 2.5 mg
Nicotinic acid 25.0 mg
Thiamine-HCl 12.5 mg
p-Aminobenzoic acid 12.5 mg
Calcium pantothenate 6.5 mg
Pyridoxine-HCl 62.5 mg
Distilled water 1000.0 ml

Trace element solution SL-6:

ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.3 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.02 g
Na₂MoO₄ 0.03 g
Distilled water 1000.0 ml
pH 7.2

Autoclave solutions 1 and 3 separately under N₂ at 121°C for 15 min, add 5.0 ml of the filter sterilized vitamin solution to 1000.0 ml of sterile solution 1. Solution 2 (autoclave at 121°C for 15 min) is not to be kept for long.

91. THERMODESULFOBACTERIUM MEDIUM

Solution 1:

Na₂SO₄ 3.0 g
NH₄Cl 1.0 g
MgCl₂ × 6 H₂O 0.2 g
KH₂PO₄ 0.3 g
Na₂HPO₄ × 12 H₂O 2.0 g
FeSO₄ × 7 H₂O 1.5 mg
Resazurin 1.0 mg
Distilled water 930.0 ml

Solution 2:

Trace element solution (see below) 10.0 ml

Solution 3:

Yeast extract 1.0 g

Distilled water 25.0 ml

Solution 4:

Na-lactate 4.0 g

Distilled water 25.0 ml

Solution 5:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.5 g

Distilled water 6.0 ml

Solution 6:

Vitamin solution (see below) 5.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 g

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 0.2 g

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.1 g

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.17 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 g

ZnCl_2 0.1 g

CuCl_2 0.02 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.01 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.026 g

NaCl 1.0 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ 0.02 g

Distilled water 1000.0 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

Ca DL-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 6.8-7.0

Solutions 1, 3, 4 and 5 autoclave under N₂ at 121°C for 15 min.

Solution 6 is filter sterilized. Before use, neutralize solution 5 by dropwise of 1 N HCl.

92. DESULFOVIBRIO MEDIUM WITH LACTATE

Solution 1:

K₂HPO₄ 0.5 g

NH₄Cl 1.0 g

$\text{CaCl}_2 \times 6 \text{ H}_2\text{O}$ 0.1 g

MgSO₄ × 7 H₂O 2.0 g

Na₂SO₄ 1.0 g

Na-lactate 5.0 g

Yeast extract 1.0 g

Resazurin 0.01 g

Cysteine 0.5 g

Distilled water 950.0 ml

Solution 2:

NaHCO₃ 4.0 g

Distilled water 40.0 ml

Solution 3:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 300.0 mg

Distilled water 6.0 ml

Solution 4:

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.4 g

Distilled water 10.0 ml

pH 6.8

Autoclave all solutions separately at 121°C for 15 min. Solution 1 bring to boil while simultaneously bubbling a mixture of oxygen-free gas composed of 97% N_2 and 3% H_2 through the mixture and sterilize in atmosphere of this gas mixture. Solution 3 sterilize in atmosphere of N_2 .

93. AZOSPIRILLUM BRASILIENSE MEDIUM 1

Ca-malate 10.0 g

K_2HPO_4 1.0 g

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.5 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.02 g

Distilled water 1000.0 ml

pH 6.5

Autoclave at 121°C for 15 min.

94. MEDIUM FOR CARBON MONOOXIDE OXIDIZERS

$\text{Na}_2\text{HPO}_4 \times 12 \text{ H}_2\text{O}$ 4.5 g

KH_2PO_4 0.75 g

NH_4Cl 1.5 g

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.2 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.03 g

$\text{Fe}(\text{NH}_4)\text{-citrate}$ 0.018 g

Agar (if necessary) 1.2 g

Trace element solution *SL-6* (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Distilled water 1000.0 ml

Trace element solution SL-6:

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.03 g

H_3BO_3 0.3 g

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 g

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 0.01 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.02 g

Na_2MoO_4 0.03 g

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 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
pH 7.0

Autoclave at 121°C for 15 min, add vitamin solution sterilized by filtration. For chemoautotrophic growth incubate under gas atmosphere of a) 20-80% CO₂ + 10% O₂ + 0-70% N₂ or b) 70% H₂ + 20% O₂ + 10% CO₂ adding 2.5 g NaHCO₃ per liter of medium. For chemoorganotrophic growth add 3.0 g sodium acetate and incubate under air.

95. *DESULFOTOMACULUM ACETOXIDANS* MEDIUM

Solution 1:

NaCl 1.17 g
MgCl₂ × 6 H₂O 0.4 g
KCl 0.3 g
CaCl₂ × 2 H₂O 0.15 g
NH₄Cl 0.27 g
KH₂PO₄ 0.2 g
Na₂SO₄ 2.84 g
Na-acetate 1.4 g
Na-butyrate 1.4 g
Yeast extract 1.0 g
Vitamin solution (see below) 1.0 ml
Trace element solution 1.0 ml (see below)
Distilled water 940.0 ml

Solution 2:

Na₂S × 9 H₂O 0.36 g
Distilled water 10.0 ml

Sodium bicarbonate for alkalization:

NaHCO₃ 4.5 g
Distilled water 50.0 g

Vitamin solution:

p-Aminobenzoic acid 4.0 mg
D(+)-Biotin 1.0 mg
Thiamine-HCl 10.0 mg
Distilled water 100.0 ml

Trace element solution:

FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 68.0 mg
MnCl₂ × 4 H₂O 100.0 ml
H₃BO₃ 62.0 mg
CoCl₂ × 6 H₂O 120.0 ml
Na₂MoO₄ × 2 H₂O 24.0 mg
HCl (0.05 M) 1000.0 ml
pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9 - 7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized.

96. *DESULFONEMA LIMICOLA* MEDIUM

Solution 1:

Na₂SO₄ 3.0 g
NaCl 13.0 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 2.2 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.15 g

KCl 0.5 g

KH_2PO_4 0.2 g

NH_4Cl 0.3 g

Distilled water 850.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO_3 5.0 g

Distilled water 100.0 ml

Solution 4:

Na-acetate $\times 3 \text{ H}_2\text{O}$ 2.5 g

Distilled water 10.0 ml

Solution 5:

Disodium succinate 0.1 g

Distilled water 1.0 ml

Solution 6:

Vitamin solution (see below) 5.0 ml

Solution 7:

$\text{AlCl}_3 \times 6 \text{ H}_2\text{O}$ 245.0 mg

Distilled water 5.0 ml

Solution 8:

Na_2CO_3 170.0 mg

Distilled water 1.6 ml

Solution 9:

Rumen fluid, clarified 20.0 ml

Solution 10:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.4 mg

Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg

Distilled water 990.0 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

Ca DL-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

pH 7.6

The trace element solution preparation: $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$ is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated. Solution 1 is boiled for a few minutes, cooled to room temperature, gassed with 80% N_2 + 20% CO_2 gas mixture to reach a pH of around 6, then autoclaved anaerobically under the same gas. Solutions 2, 3, 5, 9, and 10 are autoclaved separately under nitrogen at 121°C for 15 min, solution 3 is filter-sterilized and flushed with 80% N_2 + 20% CO_2 to remove dissolved oxygen. Solution 6 is filter-sterilized and outgassed with N_2 . Solutions 7 and 8 are combined before autoclaving at 121°C for 15 min. Solutions 2 to 10 are added to the sterile cooled solution A in the sequence as indicated. The completed medium is distributed anaerobically under 80% N_2 + 20% CO_2 into appropriate vessels.

97. DESULFONEMA MAGNUM MEDIUM

Solution 1:

Na_2SO_4 3.0 g

NaCl 21.0 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 5.5 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 1.35 g

KCl 0.5 g

KH_2PO_4 0.2 g

NH_4Cl 0.3 g

Distilled water 850.0 ml

Solution 2:

Trace element solution (see below) 1.0 ml

Solution 3:

NaHCO_3 2.5 g

Distilled water 100.0 ml

Solution 4:

Na-benzoate 0.6 g

Distilled water 10.0 ml

Solution 5:

Disodium succinate 0.1 g

Distilled water 1.0 ml

Solution 6:

Vitamin solution (see below) 5.0 ml

Solution 7:

$\text{AlCl}_3 \times 6 \text{H}_2\text{O}$ 245.0 mg

Distilled water 5.0 ml

Solution 8:

Na_2CO_3 170.0 mg

Distilled water 1.6 ml

Solution 9:

Rumen fluid, clarified 20.0 ml

Solution 10:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 0.4 mg

Distilled water 10.0 ml

Trace element solution:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 100.0 mg

H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Na₂SeO₄ 3.0 mg
Distilled water 990.0 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
Ca DL-pantothenate 5.0 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Vitamin B₁₂ 50.0 mg
Distilled water 1000.0 ml
pH 7.6

The trace element solution preparation: FeCl₂ × 4 H₂O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated. Solution 1 is boiled for a few minutes, cooled to room temperature, gassed with 80% N₂ + 20% CO₂ gas mixture to reach a pH of around 6, then autoclaved anaerobically under the same gas. Solutions 2, 3, 5, 9, and 10 are autoclaved separately under nitrogen at 121°C for 15 min, solution 3 is filter-sterilized and flushed with 80% N₂ + 20% CO₂ to remove dissolved oxygen. Solution 6 is filter-sterilized and outgassed with N₂. Solutions 7 and 8 are combined before autoclaving at 121°C for 15 min. Solutions 2 to 10 are added to the sterile cooled solution A in the sequence as indicated. The completed medium is distributed anaerobically under 80% N₂ + 20% CO₂ into appropriate vessels.

98. WORT AGAR 7 B WITH 2% CaCO₃

Malt extract Balling 7 degrees 1000.0 ml
CaCO₃ 20 g
Agar 20.0 g
Autoclave at 111°C for 30 min.

99. MEDIUM YE

Yeast extract 30.0 g
Ethanol 20.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
pH 5.0-6.0
Autoclave at 121°C for 15 min without ethanol.
Apply filter sterilize ethanol (0.1 ml/test tube) onto the surface of agar slants.

100. SAP-2 AGAR

Tryptone 1.0 g
Yeast extract 1.0 g
Agar 20.0 g
Sea water 1000.0 g
pH 7.4

Autoclave at 121°C for 15 min.

101. SOIL AGAR

Yeast extract 2.0 g

Tryptone 1.0 g

Na-acetate 1.0 g

Soil extract 50.0 ml

Agar 20.0 g

Distilled water add to 1000.0 ml

pH 7.4

Autoclave at 121°C for 15 min.

Preparation of soil extract: dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121°C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121°C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

102. DESULFOBACTER POSTGATEI MEDIUM

Solution 1:

Na₂SO₄ 3.0 g

KH₂PO₄ 0.2 g

NH₄Cl 0.3 g

NaCl 7.0 g

MgCl₂ × 6 H₂O 1.3 g

KCl 0.5 g

CaCl₂ × 2 H₂O 0.15 g

Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g

Distilled water 100.0 ml

Solution 4:

Na-acetate × 3 H₂O 2.5 g

Distilled water 10.0 ml

Solution 5:

Vitamin solution (see below) 10.0 ml

Solution 6:

Na₂S × 9 H₂O 0.4 g

Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 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
Ca DL-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized.

103. DESULFOBULBUS MEDIUM

Solution 1:

Na₂SO₄ 3.0 g
KH₂PO₄ 0.2 g
NH₄Cl 0.3 g
NaCl 1.0 g
MgCl₂ × 6 H₂O 0.4 g
KCl 0.5 g
CaCl₂ × 2 H₂O 0.15 g
Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g
Distilled water 100.0 ml

Solution 4:

Na-propionate 1.5 g
Distilled water 10.0 ml

Solution 5:

Vitamin solution (see below) 10.0 ml

Solution 6:

Na₂S × 9 H₂O 0.4 g
Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 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
Ca DL-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized.

104. MACROMONAS MEDIUM 1

Na-acetate 1.0 g
CaCl₂ × 2 H₂O 0.1 g
Casein acidic hydrolysate 0.1 g
Yeast extract 0.1 g
FeS or CaS 0.2 g
Agar 1.0 g
Distilled water 1000.0 ml
pH 7.2-7.4

Prepare suspension of FeS separately from the equimolar solutions of Na₂S × 9 H₂O and FeSO₄, wash with freshly boiled distilled water under the flow of inert gas, sterilize separately from the main medium under inert gas at 111°C for 30 min. Main medium autoclave at 121°C for 15 min.

105. MACROMONAS MEDIUM 2

(NH₄)₂SO₄ 0.5 g
MgSO₄ × 7 H₂O 0.2 g
Casein acidic hydrolysate 1.0 g
Na-acetate × 3 H₂O 1.0 g
or succinate, or benzoate 0.5 g
Na₂S₂O₃ × 5 H₂O 0.5 g
Catalase 2.0 mg
Vitamin solution (see below) 1.0 ml
Distilled water 1000.0 ml

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 B₁₂ 1.0 mg
p-Aminobenzoic acid 50.0 mg
Distilled water 1000.0 ml

pH 7.2-7.4

Autoclave at 121°C for 15 min.

Sterilize catalase and vitamins separately from the main medium by filtration. Thiosulfate should also better be autoclaved separately at 121°C for 15 min.

106. BEGGIATOIA MEDIUM 1

(NH₄)₂SO₄ 500.0 mg

MgSO₄ × 7 H₂O 100.0 mg

CaCl₂ × 2 H₂O 50.0 mg

Na-lactate 500.0 mg

Na₂S₂O₃ × 5 H₂O 500.0 mg

K₂HPO₄ 110.0 mg

KH₂PO₄ 85.0 mg

FeCl₃ × 6 H₂O 2.0 mg

EDTA 3.0 mg

Vitamin solution (see below) 1.0 ml

Buffer HEPES 0.01 M

Distilled water 1000.0 ml

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 B₁₂ 1.0 mg

p-Aminobenzoic acid 50.0 mg

Distilled water 1000.0 ml

pH 7.2-7.5 (adjust with NaOH)

Sterilize vitamins (by filtration); main medium, thiosulfate and lactate each separately (autoclave at 121°C for 15 min) and add into the main medium prior to inoculation.

107. DESULFOSARCINA MEDIUM

Solution 1:

Na₂SO₄ 3.0 g

KH₂PO₄ 0.2 g

NH₄Cl 0.3 g

NaCl 13.5 g

MgCl₂ × 6 H₂O 2.0 g

KCl 0.5 g

CaCl₂ × 2 H₂O 0.15 g

Distilled water 870.0 ml

Solution 2:

Trace element solution (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g

Distilled water 100.0 ml

Solution 4:

Na-benzoate 0.6 g

Na-lactate 1.0 g

Distilled water 10.0 ml

Solution 5:

Vitamin solution (see below) 10.0 ml

Solution 6:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.4 g

Distilled water 10.0 ml

Trace element solution:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg

$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ 3.0 mg

Distilled water 990.0 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

Ca DL-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized.

108. BEGGIATO A MEDIUM 2

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 50.0 mg

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 30.0 mg

Na-lactate 500.0 mg

$\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ 500.0 mg

NaH_2PO_4 125.0 mg

KCl 125.0 mg

NaHCO_3 125.0 mg

Na_2SO_4 500.0 mg

NaNO_3 620.0 mg

Vitamin solution (see below) 1.0 ml

Trace element solution according to *Hogland*: (see below) 1.0 ml

Distilled water 1000.0 ml

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 B₁₂ 1.0 mg
p-Aminobenzoic acid 50.0 mg
Distilled water 1000.0 ml

Trace element solution according to Hogland:

EDTA 5.0 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ 10.0 mg
NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 20.0 mg
Distilled water 1000.0 ml
pH 7.5 (adjust with 1% HCl)

To prepare the trace element solution, preliminarily acidify water to pH 3.0-4.0 with HCl.

Sterilize thiosulfate, lactate, bicarbonate, trace elements and vitamins separately and add to the main medium prior to inoculation. Lactate and thiosulfate can be more conveniently prepared as 10% solutions; bicarbonate, as 5% solution. Sterilize the vitamin solution by filtration, others solutions and base medium by autoclaving at 121°C for 15 min.

109. DESULFOVIBRIO MEDIUM

Solution 1:

K₂HPO₄ 0.5 g
NH₄Cl 1.0 g
Na₂SO₄ 1.0 g
CaCl₂ × 2 H₂O 0.1 g
MgSO₄ × 7 H₂O 2.0 g
Na DL-lactate 2.0 g
Yeast extract 1.0 g
Resazurin 1.0 g
Distilled water 980.0 ml

Solution 2:

FeSO₄ × 7 H₂O 0.5 g
Distilled water 10.0 ml

Solution 3:

Na-thioglycollate 0.1 g
Ascorbic acid 0.1 g
Distilled water 10.0 ml

Solution 4:

Trace element solution (see below) 1.0 ml

Trace element solution:

FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 68.0 mg
MnCl₂ × 4 H₂O 100.0 ml
H₃BO₃ 62.0 mg
CoCl₂ × 6 H₂O 120.0 ml
Na₂MoO₄ × 2 H₂O 24.0 mg

HCl, 0.05 M 1000.0 ml

pH 7.4-7.8

Autoclave at 121°C for 15 min under an atmosphere of N₂. Solution 1 is boiled before sterilization, being blown with N₂.

110. DESULFOVIBRIO MEDIUM WITH 2% NaCl

Solution 1:

K₂HPO₄ 0.5 g

NH₄Cl 1.0 g

NaCl 20 g

Na₂SO₄ 1.0 g

CaCl₂ × 2 H₂O 0.1 g

MgSO₄ × 7 H₂O 2.0 g

Na DL-lactate 2.0 g

Yeast extract 1.0 g

Resazurin 1.0 mg

Distilled water 980.0 ml

Solution 2:

FeSO₄ × 7 H₂O 0.5 g

Distilled water 10.0 ml

Solution 3:

Na-thioglycollate 0.1 g

Ascorbic acid 0.1 g

Distilled water 10.0 ml

Solution 4:

Trace element solution (see below) 1.0 ml

Trace element solution:

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 68.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 62.0 mg

CoCl₂ × 6 H₂O 120.0 mg

Na₂MoO₄ × 2 H₂O 24.0 mg

HCl, 0.05 M 1000.0 ml

pH 7.4-7.8

Autoclave at 121°C for 15 min under an atmosphere of N₂. Solution 1 is boiled before sterilization, being blown with N₂.

111. DESULFOVIBRIO MEDIUM WITH 3% NaCl

Solution 1:

K₂HPO₄ 0.5 g

NH₄Cl 1.0 g

NaCl 30 g

Na₂SO₄ 1.0 g

CaCl₂ × 2 H₂O 0.1 g

MgSO₄ × 7 H₂O 2.0 g

Na DL-Lactate 2.0 g

Yeast extract 1.0 g

Resazurin 1.0 mg

Distilled water 980.0 ml

Solution 2:

FeSO₄ × 7 H₂O 0.5 g

Distilled water 10.0 ml

Solution 3:

Na-thioglycollate 0.1 g

Ascorbic acid 0.1 g

Distilled water 10.0 ml

Solution 4:

Trace element solution (see below) 1.0 ml

Trace element solution:

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 68.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 62.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 120.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 24.0 mg

HCl, 0.05 M 1000.0 ml

pH 7.4-7.8

Autoclave at 121°C for 15 min under an atmosphere of N_2 . Solution 1 is boiled before sterilization, being blown with N_2 .

112. GLUCOSE YEAST EXTRACT AGAR

Glucose 20.0 g

Yeast extract 10.0 g

CaCO_3 20.0 g

Agar 17.0 g

Distilled water 1000.0 ml

Autoclave at 111°C for 30 min.

113. *DESULFOVIBRIO BAARSII* MEDIUM

Solution 1:

Na_2SO_4 3.0 g

KH_2PO_4 0.2 g

NH_4Cl 0.3 g

NaCl 1.0 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.4 g

KCl 0.5 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.15 g

Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO_3 5.0 g

Distilled water 100.0 ml

Solution 4:

Na-butyrate 0.7 g

Na-caproate 0.3 g

Na-octanoate 0.15 g

Distilled water 10.0 ml

Solution 5:

Vitamin solution (see below) 10.0 ml

Solution 6:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.4 g

Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 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

Ca DL-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 6.8-7.0

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized.

114. AZOSPIRILLUM BRASILIENSE MEDIUM 2

Ca-malate 10.0 g or

Glucose 20.0 g

K₂HPO₄ 0.1 g

KH₂PO₄ 0.2 g

MgSO₄ × 7 H₂O 0.5 g

FeCl₃ × 6 H₂O 0.1 g

CaCl₂ × 2 H₂O 0.02 g

Na₂MoO₄ × 2 H₂O 0.02 mg

Yeast extract 0.1 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 6.9

Autoclave at 111°C for 30 min.

115. METHANOBACTERIUM MEDIUM

KH₂PO₄ 0.5 g

MgSO₄ × 7 H₂O 0.4 g

NaCl 0.4 g

NH₄Cl 0.4 g

CaCl₂ × 2 H₂O 0.05 g

Trace element solution *SL-10* (see below) 1.0 ml

Vitamin solution (see below) 5.0 ml

Yeast extract 1.0 g
Na-acetate 1.0 g
Na-formate 2.0 g
NaHCO₃ 4.0 g
Resazurin 1.0 mg
L-Cysteine-HCl 0.5 g
Na₂S × 9 H₂O 0.5 g
Fatty acid mixture (see below) 20.0 ml
Sludge water: (see below) 50.0 ml
Distilled water 940.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Fatty acid mixture:

Valeric acid 0.5 g
Isovaleric acid 0.5 g
 α -Methylbutyric acid 0.5 g
Isobutyric acid 0.5 g
Distilled water 20.0 ml

pH 7.5 (adjust with concentrated NaOH)

Sludge water: to sludge from an anaerobic digester add 0.4% yeast extract and after gassing with N₂ gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13,000 g and autoclave the resulting clear supernatant under N₂ gas at 121°C for 15 min (pH 6.7-7.0).

Base medium and trace element solution autoclave at 121°C for 15 min. Vitamin solution is filter sterilized. Prepare the medium anaerobically under a gas atmosphere of 80% H₂ and 20% CO₂.

116. THERMUS RUBER MEDIUM

Pepton 5.0 g
Yeast extract 1.0 g
Starch (soluble) 1.0 g
Agar 12.0 g
Distilled water 1000.0 ml

pH 8.0
Autoclave at 121°C for 15 min.

117. METHANOSARCINA MEDIUM

Solution 1:

NaCl 0.9 g
MgCl₂ × 6 H₂O 0.2 g
CaCl₂ × 2 H₂O 0.1 g
NH₄Cl 1.0 g
Yeast extract 2.0 g
Resazurin 10.0 mg
Methanol 10% 10.0 ml
Trace element solution (see below) 10.0 ml
Vitamin solution (see below) 5.0 ml

Distilled water 965.0 ml

Solution 2 (reducing agents):

L-Cysteine-HCl 0.5 g
Na₂S × 9 H₂O 0.5 g
Distilled water 10.0 ml

Buffer solutions:

a) K₂HPO₄ 29.0 g
Distilled water 100.0 ml
b) KH₂PO₄ 15.0 g
Distilled water 100.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 g
FeSO₄ × 7 H₂O 0.1 g
MnCl₂ × 6 H₂O 0.1 g
CoCl₂ × 2 H₂O 0.17 g
CaCl₂ × 2 H₂O 0.1 g
ZnCl₂ 0.1 g
CuCl₂ × 2 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NaCl 1.0 g
Na₂SeO₄ 0.017 g
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N₂ without O₂ up to sterilization.
Solutions of reducing agents (10.0 ml) and of buffer (per 1 ml) add to base medium after

separate sterilization. Base medium, trace elements, buffer solutions and reducing agents autoclave at 121°C for 15 min. Vitamin solution is filter sterilized.

118. PLATE COUNT AGAR

Tryptone 5.0 g
Yeast extract 2.5 g
Glucose 1.0 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 7.0±0.2
Autoclave at 121°C for 15 min.

119. NITROSOLOBUS MEDIUM 2

(NH₄)₂SO₄ 0.5 g
KH₂PO₄ 0.2 g
CaCl₂ × 2 H₂O 0.02 g
MgSO₄ × 7 H₂O 0.04 g
EDTA FeNa 3.8% 0.1 ml
Phenol red 0.05% 2.0 ml
Distilled water 1000.0 ml
pH 8.0 (adjust with 6% Na₂CO₃)
Autoclave at 121°C for 15 min.

120. MANNITOL AGAR WITH PEPTONE

Yeast extract 5.0 g
Mannitol 25.0 g
Peptone 3.0 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 5.0-6.0
Autoclave at 121°C for 15 min.

121. THIOBACILLUS THIOOXIDANS MEDIUM (WAKSMAN MEDIUM)

(NH₄)₂SO₄ 300.0 mg
MgSO₄ × 7 H₂O 500.0 mg
KH₂PO₄ 3.5 g
CaCl₂ × 2 H₂O 250.0 mg
FeSO₄ × 7 H₂O 18.0 mg
Finely dispersed sulfur 5.0 g
Distilled water 1000.0 ml
pH 4.5
Autoclave at 121°C for 15 min.

122. THIOBACILLUS FERROOXIDANS MEDIUM (LEATHEN MEDIUM)

(NH₄)₂SO₄ 150.0 mg
KCl 50.0 mg
MgSO₄ × 7 H₂O 500.0 mg
KH₂PO₄ 100.0 mg
Ca(NO₃)₂ × 4 H₂O 10.0 mg
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.

After sterilization of the medium, add 10.0 ml of 10% $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ preliminarily acidified to pH 3.5 and autoclaved separately in sealed ampoules under nitrogen. pH 4.0 (adjust after sterilization and addition of iron solution).

123. MEDIUM FOR MARINE *NITROBACTER*

Solution 1 (see below) 0.5 ml

Solution 2 (see below) 0.5 ml

Solution 3 (see below) 1.0 ml

Solution 4 (see below) 0.5 ml

Solution 5 (see below) 0.5 ml

Solution 6 (see below) 0.1 ml

Distilled water 300.0 ml

Sea water 700.0 ml

Solution 1:

CaCl_2 2.0 g

Distilled water 100.0 ml

Solution 2:

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 20.0 g

Distilled water 100.0 ml

Solution 3:

Chelated iron 0.1 g

Distilled water 100.0 ml

Solution 4:

Na_2MoO_4 0.1 g

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 0.2 g

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 0.02 g

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 0.02 g

Distilled water 1000.0 ml

Solution 5:

NaNO_3 41.4 g

Distilled water 100.0 ml

Solution 6:

K_2HPO_4 1.74 g

Distilled water 100.0 ml

pH 8.6 (adjust with NaOH or KOH)

Autoclave all solutions separately at 121°C for 15 min. Leave the medium to stand for 2–3 days so that pH can adjust itself to pH 7.4–7.6.

124. *AZOSPIRILLUM* MEDIUM

Solution 1:

Yeast extract 0.05 g

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 0.01 g

K_2HPO_4 0.25 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 1.0 mg

$\text{MnSO}_4 \times \text{H}_2\text{O}$ 2.0 mg

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.2 g

NaCl 0.1 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.02 g

$(\text{NH}_4)_2\text{SO}_4$ 1.0 g

Biotin 0.1 mg

Bromothymol blue 25.0 mg

Distilled water 950.0 ml

Solution 2:

Glucose 20% 25.0 ml

Solution 3:

Na-malate 20% 25.0 ml

Dissolve bromothymol blue in diluted KOH before adding into the medium.

pH 7.1

Autoclave solutions separately at 121°C for 15 min.

125. NITROSOLOBUS MEDIUM 3

(NH₄)₂SO₄ 2.0 g

CaCl₂ × 2 H₂O 20.0 mg

MgSO₄ × 7 H₂O 200.0 mg

Chelated iron 1.0 mg

Na₂MoO₄ × 2 H₂O 100.0 mg

MnCl₂ × 4 H₂O 200.0 mg

CoCl₂ × 6 H₂O 2.0 mg

ZnSO₄ × 7 H₂O 100.0 mg

K₂HPO₄ 15.9 mg

CuSO₄ × 5 H₂O 20.0 mg

Distilled water 1000.0 ml

pH 7.5-7.8

Autoclave at 121°C for 15 min.

126. DESULFOCOCCUS NIACINI MEDIUM

Solution 1:

Na₂SO₄ 3.0 g

KH₂PO₄ 0.2 g

NH₄Cl 0.3 g

NaCl 13.5 g

MgCl₂ × 6 H₂O 2.2 g

KCl 0.5 g

CaCl₂ × 2 H₂O 0.15 g

Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g

Distilled water 100.0 ml

Solution 4:

Na-nicotinate 5.0 mM

Solution 5:

Vitamin solution (see below) 10.0 ml

Solution 6:

Na₂S × 9 H₂O 0.4 g

Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Na₂SeO₃ × 5 H₂O 40.0 mg
Distilled water 990.0 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
Ca DL-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Trace element solution is autoclaved at 121°C for 15 min. Vitamin solution is filter sterilized.

Final pH of the complete medium 7.4

127. SELENITE CONTROL MEDIUM

Solution 1:

Peptone 5.0 g
Na₂HPO₄ 7.0 g
NaH₂PO₄ 3.0 g
Lactose 4.0 g
Distilled water 960.0 ml
pH 6.9-7.1

Solution 2:

10% Na₂SeO₃ × 5 H₂O 40.0 ml
Autoclave at 111°C for 30 min.

128. HIRSCH MEDIUM

KH₂PO₄ 1.36 g
Na₂HPO₄ 2.15 g
MgSO₄ × 7 H₂O 0.2 g
MnSO₄ × 5 H₂O 1.05 g
(NH₄)₂SO₄ 0.5 g
CaCl₂ × 2 H₂O 5.97 mg
Na₂MoO₄ × 2 H₂O 1.5 mg
Methanol 5.0 ml
Distilled water to 1000.0 ml
pH 7.0

Autoclave at 121°C for 15 min. Sterilize methanol by filtration and add to the medium after autoclaving.

129. CONTROL MEDIUM C-1

KH₂PO₄ 2.0 g
K₂HPO₄ 2.0 g
MgSO₄ × 7 H₂O 0.2 g
NaCl 5.0 g
Raffinose 2.0 g
Bromothymol blue (1.6% alkaline) 2.0 ml
Crystal violet (0.01%) 20.0 ml
50% Urea 4.0 ml
Distilled water 975.0 ml
Autoclave at 111°C for 30 min. Urea solution is filter sterilized.

130. POTATO–GLUCOSE AGAR

Potato 200.0 g
Glucose 10.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.0
Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.
Autoclave at 111°C for 30 min.

131. MEDIUM Q MOD FOR *FRANKIA*

K₂HPO₄ 300.0 mg
NaH₂PO₄ 200.0 mg
MgSO₄ × 7 H₂O 200.0 mg
KCl 200.0 mg
Yeast extract 500.0 mg
Peptone 5.0 g
Glucose 10.0 g
Fe-citrate 1.0 ml
Trace element solution (see below) 1.0 ml
CaCO₃ 100.0 mg
Tween-80 2.0 g
Agar 15.0 g
Distilled water 1000.0 ml
Trace element solution:
H₃BO₃ 1.5 g
MnSO₄ × 7 H₂O 0.8 g
ZnSO₄ × 7 H₂O 0.6 g
CuSO₄ × 5 H₂O 0.1 g
(NH₄)₆Mo₇O₂₄ × 4 H₂O 0.2 g
CoSO₄ 0.01 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 111°C for 30 min.

132. *THIOBACILLUS FERROOXIDANS* MEDIUM 9K

Solution 1:
KCl 0.1 g
MgSO₄ × 7 H₂O 0.5 g
K₂HPO₄ 0.5 g

$\text{Ca}(\text{NO}_3)_2 \times 4 \text{H}_2\text{O}$ 0.01 g

Distilled water 700.0 ml

Solution 2:

$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ 63.0 g

H_2SO_4 (10 N) 1.0 ml

Distilled water 300.0 ml

pH 3.5

Autoclave solution 1 at 121°C for 15 min, solution 2 at 111°C 30 min. Mix the solutions before inoculation.

133. COLBY AND ZATMAN MEDIUM WITH METHANOL

K_2HPO_4 1.2 g

KH_2PO_4 0.62 g

$\text{CaCl}_2 \times 6 \text{H}_2\text{O}$ 0.05 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.2 g

NaCl 0.1 g

$\text{FeCl}_3 \times 6 \text{H}_2\text{O}$ 1.0 mg

$(\text{NH}_4)_2\text{SO}_4$ 0.5 g

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 5.0 µg

$\text{MnSO}_4 \times 5 \text{H}_2\text{O}$ 10.0 µg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 10.0 µg

H_3BO_3 10.0 µg

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 70.0 µg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 5.0 µg

Purified agar 15.0 g

Methanol 1.0 ml

Distilled water 1000.0 ml

pH 6.8

Autoclave at 121°C for 15 min. Methanol is filter sterilized.

134. METHYLOTROPH MEDIUM 1

KH_2PO_4 2.0 g

$(\text{NH}_4)_2\text{SO}_4$ 2.0 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.025 g

NaCl 0.5 g

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 0.02 g

Methanol 5.0 ml

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min. Methanol is filter sterilized. Methanol may be replacement 30 ml 10 % solution of methylamine, autoclave at 111°C for 30 min.

135. METHYLOTROPH MEDIUM 2

KH_2PO_4 0.8 g

$\text{Na}_2\text{HPO}_4 \times 12 \text{H}_2\text{O}$ 3.0 g

$(\text{NH}_4)_2\text{SO}_4$ 0.8 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 10.0 mg

Methanol 5.0 ml

Trace element solution (see below) 1.0 ml

Distilled water 1000.0 ml

Trace element solution:

CaCl₂ × 2 H₂O 1.25 g
CoCl₂ × 6 H₂O 1.25 g
MnSO₄ × 4 H₂O 0.1 g
ZnSO₄ × 7 H₂O 1.25 g
Na₂MoO₄ × 2 H₂O 50.0 mg
CuCl₂ × 2 H₂O 250.0 mg
Distilled water 250.0 ml
pH 7.0-7.2

Autoclave at 121°C for 15 min. Methanol is filter sterilized. Methanol may be replacement 30 ml 10 % solution of methylamine, autoclave at 111°C for 30 min.

136. MEDIUM FOR PERCHLORATE-REDUCING BACTERIA

Solution 1:

NH₄Cl 0.1 g
NaCl 0.02 g
KH₂PO₄ 0.1 g
K₂HPO₄ 0.4 g
MgSO₄ × 7 H₂O 0.1 g
Tap water 1000.0 ml

Solution 2:

HCl, to dissolve the precipitate in solution 1

Solution 3:

pH 6.9-7.2 (adjust with 5% NaOH)

Solution 4:

5% NH₄ClO₄ 5.0 ml

Solution 5:

5% Na-acetate 5.0 ml

Solution 6:

Trace element solution according to *Hogland* (see below) 0.5 ml

Solution 7:

Vitamin B₁₂ (dispensary solution) 0.2 ml

Solution 8:

96° ethanol 0.5 ml

Trace element solution according to Hogland:

EDTA 5.0 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ × 2 H₂O 10.0 mg
NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 20.0 mg
Distilled water 1000.0 ml

Sterilize ethanol by filtration, others solutions separately autoclave at 121°C for 15 min. Add solutions and additions to the main medium in the order of their enumeration.

137. MEDIUM FOR CHROMATE-REDUCING BACTERIA

Solution 1:

NH₄Cl 0.3 g
NaCl 0.1 g
KH₂PO₄ 0.5 g

K₂HPO₄ 0.3 g
MgSO₄ × 7 H₂O 0.1 g
CaCO₃ 0.05 g
FeCl₃ × 6 H₂O 0.05 g
K₂CrO₄ 0.1 g
Paper-filtered pond water 900.0 ml

Solution 2:

Peptone meat broth 100.0 ml

Solution 3:

5% Na-acetate 5.0 ml

Solution 4:

Trace element solution according to *Hogland* (see below) 0.5 ml

Solution 5:

Vitamin B₁₂ (dispensary solution) 0.2 ml

Trace element solution according to Hogland:

EDTA 5.0 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ × 2 H₂O 10.0 mg
NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 20.0 mg
Distilled water 1000.0 ml
pH 7.0-7.2

Autoclave solutions separately at 121°C for 15 min. Add solutions and additions to the main medium in the order of their enumeration.

138. LARSEN PHOTOTROPH MEDIUM

NH₄Cl 0.5 g
KH₂PO₄ 1.0 g
MgCl₂ 0.5 g
CaCl₂ × 2 H₂O 0.1 g
Trace element solution SL-12B (see below) 1.0 ml
NaHCO₃ 5.0 g
Na-acetate × 3 H₂O 2.0 g
Na₂S₂O₃ 0.4-0.6 g
Na₂S × 9 H₂O 0.1 g
Fe-citrate – traces
Distilled water 1000.0 ml

Trace element solution SL-12B:

EDTA Na 3.0 g
FeSO₄ × 7 H₂O 1.1 g
CoCl₂ × 6 H₂O 190.0 mg
MnCl₂ × 2 H₂O 50.0 mg
ZnCl₂ 42.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 18.0 mg
H₃BO₃ 300.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Distilled water 1000.0 ml

pH of the trace element solution 6.0

pH 8.4

Autoclave solutions separately at 121°C for 15 min.

139. POSTGATE MEDIUM B FOR SULFATE REDUCERS

Solution 1:

NaCl 1.0 g

KH₂PO₄ 0.5 g

NH₄Cl 1.0 g

CaSO₄ × 2 H₂O 1.0 g

MgSO₄ × 7 H₂O 2.0 g

Na-lactate 3.5 g

Yeast extract 1.0 g

Tap water 980.0 ml

Dissolve ingredients and sparge medium with O₂ free N₂ gas for 10-15 minutes

Solution 2:

Ascorbic acid 1.0 g

Thioglycolic acid 1.0 g

Distilled water 10.0 ml

Solution 3:

FeSO₄ × 7 H₂O 0.5 g

Distilled water 10.0 ml

pH 7.4

Autoclave solution 1 at 121°C for 15 min, solutions 2 and 3 separately autoclave at 111°C for 30 min.

140. DILUTED BRAIN HEART INFUSION MEDIUM (DILUTED BHI MEDIUM)

BHI broth (Pronadisa 1400) 3.7 g or

Peptone mixture 1.0 g

Beef heart infusion 1.0 g

Calf brain infusion 0.75 g

Glucose 0.2 g

Na₂HPO₄ 0.25 g

NaCl 0.5 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.4 ± 0.2

Autoclave at 121°C for 15 min.

141. CITRATE AGAR

NaCl 5.0 g

MgSO₄ × 7 H₂O 0.2 g

NH₄H₂PO₄ 1.0 g

K₂HPO₄ 1.0 g

Na-citrate 3.0 g

Bromothymol blue 0.08 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 121°C for 15 min.

142. PPYA

Potato decoction (see below) 200.0 ml

Peptone 5.0 g

Yeast extract 1.0 g

Agar 25.0 g

Distilled water to 1000.0 ml

pH 8.0.

Autoclave at 121°C for 15 min

Preparation of potato decoction: boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume. Do not use new potatoes.

143. PEPTONE MEAT AGAR WITH 1% GLYCEROL

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Glycerol 10.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0-7.2

Autoclave at 121°C for 15 min.

144. PEPTONE MEAT AGAR WITH 0.5% GLUCOSE

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Glucose 5.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0-7.2

Autoclave at 111°C for 30 min

145. PSEUDOMONAS SP. (ARTHROBACTER GLOBIFORMIS) MEDIUM

Glucose 10.0 g

Maize extract 10.0 g

Tap water 1000.0 ml

pH 7.8 (adjust with NH_4OH); paper filter.

Autoclave at 111°C for 30 min.

146. DILUTED TRYPTICASE SOY MEDIUM

Trypticase Soy Broth (BBL 11768, Oxoid CM129 or Merck 5459) 3.0 g or

Pancreatic digest of casein 1.8 g

Papaic digest of soyabean 0.6 g

NaCl 0.6 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.3

Autoclave at 121°C for 15 min.

147. HYPHOMICROBIUM MEDIUM

NaNO_3 1.0 g

NaCl 0.5 g

K_2HPO_4 1.0 g

MgSO₄ × 7 H₂O 0.2 g
Methanol 5.0-10.0 ml
Distilled water 1000.0 ml
pH 6.8-7.0
Autoclave at 121°C for 15 min. Methanol is filter sterilized.
Grow in an exicator in the presence of methanol vapors.

148. GLUCOSE YEAST CHALK MEDIUM

Beef extract 3.0 g
Yeast extract 10.0 g
Glucose 50.0 g
CaCO₃ 30.0 g
Agar 20.0 g
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.

149. CLAVIBACTER XYLI MEDIUM

Solution 1:

Flour agar 5.0 g
Papaya hydrolysate of soybean meal 8.0 g
K₂HPO₄ 1.0 g
KH₂PO₄ 1.0 g
MgSO₄ × 7 H₂O 0.2 g
Distilled water 965.0 ml

Solution 2:

Bovine hemine chloride (0.1% in 0.05 N NaOH) 15.0 ml

Solution 3:

Bovine serum albumin, fraction 5 (20%) 10.0 ml

Solution 4:

Glucose (50%) 1.0 ml

Solution 5:

Cysteine (10%) 10.0 ml

pH 6.6

Autoclave solution 1 at 121°C for 15 min, cool to 50 C and add the filter-sterilized solutions 2-5.

150. GETCHINSON MEDIUM WITH FILTER PAPER

K₂HPO₄ 1.3 g
MgSO₄ × 7 H₂O 0.3 g
CaCl₂ × 6 H₂O 0.1 g
FeCl₃ × 6 H₂O 0.01 g
NaNO₃ 2.5 g
Distilled water 1000.0 ml
pH 7.2-7.3.

Autoclave at 121°C for 15 min. Cut filter paper into strips, sterilize by dry heat and immerse into the medium so that they are not completely in the liquid medium.

151. MUNZ MEDIUM FOR METHANE-OXIDIZING BACTERIA

K₂HPO₄ 0.5 g
KH₂PO₄ 0.5 g
MgSO₄ × 7 H₂O 0.5 g
NH₄Cl 1.0 g
Tap water 1000.0 ml

pH 6.8
Autoclave at 121°C for 15 min.
Cultivate in the mixed atmosphere of air and methane (2:1).

152. TRYPTONE THIOGLYCOLATE MEDIUM

Solution 1:

K₂HPO₄ 5.45 g
KH₂PO₄ 1.2 g
MgSO₄ × 7 H₂O 25.0 mg
CaCl₂ × 2 H₂O 15.0 mg
FeSO₄ × 7 H₂O 10.0 mg
MnCl₂ × 4 H₂O 2.0 mg
CoCl₂ × 6 H₂O 2.5 mg
Na₂MoO₄ × 2 H₂O 2.5 mg
Peptone 2.0 g
Tryptone 2.0 g
Yeast extract 6.0 g
Na-thioglycolate 0.5 g
Distilled water 950.0 ml

Solution 2:

Glucose 20.0 g
Distilled water 50.0 ml
pH 7.5

Autoclave solution 1 at 121°C for 15 min, solution 2 at 111°C for 30 min.

153. MEDIUM P-2 FOR THERMOPHILIC ANAEROBIC BACTERIA

K₂HPO₄ 3.0 g
KH₂PO₄ 2.0 g
NH₄Cl 2.0 g
MgCl₂ × 6 H₂O 0.2 g
CaCl₂ × 2 H₂O 0.05 g
Tryptone 10.0 g
Glucose 5.0 g
Yeast extract 5.0 g
Resazurin 0.01 g
Distilled water 1000.0 ml
pH 7.0-7.2

Prepare medium anaerobically under N₂ and autoclave at 121°C for 15 min. Autoclave glucose separately under N₂ at 111°C for 30 min.

154. DESULFOVIBRIO SULFODISMUTANS MEDIUM

Solution 1:

KH₂PO₄ 0.2 g
NH₄Cl 0.3 g
NaCl 1.0 g
MgCl₂ × 6 H₂O 0.4 g
KCl 0.5 g
CaCl₂ × 2 H₂O 0.15 g
Distilled water 920.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 2.5 g

Distilled water 50.0 ml

Solution 4:

Na-acetate × 3 H₂O 0.3 g

Distilled water 10.0 ml

Solution 5:

D(+)-Biotin 10.0 mg

Ca-D(+)-Pantothenate 50.0 mg

Distilled water 1.0 ml

Solution 6:

Na₂S × 9 H₂O 0.4 g

Distilled water 10.0 ml

Solution 7:

Na₂S₂O₃ (0.5 M) 10.0 ml

pH 7.5-8.0 (adjust with NaOH)

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically at under 80% N₂ + 20% CO₂ at 121°C for 15 min. Solutions 2, 4, 6 and 7 are gassed with N₂ and autoclaved separately at 121°C for 15 min.

Solution 3 (gassed with N₂ + CO₂) and solution 5 (gassed with N₂) are filter-sterilized. Solutions with 2 to 7 are added to the sterile, cooled solution 1 in the sequence as indicated. When growth has started feed culture again with same amount of solution 7. After a further two days repeat feeding once more.

155. SCHATZ AND BOVELL MEDIUM FOR HYDROGEN-OXIDIZING BACTERIA

KH₂PO₄ 1.0 g

NH₄NO₂ 1.0 g

MgSO₄ × 7 H₂O 0.2 g

FeSO₄ × 7 H₂O 0.01 g

CaCl₂ × 2 H₂O 0.01 g

NaHCO₃ 0.5 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 6.8-7.2

Autoclave at 121°C for 15 min. Cultivate in a gas mixture of carbon dioxide, air and hydrogen (1:3:6).

156. MEDIUM FOR MYXOBACTERIA

Casein hydrolysate 2.5 g

Asparagine 2.5 g

K₂HPO₄ 2.0 g

NaCl 1.0 g

MgSO₄ × 7 H₂O 0.1 g
FeSO₄ × 7 H₂O 0.03 g
CaCl₂ × 2 H₂O 0.01 g
Distilled water 1000.0 ml
pH 7.4
Autoclave at 121°C for 15 min.

157. EMERSON STARCH YEAST EXTRACT AGAR

Yeast extract 4.0 g
Starch (soluble) 15.0 g
KH₂PO₄ 1.0 g
MgSO₄ × 7 H₂O 0.5 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

158. GLYCEROL YEAST AGAR

Yeast extract 5.0 g
Glycerol 50.0 g
CaCO₃ 1.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0-7.2
Autoclave at 121°C for 15 min.

159. 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 at 121°C for 15 min. Cool the slants in a sloping position. Cover solid slants with 2 ml sterile tap water. Inoculate into the covering tap water.

160. MEAT GLUCOSE MEDIUM

Peptone 10.0 g
Glucose 10.0 g
Beef extract 3.0 g
NaCl 5.0 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 111°C for 30 min.

161. RHIZOBIUM MEDIUM

Yeast extract 1.0 g
Mannitol 10.0 g
Agar 15.0 g
Soil extract (see below) 200.0 ml
Distilled water 800.0 ml
Soil extract:
Air-dried garden soil 80.0 g
Na₂CO₃ 0.2 g

Distilled water 200.0 ml
Soil extract autoclave at 121°C for 1 h. filter and make up to 200 ml.
pH 7.2
Autoclave at 121°C for 15 min.

162. *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
pH 7.0-7.2
Autoclave at 111°C for 30 min.

163. *MICROCYCLUS* MEDIUM

Glucose 5.0 g
Peptone 5.0 g
Yeast extract 5.0 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 6.8
Autoclave at 111°C for 30 min.

164. PD BROTH FOR *FLEXIBACTER*

Peptone 1.0 g
KNO₃ 100.0 mg
Yeast extract 100.0 mg
K₂HPO₄ 66.7 mg
MgSO₄ × 7 H₂O 33.3 mg
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml
Trace element solution:
Zn SO₄ × 7 H₂O 22.0 mg
MnSO₄ × 7 H₂O 1.81 g
CuSO₄ × 5 H₂O 79.0 mg
NaBO₃ × 4 H₂O 1.0 g
(NH₄)₆Mo₇O₂₄ × 4 H₂O 9.3 g
FeSO₄ × 7 H₂O 20.0 mg
Co(NO₃)₂ × H₂O 20.0 mg
Trilon B 10.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 111°C for 30 min.

165. INDICATOR MEDIUM WITH MALONATE

Yeast extract 1.0 g
(NH₄)₂SO₄ 2.0 g
KH₂PO₄ 0.4 g
K₂HPO₄ 0.6 g
NaCl 2.0 g
Na-malonate 3.0 g
Glucose 0.25 g

Bromothymol blue (0.2%) 12.0 ml

Distilled water 1000.0 ml

Dissolve components of the medium in boiling water in the specified sequence (except the indicator). Then filter through a cotton wool-gauze filter to remove the possible precipitate, bring to the initial volume, cool, adjust pH 6.7, add the indicator. Autoclave at 121°C for 15 min.

166. 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

pH 7.2-7.4

Autoclave at 111°C for 30 min.

167. PEPTONE MEAT AGAR WITH 3% NaCl

Peptone 10.0 g

Beef extract 3.0 g

NaCl 30.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2-7.4

Autoclave at 121°C for 15 min.

168. CORYNEBACTERIUM MEDIUM WITH 6% NaCl

Casein peptone, tryptic digest 10.0 g

Yeast extract 5.0 g

Glucose 5.0 g

NaCl 60.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2-7.4

Autoclave at 121°C for 15 min.

169. HETEROTROPHIC MEDIUM H3P

Solution 1:

KH₂PO₄ 2.3 g

Na₂HPO₄ × 2 H₂O 2.9 g

Distilled water 50.0 ml

Solution 2:

NH₄Cl 1.0 g

MgSO₄ × 7 H₂O 0.5 g

CaCl₂ × 2 H₂O 0.01 g

MnCl₂ × 4 H₂O 0.05 g

NaVO₃ × H₂O 0.05 g

Trace element solution SL-6 (see below) 5.0 ml

Agar (if necessary) 15.0 g

Distilled water 850.0 ml

Solution 3:

Fe(NH₄)-citrate 0.05 g

Distilled water 20.0 ml

Solution 4:

Yeast extract 1.0 g
Na-acetate $\times 3 \text{ H}_2\text{O}$ 1.0 g
Na₂-succinate 1.0 g
DL-Malate 1.0 g
Distilled water 30.0 ml
pH 7.0

Solution 5:

Na-lactate 1.0 g
Na-pyruvate 1.0 g
D-Mannitol 1.0 g
D-Glucose 2.0 g
Distilled water 50.0 ml
pH 7.0

Trace element solution SL-6:

ZnSO₄ $\times 7 \text{ H}_2\text{O}$ 0.1 g
MnCl₂ $\times 4 \text{ H}_2\text{O}$ 0.03 g
H₃BO₃ 0.3 g
CoCl₂ $\times 6 \text{ H}_2\text{O}$ 0.2 g
CuCl₂ $\times 2 \text{ H}_2\text{O}$ 0.01 g
NiCl₂ $\times 6 \text{ H}_2\text{O}$ 0.02 g
Na₂MoO₄ 0.03 g
Distilled water 1000.0 ml

Vitamin solution:

Riboflavin 10.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 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 B₁₂ 1.0 mg
Distilled water 100.0 ml

Solutions 1, 2, 3, 4 and trace element solution are autoclaved separately for 15 min at 121°C, cooled down to 50°C and then mixed aseptically with filter-sterilized solution 5 (at 50°C) and 5.0 ml of filter-sterilized standard vitamin solution. The final pH of this medium should be 6.8 without adjustment.

170. CYTOPHAGA MEDIUM

Yeast extract 10.0 g
NH₄Cl 1.0 g
MgSO₄ $\times 7 \text{ H}_2\text{O}$ 1.0 g
K₂HPO₄ 0.2 g
NaCl 20.0 g
FeCl₃ $\times 6 \text{ H}_2\text{O}$ Traces
Agar (if necessary) 2.0-3.0 g
Distilled water 1000.0 ml
pH 7.5
Autoclave at 121°C for 15 min.

171. YEAST AGAR

K₂HPO₄ 1.0 g

MgSO₄ × 7 H₂O 0.5 g
Yeast extract 10.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.0-7.2
Autoclave at 121°C for 15 min.

172. PEPTONE SUCCINATE AGAR

(NH₄)₂SO₄ 1.0 g
MgSO₄ × 7 H₂O 1.0 g
MnSO₄ × 5 H₂O 2.0 mg
FeCl₃ × 6 H₂O 2.0 mg
Succinic acid 1.68 g
Peptone 5.0 g
Agar 1.5 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

173. SPIRILLUM GRACILLE MEDIUM

Peptone 5.0 g
Yeast extract 0.5 g
Tween 80 0.02 g
K₂HPO₄ 0.1 g
Agar (if needed) 15.0 g
Tap water 1000.0 ml
pH to 7.2
Autoclave at 121°C for 15 min.

174. MEDIUM FOR DENTRIFYING BACTERIA (GILTAY MEDIUM)

Solution 1:

KNO₃ 1.0 g
Asparagine 1.0 g
Distilled water 250.0 ml

Solution 2:

Ca-citrate 8.5 g
KH₂PO₄ 1.0 g
Distilled water 500.0 ml

Solution 3:

MgSO₄ × 7 H₂O 1.0 g
FeCl₂ × 4 H₂O traces
CaCl₂ × 2 H₂O 0.2 g
Distilled water 250.0 ml

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

175. AQUASPIRILLUM MEDIUM 1

Peptone 2.0 g
Succinic acid 1.0 g
(NH₄)₂SO₄ 1.0 g
MgSO₄ × 7 H₂O 1.0 g
CaCl₂ × 2 H₂O 0.03 g
Vitamin solution (see below) 1.0 ml

Trace elements (see below) 1.0 ml

Agar (if needed) 15.0 g

Distilled water 1000.0 ml

Adjust pH to 7.4-7.6. Autoclave main medium and trace elements solution separately at 121°C for 15 min and add 1.0 ml/l vitamin solution from a filter sterilized stock solution.

Trace elements solution (Pfennig & Lippert, 1966):

EDTA 5.0 g

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 2.2 g

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.03 g

H_3BO_3 0.03 g

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 g

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 0.03 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.03 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.03 g

Distilled water 1000.0 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 B₁₂ 1.0 mg

p-Aminobenzoic acid 50.0 mg

Distilled water 1000.0 ml

176. METHANOBACTERIUM MEDIUM

Solution 1:

NaCl 0.9 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 g

NH_4Cl 1.0 g

Yeast extract 2.0 g

Resazurin 0.01 g

Trace element solution (see below) 10.0 ml

Vitamin solution (see below) 5.0 ml

Buffer solution a (see below) 10.0 ml

Buffer solution b (see below) 10.0 ml

Distilled water 965.0 ml

Solution 2 (reducing agents):

L-Cysteine-HCl $\times \text{H}_2\text{O}$ 0.5 g

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.5 g

Distilled water 10.0 ml

Buffer solutions:

a) K_2HPO_4 29.0 g

Distilled water 100.0 ml

b) KH_2PO_4 15.0 g

Distilled water 100.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 mg
FeSO₄ × 7 H₂O 0.1 mg
MnCl₂ × 6 H₂O 0.1 mg
CoCl₂ × 2 H₂O 0.17 mg
CaCl₂ × 2 H₂O 0.1 mg
ZnCl₂ 0.1 mg
CuCl₂ × 2 H₂O 0.02 mg
H₃BO₃ 0.01 mg
Na₂MoO₄ × 2 H₂O 0.01 mg
NaCl 1.0 mg
Na₂SeO₄ 0.017 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N₂ without O₂ up to sterilization. Solutions of reducing agents (10.0 ml) and others solutions add to base medium after separate autoclaving at 121°C for 15 min. Sterilize vitamin solution by filtration. Cultivate in a gas mixture of 80% H₂ and 20% CO₂.

177. CYTOPHAGA LYTICA MEDIUM

Starch (soluble) 10.0 g
Yeast extract 1.0 g
Beef extract 1.0 g
Pancreatic casein hydrolysate 2.0 g
CoCl₂ × 6 H₂O 0.02 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.3
Autoclave at 121°C for 15 min.

178. MEDIUM FOR MARINE CYTOPHAGA

Solution 1:

Yeast extract 1.0 g
Tryptone 1.0 g
KCl 0.7 g
NaCl 24.7 g
MgSO₄ × 7 H₂O 6.3 g
MgCl₂ × 6 H₂O 4.6 g
Agar 15.0 g
Distilled water 950.0 ml
Solution 2:

CaCl₂ × 2 H₂O 1.2 g

Distilled water 25.0 ml

Solution 3:

NaHCO₃ 0.2 g

Distilled water 25.0 ml

pH 7.2

Autoclave solutions separately at 121°C for 15 min and mix aseptically.

179. SELENOMONAS RUMINANTIUM MEDIUM

Glucose 1.0 g

Trypticase 5.0 g

KH₂PO₄ 1.0 g

Na-acetate × 3 H₂O 4.0 g

Yeast extract 2.0 g

Valeric acid 0.1 ml

Resazurin 1.0 mg

Na₂CO₃ 4.0 g

L-Cysteine-HCl × H₂O 0.5 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 111°C for 30 min.

Gas atmosphere: 100% CO₂.

180. DESULFOVIBRIO CARBINOLICUS MEDIUM

Solution 1:

Na₂SO₄ 3.0 g

KH₂PO₄ 0.2 g

NH₄Cl 0.3 g

NaCl 1.0 g

MgCl₂ × 6 H₂O 0.4 g

KCl 0.5 g

CaCl₂ × 2 H₂O 0.15 g

Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g

Distilled water 100.0 ml

Solution 4:

Ethanol 0.7 g

Casamino acids 0.1 g

Yeast extract 0.1 g

Distilled water 10.0 ml

Solution 5:

Vitamin solution (see below) 10.0 ml

Solution 6:

Na₂S × 9 H₂O 0.4 g

Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 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
Ca DL-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized. Others solutions autoclave at 121°C for 15 min and add to base medium aseptically.

Final pH of the complete medium 7.1-7.4

181. CASEIN-CITRATE AGAR

Casein hydrolysate 7.5 g
Yeast extract 10.0 g
MgSO₄ × 7 H₂O 20.0 g
Na-citrate 3.0 g
KCl 2.0 g
NaCl 200.0 g
4.98% FeSO₄ in 0.01 N HCl 1.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.4
Autoclave at 121°C for 15 min.

182. DESULFOBACTERIUM MEDIUM

Solution 1:

Na₂SO₄ 3.0 g
KH₂PO₄ 0.2 g
NH₄Cl 0.3 g
NaCl 21.0 g
MgCl₂ × 6 H₂O 3.0 g
KCl 0.5 g
CaCl₂ × 2 H₂O 0.15 g
Resazurin 1.0 mg
Distilled water 930.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

Vitamin solution (see below) 10.0 ml

Solution 4:

NaHCO₃ 2.5 g

Distilled water 50.0 ml

Solution 5:

Na₂SeO₃ × 5 H₂O (3 mg in 1000.0 ml 0.01 M NaOH) 1.0 ml

Solution 6:

Na₂S × 9 H₂O 0.4 g

Distilled water 10.0 ml

Solution 7:

Substrate - depending on the species of bacteria:

25% Na-acetate 10.0 ml or indole 0.3 g

NaCl 2.1 g

MgCl₂ × 6 H₂O 0.3 g

Distilled water 100.0 ml

Phenol 40.0 mg

or Na-benzoate 400.0 mg

Distilled water 4.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

p-Aminobenzoic acid 4.0 mg

D(+)-Biotin 1.0 mg

Thiamine-HCl 10.0 mg

Distilled water 100.0 ml

pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically under 80% N₂ + 20% CO₂ at 121°C for 15

min. Solutions 2, 4, 5 and 6 are gassed with N₂ and autoclaved separately at 121°C for 15 min.

Solution 3 (gassed with N₂ + CO₂) and solution 7 (gassed with N₂) are filter-sterilized.

183. BENETT MEDIUM

Yeast extract 1.0 g

Meat extract 1.0 g

Fermentative casein hydrolysate 2.0 g

Glucose 10.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 111°C for 30 min.

184. ISP 2 MEDIUM

Glucose 4.0 g

Yeast extract 4.0 g
Malt extract 10.0 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 111°C for 30 min.

185. HALOBACTERIUM MEDIUM 4

Yeast extract 5.0 g
Casamino acids 5.0 g
Na-glutamate 1.0 g
KCl 2.0 g
Na-citrate 3.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 20.0 g
NaCl 200.0 g
 $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$ 36.0 mg
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 0.36 mg
Agar 20.0 g
Distilled water to 1000.0 ml
pH 7.0-7.2
Autoclave at 111°C for 30 min.

186. HALOBACTERIUM MEDIUM 5

Solution 1:

Casamino acids 7.5 g
Yeast extract 10.0 g
Na-citrate 3.0 g
KCl 2.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 20.0 g
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 0.05 g
 $\text{MnSO}_4 \times \text{H}_2\text{O}$ 0.2 g
NaCl 250.0 g
Distilled water 750.0 ml

Solution 2:

Agar 20.0 g
Distilled water 250.0 ml
pH 7.4
Autoclave separately at 121°C for 15 min and mix aseptically.

187. HALOCOCCUS MEDIUM

Solution 1:

Skim milk 50.0 g
Distilled water 500.0 ml

Solution 2:

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 10.0 g
 KNO_3 2.0 g
NaCl 200.0 g
Fe-citrate traces
Distilled water 100.0 ml

Solution 3:

Neopeptone 5.0 g
Glycerol 10.0 g

Agar 25.0 g
Distilled water 400.0 ml
Autoclave of solution 1 at 111°C for 15 min. Mix together heated solutions 2 and 3, adjust pH of the mixture to 8.4 and autoclave at 121°C for 15 min.

188. NATRONOBACTERIUM MEDIUM

Solution 1:

KH₂PO₄ 1.0 g
KCl 1.0 g
NH₄Cl 1.0 g
MgSO₄ × 7 H₂O 0.24 g
CaSO₄ × 2 H₂O 0.17 g
Trace element solution *SL-10* (see below) 1.0 ml
NaCl 200.0 g
Glutamate 1.0 g
Yeast extract 5.0 g
Casamino acids 5.0 g
Agar, if necessary (heat and dissolve it before adding sodium chloride) 20.0 g
Distilled water to 1000.0 ml
pH 6.5

Solution 2:

Na₂CO₃ 5.0 g
Distilled water 50.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml
pH 9.0-9.5

Autoclave separately at 121°C for 15 min and mix aseptically.

189. HALOBACTERIUM MEDIUM 6

NaCl 156.0 g
MgCl₂ × 6 H₂O 13.0 g
MgSO₄ × 7 H₂O 20.0 g
CaCl₂ × 2 H₂O 1.0 g
KCl 4.0 g
NaHCO₃ 0.2 g
NaBr 0.5 g
Yeast extract 5.0 g
Glucose 1.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 111°C for 30 min.

190. PRAUSER MEDIUM 79

Glucose 10.0 g
Peptone 10.0 g
Yeast extract 2.0 g
Casamino acids 2.0 g
NaCl 6.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.5
Autoclave at 111°C for 30 min.

191. STARCH-YEAST AGAR

Yeast extract 2.0 g
Starch (soluble) 10.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.3
Autoclave at 121°C for 15 min.

192. MYA-AGAR

Glucose 2.0 g
L-Asparagine 1.0 g
K₂HPO₄ 0.5 g
MgSO₄ × 7 H₂O 0.5 g
Trace element solution (see below) 1.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
Trace element solution:
FeSO₄ × 7 H₂O 0.1 g
CuSO₄ × 5 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.1 g
Distilled water 100.0 ml
pH 7.4
Autoclave base medium at 111°C for 30 min, trace element solution at 121°C for 15 min.

193. ACETATE AGAR

NaCl 5.0 g
K₂HPO₄ 1.0 g
NH₄H₂PO₄ 1.0 g
MgSO₄ × 7 H₂O 0.2 g
Na-acetate × 3 H₂O 2.0 g
Bromothymol blue (0.2%) 40.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2
Add the indicator last, after pH is set and the possible precipitate is separated by filtration through a cotton wool-gauze filter.
Autoclave at 121°C for 15 min.

194. INMI MEDIUM 3

NaCl 250.0 g
KCl 2.0 g

Na-citrate 3.0 g
MgSO₄ × 7 H₂O 20.0 g
Casamino acids 5.0 g
Yeast extract 2.5 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 121°C for 15 min.

195. INMI MEDIUM 4

Yeast extract 2.5 g
Casamino acids 5.0 g
pH 9.5
Autoclave at 121°C for 15 min.

196. MEDIUM FOR PURPLE BACTERIA (VAN NIEL MEDIUM)

NH₄Cl 1.0 g
K₂HPO₄ 0.5 g
MgCl₂ × 6 H₂O 0.2 g
NaHCO₃ 1.0 g
Na₂S × 9 H₂O 1.0 g
Tap water 1000.0 ml
pH 7.6
Autoclave base medium and sulfide separately at 121°C for 15 min.

197. MEDIUM FOR RHODOSPIRILLUM (PFENNIG MEDIUM)

NH₄Cl 0.4 g
KH₂PO₄ 0.5 g
MgSO₄ × 7 H₂O 0.2 g
NaCl 0.4 g
CaCl₂ × 2 H₂O 0.05 g
Acetate, or butyrate, or propionate, or succinate 1.0 g
Yeast extract 0.2 g
Fe-citrate (0.1%) 5.0 ml
Trace element solution according to *Pfennig* (see below) 1.0 ml
Vitamin B₁₂ (dispensary solution, 0,01%) 1.0 ml
Distilled water 1000.0 ml
Trace element solution according to Pfennig:
EDTA 1.5 g
Trace element solution according to *Hogland* (see below) 6.0 ml
FeSO₄ × 7 H₂O 0.2 g
MnCl₂ × 4 H₂O 0.02 g
ZnSO₄ × 7 H₂O 0.1 g
Distilled water 1000.0 ml
Trace element solution according to Hogland:
EDTA 5.0 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ × 2 H₂O 10.0 mg

NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 20.0 mg
Distilled water 1000.0 ml
Autoclave base medium and trace element solution separately at 121°C for 15 min.

198. THIOBACILLUS DENITRIFICANS MEDIUM (TAYLOR MEDIUM)

KNO₃ 2.0 g
NH₄Cl 1.0 g
KH₂PO₄ 2.0 g
NaHCO₃ 2.0 g
MgSO₄ × 7 H₂O 0.8 g
Na₂S₂O₃ × 5 H₂O 5.0 g
Trace element solution according to *Pfennig* (see below) 1.0 ml
Distilled water 1000.0 ml
Trace element solution according to Pfennig:
EDTA 1.5 g
Trace element solution according to *Hogland* (see below) 6.0 ml
FeSO₄ × 7 H₂O 0.2 g
MnCl₂ × 4 H₂O 0.02 g
ZnSO₄ × 7 H₂O 0.1 g
Distilled water 1000.0 ml
Trace element solution according to Hogland:
EDTA 5.0 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ × 2 H₂O 10.0 mg
NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 20.0 mg
Distilled water 1000.0 ml
Autoclave base medium and trace element solution separately at 121°C for 15 min.

199. THIOBACILLUS DENITRIFICANS MEDIUM (BAALSRUD MEDIUM)

KNO₃ 2.0 g
NH₄Cl 0.5 g
KH₂PO₄ 2.0 g
NaHCO₃ 1.0 g
MgCl₂ × 6 H₂O 0.5 g
Na₂S₂O₃ × 5 H₂O 5.0 g
FeSO₄ × 7 H₂O 0.01 g
Distilled water 1000.0 ml
pH 7.0
Autoclave stock solutions of iron, phosphorus and bicarbonate salts separately at 121°C for 15 min.

200. THIOBACILLUS DENITRIFICANS MEDIUM (LIESKE MEDIUM)

KNO₃ 5.0 g
KH₂PO₄ 0.2 g
NaHCO₃ 1.0 g
MgCl₂ × 6 H₂O 0.1 g

$\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ 5.0 g
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ Traces
 $\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$ Traces
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.

201. MODIFIED BROCK MEDIUM FOR SULFUROXIDIZING BACTERIA

$(\text{NH}_4)_2\text{SO}_4$ 1.3 g
 KH_2PO_4 0.37 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.25 g
 $\text{CaCl}_2 \times 6 \text{ H}_2\text{O}$ 0.07 g
Distilled water 1000.0 ml
pH 2.4 (range: 2.3-3.0; adjust with H_2SO_4).
Additions to the medium
Trace element solution according to *Pfennig* (see below) 1.0 ml
Yeast extract 0.2 g
Element sulfur 10.0 g
Chalk (CaCO_3) 10.0 g

For cultivation of heterotrophic representatives of the group the medium after sterilization is to be also supplemented with

Peptone 1.75 g
Sucrose 0.25 g

Trace element solution according to Pfennig:

EDTA 1.5 g
Trace element solution according to *Hogland* (see below) 6.0 ml
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.2 g
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.02 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
Distilled water 1000.0 ml

Trace element solution according to Hogland:

EDTA 5.0 g
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 2.0 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 100.0 mg
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 30.0 mg
 H_3BO_3 300.0 mg
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 200.0 mg
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 10.0 mg
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 20.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 20.0 mg
Distilled water 1000.0 ml

Autoclave base medium and others components of medium separately at 121°C for 15 min.

202. MEDIUM FOR MAGNETIC BACTERIA

Tartaric acid 0.37 g
Succinic acid 0.37 g
 $\text{Na-acetate} \times 3 \text{ H}_2\text{O}$ 0.05 g
 KH_2PO_4 0.68 g
 NaNO_3 0.12 g
Vitamin solution (see below) 10.0 ml
Trace element solution (see below) 5.0 ml
Fe-quinat 2.0 ml
Na-thioglycolate 0.05 g

Resazurin 0.5 mg
Agar 1.3 g
Bidistilled water 1000.0 ml
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 B₁₂ 1.0 mg
p-Aminobenzoic acid 50.0 mg
Distilled water 1000.0 ml

Trace element solution:
Nitrilotriacetic acid 12.8 g
FeCl₂ × 4 H₂O 0.2 g
MnCl₂ × 4 H₂O 0.1 g
CoCl₂ × 6 H₂O 0.17 g
CaCl₂ × 2 H₂O 0.1 g
ZnCl₂ 0.1 g
CuCl₂ × 2 H₂O 0.02 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.026 g
NaCl 1.0 g
Na₂SeO₃ × 5 H₂O 0.02 g
Distilled water 1000.0 ml
pH 6.75 (adjust with NaOH)

Prior to sterilization the medium is to be blown down by the flow of N₂ and autoclaved in the nitrogen atmosphere at 121°C for 15 min. Vitamin solution is filter sterilized. Trace element solution autoclave at 121°C for 15 min and add to base medium aseptically.

203. MEDIUM FOR *FLEXIBACTER* (LEWIN MEDIUM)

Na-glycerophosphate 0.1 g
KNO₃ 0.1 g
MgSO₄ × 7 H₂O 0.1 g
Trace element solution (see below) 1.0 ml
Vitamin B₁₂ 1.0 mg
CaCl₂ × 2 H₂O 0.1 g
Tris 1.0 g
Thiamine 1.0 mg
Casamino acids 1.0 g
Distilled water 1000.0 ml
Trace element solution:
Nitrilotriacetic acid 12.8 g
FeCl₂ × 4 H₂O 0.2 g
MnCl₂ × 4 H₂O 0.1 g
CoCl₂ × 6 H₂O 0.17 g
CaCl₂ × 2 H₂O 0.1 g
ZnCl₂ 0.1 g
CuCl₂ × 2 H₂O 0.02 g
Na₂MoO₄ × 2 H₂O 0.01 g

NiCl₂ × 6 H₂O 0.026 g
NaCl 1.0 g
Na₂SeO₃ × 5 H₂O 0.02 g
Distilled water 1000.0 ml
pH 7.5

Autoclave base medium and trace element solution separately at 121°C for 15 min.

204. MEDIUM FOR *CLOSTRIDIUM*

Glucose 10.0 g
Peptone 12.0 g
NaCl 2.0 g
Agar 16.0 g
Distilled water 1000.0 ml
The medium can be used with chalk addition as buffer against acidification of the medium during cultivation.
pH to 6.8-7.2
Autoclave at 111°C for 30 min.

205. OATMEAL AGAR A

Oatmeal or oat flakes 20.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH to 7.2
Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.
Autoclave at 121°C 15 min.

206. ISP MEDIUM 3

Oatmeal 20.0 g
Salt solution A (see below) 1.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
Salt solution A:
FeSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.1 g
Distilled water 100.0 ml
Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.
pH 7.2
Autoclave separately at 121°C 15 min.

207. MINERAL AGAR 1

Starch (soluble) 20.0 g
K₂HPO₄ 0.5 g
MgSO₄ × 7 H₂O 0.5 g
KNO₃ 1.0 g
NaCl 0.5 g
FeSO₄ × 7 H₂O 0.01 g
Agar 30.0 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C 15 min.

208. GLUCOSE ASPARAGINE AGAR

Glucose 10.0 g
L-Asparagine 0.5 g
 K_2HPO_4 0.5 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 6.8.
Autoclave at 111°C for 30 min.

209. MODIFICATION OF THE CZAPEK MEDIUM WITH STARCH

Starch (soluble) 20.0 g
 K_2HPO_4 0.5 g
 KNO_3 1.0 g
NaCl 0.2 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.3 g
 CaCO_3 0.5 g
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.01 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 121°C 15 min.

210. GLYCEROL-ASPARAGINE AGAR

L-Asparagine 1.0 g
Glycerol 10.0 g
 K_2HPO_4 1.0 g
Salt solution A (see below) 1.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
Salt solution A:
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.1 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
Distilled water 100.0 ml
pH 7.0-7.4
Autoclave separately at 121°C 15 min.

211. OATMEAL AGAR WITH 0.1% YEAST EXTRACT

Oatmeal 20.0 g
Yeast extract 1.0 g
Agar 20.0 g
Tap water 1000.0 ml
pH 7.2
Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.
Autoclave at 121°C 15 min.

212. MODIFICATION OF CZAPEK MEDIUM WITH GLUCOSE

Glucose 20.0 g
 K_2HPO_4 0.5 g
 KNO_3 1.0 g
NaCl 0.2 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.3 g

CaCO₃ 0.5 g
FeSO₄ × 7 H₂O 0.01 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2-7.4
Autoclave at 111°C for 30 min.

213. ORGANIC AGAR 2

Hottinger broth (HiMedia M1425) 0.69 g
Peptone 5.0 g
NaCl 5.0 g
Glucose 10.0 g
Agar 30.0 g
Tap water 1000.0 ml
pH 7.0-7.2
Autoclave at 111°C for 30 min.

214. STARCH AMMONIA AGAR

Starch (soluble) 10.0 g
K₂HPO₄ 1.0 g
MgSO₄ × 7 H₂O 1.0 g
NaCl 1.0 g
(NH₄)₂SO₄ 2.0 g
CaCO₃ 2.0 g
Salt solution A (see below) 1.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
Salt solution A:
FeSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.1 g
Distilled water 100.0 ml
pH 7.0-7.4
Autoclave separately at 121°C 15 min.

215. GLYCEROL NITRATE AGAR

Glycerol 30.0 g
NaNO₃ 2.0 g
K₂HPO₄ 1.0 g
MgSO₄ × 7 H₂O 0.5 g
KCl 0.5 g
FeSO₄ × 7 H₂O 0.01 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0-7.2.
Autoclave at 121°C for 15 min.

216. TETRATHIONATE BROTH (MULLER MEDIUM)

Solution 1:

Hottinger broth (HiMedia M1425) 20.24 g
Distilled water 880.0 ml

Solution 2:

Lugol solution (see below) 20.0 ml

Solution 3:

50% $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ 100.0 ml

Lugol solution:

KI 20.0 g

I_2 25.0 g

Distilled water 100.0 ml

pH 7.2 - 7.4

Autoclave solution 3 at 121°C 15 min. Lugol solution is filter sterilized. Pour the medium into sterile vials with CaCO_3 (25 g CaCO_3 per 1000.0 ml of medium). Sterilize the flasks with CaCO_3 with dry heat (170 C for 60 min).

217. MEDIUM FOR MICROMONOSPORES

Glucose 10.0 g

Starch (soluble) 20.0 g

Yeast extract 5.0 g

Fermentative casein hydrolysate 5.0 g

CaCO_3 1.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 111°C for 30 min.

218. PYA WITH MARINE WATER

Peptone 5.0 g

Yeast extract 3.0 g

Agar 12.0 g

Distilled water 250.0 ml

Aged filtered sea water 750.0 ml

pH 7.5-7.6

Autoclave at 121°C 15 min.

219. MEDIUM FOR HALOPHILIC BACILLI

NaCl 100.0 g

NaHCO_3 10.0 g

Na_2CO_3 10.0 g

Nutrient broth 1000.0 ml

pH 9.5

Autoclave at 121°C 15 min.

220. PYEA MEDIUM

Peptone 10.0 g

Yeast extract 10.0 g

NaCl 5.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Autoclave at 121°C 15 min.

221. MEDIUM FOR METHANOTROPHIC BACTERIA

KNO_3 1.0 g

KH_2PO_4 0.7 g

MgSO₄ × 7 H₂O 0.2 g
CaCl₂ × 2 H₂O 0.02 g
Na₂HPO₄ × 12 H₂O 1.5 g
EDTA 5.0 mg
FeSO₄ × 7 H₂O 2.0 mg
ZnSO₄ × 7 H₂O 0.1 mg
MnCl₂ × 4 H₂O 0.03 mg
CoCl₂ × 6 H₂O 0.2 mg
CuCl₂ × 2 H₂O 0.1 mg
NiCl₂ × 6 H₂O 0.02 mg
Na₂MoO₄ × 2 H₂O 0.03 mg
Distilled water 1000.0 ml
pH 6.7-7.1
Autoclave at 121°C 15 min.
Cultivation under mixture of methane and air (1:1).

222. MEDIUM FOR MARINE METHYLOTROPHIC BACTERIA

KH₂PO₄ 2.0 g
(NH₄)₂SO₄ 2.0 g
NaCl 30.0 g
MgSO₄ × 7 H₂O 0.025 g
FeSO₄ × 7 H₂O 0.02 g
Yeast extract 0.1 g
Methanol 5.0 ml
Biotin 0.01 mg
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C 15 min. After autoclaving add filter sterilize methanol and biotin.

223. MEDIUM FOR OLIGOCARBOPHILIC BACTERIA

Solution 1 (basic solution):

Peptone 0.25 g
Yeast extract 0.25 g
Agar 5.0 g
Solution 2 20.0 ml
Distilled water 965.0 ml
Glucose (2.5 %) 10.0 ml
Solution 3 5.0 ml
pH 7.5

Solution 2 (trace elements):

Nitrilotriacetic acid 10.0 g
MgSO₄ × 7 H₂O 29.7 g
CaCl₂ × 2 H₂O 3.34 g
Na₂MoO₄ × 2 H₂O 12.67 mg
FeSO₄ × 7 H₂O 350.0 mg
Na-EDTA 125.0 mg
ZnSO₄ × 7 H₂O 548.0 mg
MnSO₄ × H₂O 77.0 mg
CuSO₄ × 5 H₂O 20.0 mg
Co(NO₃)₂ × 6 H₂O 12.4 mg
Na₂B₄O₇ × 10 H₂O 8.8 mg
Distilled water 950.0 ml

Dissolve nitrilotriacetic acid first by neutralizing with KOH, then add other salts. Adjust volume to 1000.0 ml.

Adjust pH to 7.2

Solution 3 (vitamin solution):

Biotin 4.0 mg

Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg

Riboflavine 10.0 mg

Thiamine-HCl 10.0 mg

Nicotin amide 10.0 mg

Ca-D-pantothenate 10.0 mg

Vitamin B₁₂ 0.2 mg

p-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

pH 6.8–7.0

Autoclave glucose stock solution at 111°C for 30 min, solutions 1 and 2 at 121°C 15 min.

Vitamin solution is filter sterilized. Store in refrigerator at +5 C.

224. MEDIUM FOR *DESULFOTOMACULUM ALKALIPHILUM*

Solution 1 (basic solution):

Na₂CO₃ 0.5 g

Na₂SO₄ 5.0 g

NaCl 5.0 g

Na formate 5.0 g

Yeast extract 1.0 g

Solution 2 (see below) 10.0 ml

Solution 3 (see below) 2.0 ml

Solution 4 (see below) 1.0 ml

Rezazurine traces

Distilled water 1000.0 ml

NaHCO₃ final concentration 8.0 g /1000.0ml

Na₂S × 9 H₂O final concentration 0.5 g/1000.0 ml

Solution 2:

KH₂PO₄ 0.2 g

MgCl₂ × 6 H₂O 0.1 g

NH₄Cl 1.0 g

KCl 0.2 g

Distilled water 1000.0 ml

Solution 3 (vitamin solution):

Biotin 10.0 mg

Folic acid 10.0 mg

Pyridoxine-HCl 50.0 mg

Riboflavine 25.0 mg

Thiamine-HBr 25.0 mg

Nicotin amide 25.0 mg

D-pantothenate 25.0 mg

Vitamin B₁₂ 0.5 mg

p-Aminobenzoic acid 25.0 mg

Thioctic acid 25.0 mg

Distilled water 500.0 ml

Store in refrigerator at +5°C.

Solution 4 (trace elements):

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 20.0 mg
 $\text{Fe} (\text{NH}_4)_2(\text{SO}_4)_2 \times 6 \text{ H}_2\text{O}$ 400.0 mg
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 200.0 mg
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 200.0 mg
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 720.0 mg
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 100.0 mg
 $\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$ 20.0 mg
 $\text{AlK}(\text{SO}_4)_2 \times 12 \text{ H}_2\text{O}$ 20.0 mg
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 200.0 mg
 H_3BO_3 20.0 mg
 HCl 5.0 ml
Distilled water 200.0 ml
pH of the autoclaved medium is 8.7-9.0
Vitamin solution is filter sterilized. Others solutions autoclave separately at 121°C for 15 min and add to the Solution 1 after cooling.

225. 1/5 STARCH-YEAST AGAR

Yeast extract 0.4 g
Soluble starch 2.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.3
Autoclave at 121°C 15 min.

226. MEDIUM FOR *ACTINOPOLYSPORA MORTIVALLIS*

Bacto vitamin assay casamino acids 7.5 g
Yeast extract 10.0 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 20.0 g
Trisodium citrate $\times 2 \text{ H}_2\text{O}$ 3.0 g
 KCl 2.0 g
 NaCl 150.0 g
4.98% FeSO_4 in 0.01 N HCl 1.0 ml
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.4
Autoclave at 121°C 15 min.

227. *MICROLUNATUS* MEDIUM

Glucose 0.5 g
Peptone 0.5 g
Yeast extract 0.5 g
Na- glutamate 0.5 g
 KH_2PO_4 0.44 g
 $(\text{NH}_4)_2\text{SO}_4$ 0.1 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 111°C for 30 min.

228. *MICROCOCCUS HALOPHILUS* MEDIUM

Peptone 10.0 g
Yeast extract 5.0 g

Malt extract 5.0 g
Casamino acids 5.0 g
Meat extract 2.0 g
Glycerol 2.0 g
Tween-80 50.0 mg
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 1.0 g
NaCl 50.0 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 121°C 15 min.

229. ALKALIBACTER MEDIUM

$(\text{NH}_4)_2\text{SO}_4$ 1.0 g
 NH_4Cl 0.4 g
 $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ 0.1 g
 K_2HPO_4 0.5 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.05 g
NaCl 10.0 g
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 2.0 mg
Trace element solution (see below) 10.0 ml
Resazurin 0.01 g
Yeast extract 0.25 g
Tryptone 2.0 g
Glucose 5.0 g
Vitamin solution (see below) 10.0 ml
L-cysteine 0.5 g
 Na_2CO_3 (5% w/v) 50.0 ml
Distilled water 930.0 ml
Trace element solution:
Nitrilotriacetic acid 12.8 mg
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 mg
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.1 mg
 $\text{CoCl}_2 \times 2 \text{ H}_2\text{O}$ 0.17 mg
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 mg
 ZnCl_2 0.1 mg
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 0.02 mg
 H_3BO_3 0.01 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.01 mg
NaCl 1.0 mg
 Na_2SeO_4 0.017 mg
Distilled water 1000.0 ml
Vitamin solution:
Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg

Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
Basal medium autoclave at 111°C for 30 min. Vitamin solution is filter sterilized. Others solutions autoclave at 121°C for 15 min (Na₂CO₃ is autoclaved under 100% N₂).
Final pH 9.0

230. CALDITHRIX ABYSSI MEDIUM

Sea salt 37.9 g
NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
Resazurin 0.5 mg
Yeast extract (20% w/v) 15.0 ml
Trace element solution (see below) 10.0 ml
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O (3% w/v) 20.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Distilled water 900.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 mg
FeSO₄ × 7 H₂O 0.1 mg
MnCl₂ × 4 H₂O 0.1 mg
CoCl₂ × 2 H₂O 0.17 mg
CaCl₂ × 2 H₂O 0.1 mg
ZnCl₂ 0.1 mg
CuCl₂ × 2 H₂O 0.02 mg
H₃BO₃ 0.01 mg
Na₂MoO₄ × 2 H₂O 0.01 mg
NaCl 1.0 mg
Na₂SeO₄ 0.017 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3 mg
Na₂WO₄ × 2 H₂O 4 mg
Distilled water 1000.0 ml

Prepare medium anaerobically under 80% N₂ + 20% CO₂ gas mixture. After autoclaving add from separately prepared, sterile anaerobic stock solutions NaHCO₃ (5% w/v) 50.0 ml. Vitamin solution is filter sterilized. Others solutions autoclave separately at 121°C for 15 min.
Final pH of the medium to 6.8

231. CARBOXYDOCELLA SPOROPRODUCENS MEDIUM

KCl 0.33 g
MgCl₂ × 6 H₂O 0.52 g
CaCl₂ × 2 H₂O 0.29 g
NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
NaHCO₃ 1.0 g
Trace element solution *SL-4* (see below) 10.0 ml

Resazurin 0.5 mg
Vitamin solution (see below) 10.0 ml

Pyruvate 2.5 g
Yeast extract 0.05 g
Na₂S × 9 H₂O 0.3 g
Distilled water 1000.0 ml

Trace element solution SL-4:

EDTA 0.5 g
FeSO₄ × 7 H₂O 0.2 g
Trace element solution *SL-6* (see below) 100.0 ml
Distilled water 900.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Trace element solution SL-6:

ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.3 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.02 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, yeast extract, vitamins, pyruvate and sulfide, boil medium for 1 min, then cool to room temperature under N₂ gas atmosphere. Dispense medium under same gas atmosphere in tubes or serum bottles and autoclave at 121°C for 15 min. Add vitamins (sterilized by filtration), yeast extract, pyruvate and sulfide from sterile anoxic stock solutions prepared under N₂ gas atmosphere and bicarbonate from a sterile anoxic solution prepared under 80% N₂ and 20% CO₂ gas mixture (all solutions autoclave separately at 121°C 15 min). Adjust pH to 7.0 with a sterile anoxic solution of 10% (w/v) NaHCO₃. Inoculated vessels are pressurized with carbon monoxide gas to 2 bar overpressure.

232. CARBOXYDOTHERMUS FERRIREDUCENS MEDIUM

KH₂PO₄ 0.33 g

NH₄Cl 0.33 g
KCl 0.33 g
MgCl₂ × 6 H₂O 0.33 g
CaCl₂ × 2 H₂O 0.33 g
NaHCO₃ 2.0 g
Glycerol (87%) 3.0 ml
Vitamin solution (see below) 10.0 ml
Trace element solution *SL-10* (see below) 1.0 ml
NiCl₂ × 6 H₂O 200.0 µg
Na₂SeO₃ × 5 H₂O 120.0 µg
Na₂WO₄ × 2 H₂O 30.0 µg
Yeast extract 1.0 g
Na₂-9,10-anthraquinone-2,6-disulfonate (Sigma A9706) 8.25 g
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Dissolve ingredients (except CaCl₂ × 2 H₂O, NaHCO₃, and vitamins), boil medium for some minutes to dissolve the anthraquinone, then cool under 80% N₂ and 20% CO₂ gas atmosphere to room temperature. Add solid NaHCO₃ and adjust medium pH to 6.8 with NaOH. Dispense medium in tubes or bottles under same gas. Autoclave at 121°C for 15 min. Before use, add CaCl₂ × 2 H₂O and vitamins from anoxic, sterile stock solutions. Vitamin solution is filter sterilized. CaCl₂ × 2 H₂O and trace element solution Autoclave at 121°C for 15 min

233. CLOSTRIDIUM ALKALICELLULOSI MEDIUM

NH₄Cl 0.5 g
KH₂PO₄ 0.2 g
MgCl₂ × 6 H₂O 0.1 g
KCl 0.2 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Na₂CO₃ 1.0 g

NaHCO₃ 7.6 g
NaCl 10.0 g
Yeast extract 0.2 g
Cellobiose 3.0 g
Na₂S × 9 H₂O 0.5 g

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3 mg
Na₂WO₄ × 2 H₂O 4 mg
Distilled water 1000.0 ml

Dissolve ingredients except cellobiose and sulfide, flush medium with N₂ gas for 30–60 min, dispense under N₂ gas atmosphere and autoclave at 121°C 15 min. Add cellobiose after autoclaving from an anoxic stock solution sterilized by filtration and sulfide from a sterile (Autoclave at 121°C 15 min), anoxic stock solution prepared under N₂.
Final pH of the medium 8.8-9.0.

234. DESULFOHALOBIUM UTAHENSE MEDIUM

NaCl 100.0 g
MgSO₄ × 7 H₂O 10.0 g
KCl 6.0 g
CaCl₂ × 2 H₂O 0.4 g
NH₄Cl 1.0 g
KH₂PO₄ 0.1 g
Yeast extract 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
NaHCO₃ 4.0 g
Na-(L)-lactate 2.5 g
Resazurin 0.5 mg
Na₂S × 9 H₂O 0.3 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ 3 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 4 mg

Distilled water 1000.0 ml

Dissolve ingredients (except lactate, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Dispense under same gas atmosphere in culture vessels and autoclave at 121°C 15 min. Add sodium lactate and sulfide from sterile anoxic stock solutions prepared under N_2 and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 (all solutions sterilize separately at 121°C 15 min).

Final pH of the medium 7.0-7.2

235. *DESULFONATRONUM COOPERATIVUM* MEDIUM

KH_2PO_4 0.2 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.1 g

NH_4Cl 1.0 g

KCl 0.2 g

Na_2SO_4 5.0 g

NaCl 10.0 g

Na_2CO_3 3.5 g

Trace element solution *SL-10* (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Yeast extract 1.0 g

Resazurin 0.5 mg

Na-formate 4.0 g

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 10.0 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

p-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

Vitamin B_{12} 0.1 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve the ingredients (except formate, vitamin solution and sulfide) and flush medium with 100 % N₂ for 30 min. Add the sodium sulfide, adjust the pH to 8.8-9.0, dispense in Hungate tubes under N₂, and autoclave at 121°C 15 min. Before use add sodium formate and vitamin solution from a sterile, anaerobic stock solution (autoclave sodium formate 121°C 15 min and vitamin solution by filtration).

236. *DESULFOTOMACULUM CARBOXYDIVORANS* MEDIUM

Solution 1:

NaCl 1.17 g
MgCl₂ × 6 H₂O 0.4 g
KCl 0.3 g
CaCl₂ × 2 H₂O 0.15 g
NH₄Cl 0.27 g
KH₂PO₄ 0.2 g
Na₂SO₄ 2.84 g
Na-pyruvate 2.2 g
Yeast extract 0.5 g
Vitamin solution (see below) 1.0 ml
Trace element solution 1.0 ml (see below)
Distilled water 940.0 ml

Solution 2:

Na₂S × 9 H₂O 0.36 g
Distilled water 10.0 ml

Sodium bicarbonate for alkalization:

NaHCO₃ 4.5 g
Distilled water 50.0 g

Vitamin solution:

p-Aminobenzoic acid 4.0 mg
D(+)-Biotin 1.0 mg
Thiamine-HCl 10.0 mg
Distilled water 100.0 ml

Trace element solution:

FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 68.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 62.0 mg
CoCl₂ × 6 H₂O 120.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
HCl (0.05 M) 1000.0 ml
pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9 - 7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution and Na-pyruvate is filter sterilized.

237. *DESULFUROCOCCUS FERMENTANS* MEDIUM

NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.44 g
MgCl₂ × 6 H₂O 0.7 g
NaCl 0.5 g

Trace elements *SL-10* (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Yeast extract 0.2 g

Starch 5.0 g

Resazurin 1.0 mg

NaHCO₃ 0.8 g

Na₂S × 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 10.0 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

p-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

Vitamin B₁₂ 0.1 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except vitamins, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N₂ and 20% CO₂ gas atmosphere. Adjust pH to 6.2-6.4 and autoclave at 121°C 15 min. After autoclaving add vitamins from an anoxic stock solution sterilized by filtration and bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture (autoclave at 121°C 15 min). Prior to inoculation reduce medium by adding sulfide from a sterile, anoxic stock solution prepared under N₂ (autoclave at 121°C 15 min).

238. AQUASPIRILLUM MEDIUM II

(NH₄)₂SO₄ 1.0 g

MgSO₄ × 7 H₂O 1.0 g

CaCl₂ × 2 H₂O 30.0 mg

Na₂HPO₄ 10.0 mg

Casamino acids 1.5 g

Sodium succinate (10% solution) 10.0 ml

Na₂S₂O₃ × 5 H₂O (10% solution) 1.0 ml

Vitamin solution (see below) 5.0 ml

Trace element solution *SL-10* (see below) 1.0 ml

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 B₁₂ 1.0 mg
p-Aminobenzoic acid 50.0 mg
Distilled water 1000.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml
Agar 0.5 g
Distilled water 985.0 ml
pH 7.5

Basal medium, trace element solution autoclave separately at 121°C 15 min. Vitamin solution sterilize by filtration.

239. METHYLOPHAGA ALCALICA MEDIUM

KH₂PO₄ 1.0 g
KNO₃ 1.0 g
MgSO₄ × 7 H₂O 0.22 g
NaCl 30.0 g
Na₂CO₃ 5.0 g
Trace element solution (see below) 1.0 ml
Vitamin B₁₂ 0.02 mg
Methanol 10.0 ml
Distilled water 1000.0 ml
Final pH 9.5

Trace elements solution:

Ferric citrate 30.0 mg
CaCl₂ × 2 H₂O 30.0 mg
MgCl₂ × 4 H₂O 5.0 mg
ZnSO₄ × 7 H₂O 5.0 mg
CuSO₄ × 5 H₂O 0.5 g
Distilled water 1000.0 ml

Prepare the medium without the Na₂CO₃, vitamin B₁₂ and methanol. Basal medium, trace element and Na₂CO₃ autoclave separately at 121°C 15 min. Add the vitamin B₁₂ solution from a filter-sterilized stock solution and filter-sterilized methanol to the cooled medium. When preparing liquid media cool the mineral salts solution and Na₂CO₃ to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool the Na₂CO₃ stock solution and agar to 50-55°C before mixing.

240. METHYLOTHERMUS THERMALIS MEDIUM

KNO₃ 0.25 g

NH₄Cl 0.25 g
KH₂PO₄ 0.13 g
Na₂HPO₄ × 12 H₂O 0.358 g
MgSO₄ × 7 H₂O 0.4 g
CaCl₂ × 2 H₂O 0.1 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 6.8
The gas phase methane/air mixture (4:1)
Autoclave at 121°C 15 min.

241. OCEANITHERMUS PROFUNDUS MEDIUM

NH₄Cl 0.33 g
MgCl₂ × 6 H₂O 0.33 g
CaCl₂ × 2 H₂O 0.33 g
KCl 0.33 g
KNO₃ 0.33 g
NaCl 30.0 g
HEPES 2.38 g
Yeast extract 0.2 g
Tryptone 1.0 g
Sucrose 2.0 g
Vitamin solution (see below) 1.0 ml
Trace elements (see below) 1.0 ml
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 mg
FeSO₄ × 7 H₂O 0.1 mg
MnCl₂ × 4 H₂O 0.1 mg
CoCl₂ × 2 H₂O 0.17 mg
CaCl₂ × 2 H₂O 0.1 mg
ZnCl₂ 0.1 mg
CuCl₂ × 2 H₂O 0.02 mg
H₃BO₃ 0.01 mg
Na₂MoO₄ × 2 H₂O 0.01 mg
NaCl 1.0 mg
Na₂SeO₄ 0.017 mg
Distilled water 1000.0 ml

Prepare the medium anaerobically, under N₂, omitting the CaCl₂ × 2 H₂O, MgCl₂ × 6 H₂O, KNO₃, 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 N_2 and autoclave at 121°C 15 min. To the sterile, cooled medium add, from sterile stock solutions the $CaCl_2 \times 2 H_2O$, $MgCl_2 \times 6 H_2O$, KNO_3 , tryptone, yeast extract, vitamins and sucrose. The $CaCl_2 \times 2 H_2O$, $MgCl_2 \times 6 H_2O$, KNO_3 , tryptone, and yeast extract, stock solutions should be autoclaved at 121°C 15 min, sucrose at 111° 30 min, while the vitamin solution is sterile filtered.

242. *PSYCHROBACTER* MEDIUM

Peptone 5.0 g
Yeast extract 1.0 g
Sea salts 17.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 121°C 15 min.

243. *ROSEICYCLUS* MEDIUM

KH_2PO_4 0.3 g
 $MgSO_4 \times 7 H_2O$ 2.0 g
 NH_4Cl 0.3 g
KCl 0.3 g
 $CaCl_2 \times 2 H_2O$ 0.05 g
 Na_2SO_4 15.0 g
 $NaHCO_3$ 0.5 g
Na-acetate $\times 3 H_2O$ 1.0 g
Na-malate 1.0 g
Yeast extract 1.0 g
Peptone 0.5 g
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.8-8.0
Autoclave at 121°C 15 min.

244. *SULFOBACILLUS* MEDIUM

Solution A:

$(NH_4)_2SO_4$ 3.0 g
KCl 0.1 g
 K_2HPO_4 0.5 g
 $MgSO_4 \times 7 H_2O$ 0.5 g
 $Ca(NO_3)_2$ 0.01 g
Distilled water 680.0 ml
pH 2.0-2.2 (adjust with sulfuric acid)

Solution B:

$FeSO_4 \times 7 H_2O$ 44.2 g
Distilled water 300.0 ml
 H_2SO_4 (10 N) 1.0 ml

Solution C:

Yeast extract (1% w/v in water) 20.0 ml
pH 1.9-2.4

After autoclaving at 121°C 15 min, combine the three solutions.

245. *THERMINCOLA* MEDIUM

NH_4Cl 1.0 g

MgCl₂ × 6 H₂O 0.33 g
CaCl₂ × 2 H₂O 0.1 g
KCl 0.33 g
KH₂PO₄ 0.5 g
Resazurin 0.5 mg
Wolfe's mineral elixir (see below) 1.0 ml
Vitamin solution (see below) 20.0 ml

NaHCO₃ 0.5 g
Na₂CO₃ 0.5 g
Na-acetate × 3 H₂O 0.2 g
Yeast extract 0.2 g
Na₂S × 9 H₂O 1.0 g
Distilled water 1000.0 ml

Wolfe's mineral elixir:

MgSO₄ × 7 H₂O 30.0 g
MnSO₄ × H₂O 5.0 g
NaCl 10.0 g
FeSO₄ × 7 H₂O 1.0 g
CoCl₂ × 6 H₂O 1.8 g
CaCl₂ × 2 H₂O 1.0 g
ZnSO₄ × 7 H₂O 1.8 g
CuSO₄ × 5 H₂O 0.1 g
KAl(SO₄)₂ × 12 H₂O 0.18 g
H₃BO₃ 0.1 g
Na₂MoO₄ × 2 H₂O 0.1 g
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 2.8 g
Na₂WO₄ × 2 H₂O 0.1 g
Na₂SeO₄ 0.1 g
Distilled water 1000.0 ml

First adjust pH to 1.0 with diluted H₂SO₄, then add and dissolve the salts.

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except carbonates, vitamin solution, yeast extract and sulfide, boil medium for 1 min, then cool to room temperature under N₂ gas atmosphere. Add carbonates and sulfide to the medium, dispense under CO gas atmosphere in culture vessels (e.g., 10.0 ml medium in 50 ml serum bottles) and autoclave at 121°C 15 min. Prior to inoculation add yeast extract from a sterile, anoxic stock solution prepared under N₂ and adjust pH of final medium to 8.0 with a sterile, anoxic solution of 1 N HCl (this solutions autoclave at 121°C 15 min). Add filter-sterilized vitamin solution.

246. VULCANITHERMUS MEDIUM

NH₄Cl 0.33 g

MgCl₂ × 6 H₂O 0.33 g
CaCl₂ × 2 H₂O 0.33 g
KCl 0.33 g
KNO₃ 0.33 g
NaCl 25.0 g
PIPES 3.6 g
Yeast extract 0.5 g
Tryptone 1.0 g
Sucrose 1.0 g
Vitamin solution (see below) 1.0 ml
Trace elements solution (see below) 1.0 ml
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 mg
FeSO₄ × 7 H₂O 0.1 mg
MnCl₂ × 4 H₂O 0.1 mg
CoCl₂ × 2 H₂O 0.17 mg
CaCl₂ × 2 H₂O 0.1 mg
ZnCl₂ 0.1 mg
CuCl₂ × 2 H₂O 0.02 mg
H₃BO₃ 0.01 mg
Na₂MoO₄ × 2 H₂O 0.01 mg
NaCl 1.0 mg
Na₂SeO₄ 0.017 mg
Distilled water 1000.0 ml

pH 6.8

Prepare the medium anaerobically, under N₂, omitting the CaCl₂ × 2 H₂O, MgCl₂ × 6 H₂O, KNO₃, tryptone, yeast extract, vitamins and sucrose. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of N₂ and autoclave at 121°C 15 min. To the sterile, cooled medium add, from sterile stock solutions the CaCl₂ × 2 H₂O, MgCl₂ × 6 H₂O, KNO₃, tryptone, yeast extract, vitamins and sucrose. The CaCl₂ × 2 H₂O, MgCl₂ × 6 H₂O, KNO₃, tryptone, and yeast extract, stock solutions should be autoclaved at 121°C 15 min, sucrose at 111°C 30 min, while the vitamin solution is sterile filtered.

247. RHODOBLASTUS MEDIUM

Yeast extract 0.1 g
Na₂-succinate 1.0 g
KH₂PO₄ 0.5 g
MgSO₄ × 7 H₂O 0.4 g
NaCl 0.4 g

NH₄Cl 0.4 g
CaCl₂ × 2 H₂O 0.05 g
Trace element solution SL-6 (see below) 1.0 ml
Methanol 0.01-1%
Distilled water 1000.0 ml
pH 5.7

Trace element solution SL-6:

ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.3 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.02 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml

Autoclave basal medium and trace element solution separately at 121°C 15 min, methanol sterilize by filtration.

248. PFENNIG'S MEDIUM

KH₂PO₄ 0.34 g
NH₄Cl 0.34 g
MgSO₄ × 7 H₂O 0.5 g
CaCl₂ × 2 H₂O 0.05 g
KCl 0.34 g
Trace element solution SLA (see below) 1.0 ml
Vitamin B₁₂ 20 µg
NaHCO₃ 1.5 g
Na₂S × 9 H₂O 0.4 g
Na₂S₂O₃ × 5 H₂O 0.5 g
NaCl 15.0 g
MgCl₂ × 6 H₂O 2.5 g
pH 7.5

Trace element solution SLA:

FeCl₂ × 4 H₂O 1.8 mg
CoCl₂ × 6 H₂O 250 mg
NiCl₂ × 6 H₂O 10 mg
CuCl₂ × 2 H₂O 10 mg
MnCl₂ × 4 H₂O 70 mg
ZnCl₂ 100 mg
H₃BO₃ 500 mg
Na₂MoO₄ × 2 H₂O 30 mg
Na₂SeO₃ × 5 H₂O 10 mg
Distilled water 1000.0 ml

Autoclave basal medium and trace element solution separately at 121°C 15 min, vitamin B₁₂ sterilize by filtration.

249. METHYLOPHAGA MURALIS MEDIUM

KH₂PO₄ 2.0 g
(NH₄)₂SO₄ 2.0 g
MgSO₄ × 7 H₂O 0.025 g
NaCl 30.0 g
FeSO₄ × 7 H₂O 0.02 g

Methanol 5.0 ml
Vitamin B₁₂ 20.0 µg
Distilled water 1000.0 ml
pH 8.0
Autoclave at 121°C for 15 min. Methanol and vitamin B₁₂ added from stock solution (sterilized by filtration) after autoclaving.

250. MODIFIED MEDIUM FOR MARINE METHYLOTROPHIC BACTERIA WITH YEAST EXTRACT AND BIOTIN

KH₂PO₄ 2.0 g
(NH₄)₂SO₄ 2.0 g
MgSO₄ × 7 H₂O 0.025 g
FeSO₄ × 7 H₂O 0.02 g
Yeast extract 0.1 g
Methanol 10.0 ml
Biotin 0.01 mg
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C 15 min, methanol and biotin sterilize by filtration.

251. CHELATIVORANS OLIGOTROPHICUS MEDIUM

Na₄EDTA 1.0 g
Na₂HPO₄ × 12 H₂O 4.0 g
KH₂PO₄ 0.26 g
MgSO₄ × 7 H₂O 1.0 g
CaCl₂ × 2 H₂O 0.4 g
Trace element solution (see below) 2.0 ml
Vitamin solution (see below) 1.0 ml
Distilled water 1000.0 ml
pH 7.0

Trace element solution:

FeCl₂ × 4 H₂O 1.5 g
H₃BO₃ 60.0 mg
MnCl₂ × 4 H₂O 100.0 mg
CoCl₂ × 6 H₂O 120.0 mg
ZnCl₂ 70.0 mg
NiCl₂ × 6 H₂O 25.0 mg
CuCl₂ × 2 H₂O 15.0 mg
Na₂MoO₄ × 2 H₂O 25.0 mg
HCl, 0.05 molar 1000.0 ml

Vitamin solution:

Pyridoxine-HCl 100.0 mg
Thiamine-HCl × 2 H₂O 50.0 mg
Riboflavin 50.0 mg
Nicotinic acid 50.0 mg
Pantothenic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Lipoic acid 50.0 mg
Nicotine amide 50.0 mg
Vitamin B₁₂ 50.0 µg
Biotin 20.0 mg
Folic acid 20.0 mg

Distilled water 1000.0 ml

Autoclave at 121°C 15 min, vitamin solution stir for some hours and sterilize by filtration.

252. MODIFIED METHYLOTROPH MEDIUM 1 WITH KCNS

KH₂PO₄ 2.0 g

(NH₄)₂SO₄ 2.0 g

MgSO₄ × 7 H₂O 0.025 g

NaCl 0.5 g

KCNS 0.24 g

FeSO₄ × 7 H₂O 0.02 g

Methanol 5.0 ml or

Methylamine 3.0 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C 15 min, methanol and methylamine sterilize by filtration.

253. MODIFIED MEDIUM FOR METHANOTROPHIC BACTERIA WITH ETHANOL

KNO₃ 1.0 g

KH₂PO₄ 0.7 g

MgSO₄ × 7 H₂O 0.2g

CaCl₂ × 2 H₂O 0.02 g

Na₂HPO₄ × 2 H₂O 1.5 g

Trylon B 5.0 mg

FeSO₄ × 7 H₂O 2.0 mg

ZnSO₄ × 7 H₂O 0.1 mg

MnCl₂ × 4 H₂O 0.03 mg

CoCl₂ × 6 H₂O 0.2 mg

CuCl₂ × 2 H₂O 0.1 mg

NiCl₂ × 6 H₂O 0.02 mg

Na₂MoO₄ × 2 H₂O 0.03 mg

Ethanol 5.0 ml

Distilled water 1000.0 ml

pH 6.7 - 7.1

Autoclave at 121°C 15 min, ethanol sterilize by filtration.

Cultivation under mixture of methane and air (1:1).

254. MODIFICATION OF TWEEN-80 MEDIUM FOR MILK-ACID BACTERIA (10% NaCl)

Beef extract 1.2 g

Yeast extract 5.0 g

Glucose 2.5 g

Tween-80 1.0 ml

K₂HPO₄ 2.0 g

Na-acetate × 3 H₂O 5.0 g

NaCl 60.0 g

NH₄-citrate 2.0 g

MgSO₄ × 7 H₂O 0.2 g

MnSO₄ × 4 H₂O 0.05 g

Agar 5.0 g

Distilled water 1000.0 ml

pH 6.0-6.5

Autoclave at 111°C for 30 min.

255. MINERAL MEDIUM FOR METHYLOMICROBIUM KENYENSE

Na₂CO₃ 2.3 g

NaHCO₃ 7.0 g

K₂HPO₄ × 12 H₂O 0.5 g

KNO₃ 0.5 g

MgSO₄ × 7 H₂O 0.4 g

NaCl 5.0 g

Trace element solution (see below) 1.0 ml

Distilled water 1000.0 ml

Trace elements solution:

EDTA 5.0 g

CuCl₂ × 2 H₂O 0.1 g

FeSO₄ × 7 H₂O 2.0 g

ZnSO₄ × 7 H₂O 0.1 g

NiCl₂ × 6 H₂O 0.02 g

CoCl₂ × 6 H₂O 0.2 g

Na₂MoO₄ × 2 H₂O 0.03 g

Distilled water 1000.0 ml

Prepare the medium without NaHCO₃ and Na₂CO₃. Basal medium, trace element solution, NaHCO₃ and Na₂CO₃ autoclave at 121°C 15 min. When preparing liquid media cool the mineral salts solution, NaHCO₃ and Na₂CO₃ to room temperature before mixing. For solid medium add 2.0 % agar to the mineral salt solution and autoclave. Cool the NaHCO₃ and Na₂CO₃ stock solution and agar to 50-55°C before mixing.

The pH of the complete medium should be at 10.0.

256. MODIFIED METHYLOTROPH MEDIUM 1 WITH YEAST AUTOLYZATE

KH₂PO₄ 2.0 g

(NH₄)₂SO₄ 2.0 g

MgSO₄ × 7 H₂O 0.025 g

NaCl 0.5 g

FeSO₄ × 7 H₂O 0.02 g

Methanol 5.0 ml or Methylamine 3.0 g

Yeast autolizate 1.0 g

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C 15 min, methanol and methylamine sterilize by filtration.

257. MODIFIED STARCH AMMONIA AGAR (10% NaCl)

Starch (soluble) 10.0 g

K₂HPO₄ 1.0 g

MgSO₄ × 7 H₂O 1.0g

NaCl 100.0 g

(NH₄)₂SO₄ 2.0 g

CaCO₃ 2.0 g

Salt solution (see below) 1.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

Salt solution:

FeSO₄ × 7 H₂O 0.1 g

MnCl₂ × 4 H₂O 0.1 g

ZnSO₄ × 7 H₂O 0.1 g

Distilled water 100.0 ml
pH 7.0-7.4
Autoclave separately at 121°C 15 min.

258. *NOCARDIOIDES AQUATICUS* MEDIUM

Base medium (see below) 600.0 ml
Artificial sea water (see below) 400.0 ml
pH 7.5

Base medium:

Peptone Bacto 0.25 g
Yeast extract Bacto 0.25 g
Glucose solution (2.5%) 10.0 ml
Staley's vitamin solution, double concentration (see below) 5.0 ml
Hutner's basal salts medium (see below) 20.0 ml

Agar Bacto 15.0 g
Distilled water 965.0 ml

Artificial sea water (AWS):

NaCl 23.477 g
Na₂SO₄ 3.917 g
MgCl₂ × 6 H₂O 4.981 g
CaCl₂ × 2 H₂O 1.1 g
NaHCO₃ 192.0 mg
KCl 664.0 mg
KBr 6.0 mg
H₃BO₃ 26.0 mg
SrCl₂ 24.0 g
NaF 3.0 mg

Distilled water 1000.0 ml

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g
MgSO₄ × 7 H₂O 29.7 g
CaCl₂ × 2 H₂O 3.34 g
Na₂MoO₄ × 2 H₂O 12.67 mg
FeSO₄ × 7 H₂O 99.0 mg
Metal salt solution "44" (see below) 50.0 ml
Dissolve NTA first by neutralizing with KOH, then add other salts.
pH 7.2 (adjust with KOH or H₂SO₄).
Adjust volume to 1000.0 ml with distilled water.

Metal solution "44":

Na-EDTA 250.0 mg
ZnSO₄ × 7 H₂O 1095.0 mg
FeSO₄ × 7 H₂O 500.0 mg
MnSO₄ × 7 H₂O 154.0 mg
CuSO₄ × 5 H₂O 39.0 mg
Co(NO₃)₂ × 6 H₂O 24.8 mg
Na₂B₄O₇ × 10 H₂O 17.7 mg
Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Staley's vitamin solution, double concentration:

Biotin 4.0 mg
Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg
Riboflavin 10.0 mg
Thiamine-HCl $\times$ 2 H₂O 10.0 mg
Nicotinamide 10.0 mg
D-Ca-pantothenate 10.0 mg
Vitamin B₁₂ 0.2 mg
p-Aminobenzoic acid 10.0 mg
Distilled water 1000.0 ml

Autoclave Base medium (except glucose solution and vitamin solution) and Artificial sea water separately at 121°C 15 min. After cooling to 60°C aseptically add to the Base medium glucose solution (sterilize by filtration) and vitamin solution (sterilize by filtration). Store vitamin solution in the dark and cold (5°C). Add 600.0 ml Base medium to 400 ml Artificial sea water and mix thoroughly.

259. ISP-2 MEDIUM WITH 5% NaCl

Yeast extract 4.0 g
Malt extract 10.0 g
Glucose (D-glucose) 4.0 g
Agar 15.0-20.0 g
NaCl 50.0 g
Distilled water 1000.0 ml
pH 7.2
Autoclave at 111°C for 30 min.

260. V-8 MEDIUM

Commercial V8 vegetable juice 175.0 ml
CaCO₃ 3.0 g
Agar 20.0 g
Distilled water to 1000.0 ml
pH 6.4
Autoclave at 111°C for 30 min.

261. ROSEINATRONOBACTER MONICUS MEDIUM

NH₄Cl 0.4 g
KH₂PO₄ 0.5 g
MgCl₂ $\times$ 6 H₂O 0.2 g
Na₂SO₄ 0.5 g
NaNO₃ 0.4 g
NaCl 40.0 g
KCl 0.5 g
NaHCO₃ 10.0 g
Na₂CO₃ 5.0 g
Na₂S₂O₃ $\times$ 5 H₂O 1.0 g
Vitamin B₁₂ 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 *SL-10* 1.0 ml
Distilled water 1000.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

The trace element solution preparation: FeCl₂ × 4 H₂O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Autoclave the medium, trace element solution, NaHCO₃ and Na₂CO₃ separately at 121°C for 15 min and add to the medium. Add the vitamin B₁₂ solution from a filter-sterilized stock solution to the autoclaved, cooled medium.

Final pH 9.0 - 9.5

262. MINERAL MEDIUM

KNO₃ 250.0 mg
KH₂PO₄ 100.0 mg
MgSO₄ × 7 H₂O 50.0 mg
CaCl₂ × 2 H₂O 10.0 mg
Trace elements solution 1.0 ml
Distilled water 1000.0 ml

Trace elements solution:

EDTA 5.0 g
CuCl₂ × 2 H₂O 0.1 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 0.1 g
NiCl₂ × 6 H₂O 0.02 g
CoCl₂ × 6 H₂O 0.2 g
Na₂MoO₄ × 2 H₂O 0.03 g
MnCl₂ × 4 H₂O 0.03 g
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min.
Final pH 5.5-6.0

263. SINGULISPHAERA MEDIUM

N-acetylglucosamine 1.0 g
KH₂PO₄ 0.1 g
Hutner's basal salts medium 20.0 ml
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

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g
MgSO₄ × 7 H₂O 29.7 g
CaCl₂ × 2 H₂O 3.34 g
Na₂MoO₄ × 2 H₂O 12.67 mg
FeSO₄ × 7 H₂O 99.0 mg
Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H₂SO₄).

Adjust volume to 1000.0 ml with distilled water.

Metal solution "44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × 7 H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.0 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Autoclave at 121°C 15 min.

264. ROSEOCOCCUS SUDUNTUYENSIS MEDIUM

NH₄Cl 0.4 g

KH₂PO₄ 0.5 g

MgCl₂ × 6 H₂O 0.2 g

Na₂SO₄ 0.5 g

NaCl 2.0 g

KCl 0.5 g

NaHCO₃ 5.0 g

Na₂CO₃ 0.5 g

Na₂S₂O₃ × 5 H₂O 1.0 g

Vitamin B₁₂ 10.0 µg

Na-pyruvate 1.0 g

Na-acetate × 3 H₂O 1.0 g

Peptone 1.0 g

Yeast extract 1.0 g

Trace element solution *SL-10* (see below) 1.0 ml

Final pH 8.5

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

The trace element solution preparation: FeCl₂ × 4 H₂O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Autoclave the medium, trace element solution, NaHCO₃ and Na₂CO₃ separately at 121°C for 15 min and add to the medium. Add the vitamin B₁₂ solution from a filter sterilized stock solution to the cooled medium.

265. ECTOTHIORHODOSPIRA VARIABILIS MEDIUM

NaCl 50.0 g
Na₂CO₃ 5.0 g
NaHCO₃ 15.0 g
KCl 0.1 g
K₂HPO₄ 0.5 g
MgCl₂ × 6 H₂O 0.2 g
NH₄Cl 0.5 g
Na₂S × 9 H₂O 0.5 g
Na₂S₂O₃ × 5 H₂O 0.5 g
Yeast extract 0.1 g
Na-acetate × 3 H₂O 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Vitamin B₁₂ 20.0 µg
Distilled water 1000.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

The trace element solution preparation: FeCl₂ × 4 H₂O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Prepare and boil the medium in the absence of Na₂S × 9 H₂O, NaHCO₃, vitamin B₁₂, MgCl₂ × 6 H₂O. Cool under a stream of nitrogen and add the remaining components, except vitamin B₁₂ and Na₂S₂O₃ × 5 H₂O. Dispense into tubes or bottle fitted with rubber stoppers and autoclave. Add the vitamin B₁₂ (filter sterilized) and Na₂S₂O₃ × 5 H₂O from sterile stock solutions.

Autoclave at 121°C for 15 min.

Final pH 9.0 – 9.5

266. ACIDISOMA MEDIUM

KH₂PO₄ 0.1 g
(NH₄)₂SO₄ 0.25 g
CaCl₂ × 2 H₂O 0.05 g
MgSO₄ × 7 H₂O 0.02 g
Trace elements solution (see below) 1.0 ml
Yeast extract 0.1 g
Na gluconate 0.5 g
Distilled water 1000.0 ml
Trace element solution s:
EDTA 5.0 g
CuCl₂ × 2 H₂O 0.1 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 0.1 g
NiCl₂ × 6 H₂O 0.02 g
CoCl₂ × 6 H₂O 0.2 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml

Autoclave the base medium and trace element solution separately at 121°C for 15 min. Adjust to pH 5.0 – 5.5.

267. GRANULICELLA MEDIUM

Glucose 0.5 g
Yeast extract 0.1 g
Casamino acids 0.1 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.04 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.02 g
Distilled water 1000.0 ml
The medium may be solidified with 15 g/l agar.
Final pH 4.5 – 5.2
Autoclave at 121°C for 15 min.

268. ZAVARZINELLA FORMOSA MEDIUM

KH_2PO_4 0.1 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g
 $\text{CaCl}_2 \times 6 \text{H}_2\text{O}$ 0.05 g
NaCl 0.01 g
N-acetylglucosamine 1.0 g
Glucose 0.5 g
Peptone 0.1 g
Yeast extract 0.1 g
Casamino acids 0.1 g
“Metals 44” 1.0 ml
Agar (for solid medium) 15.0 g
Distilled water 1000.0 ml
Final pH 5.8 – 6.0
Metal solution “44”:
Na-EDTA 250.0 mg
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 1095.0 mg
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 500.0 mg
 $\text{MnSO}_4 \times 7 \text{H}_2\text{O}$ 154.0 mg
 $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 39.0 mg
 $\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$ 24.8 mg
 $\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$ 17.7 mg
Distilled water 1000.0 ml
Dissolve Na-EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of heavy metal ions.
Autoclave the medium and trace element solution separately at 121°C for 15 min.

269. THIOTHRUX MEDIUM

NH_4Cl 0.2 g
 K_2HPO_4 10.0 mg
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 10.0 mg
 CaSO_4 (saturated solution) 20.0 ml
Trace element solution (see below) 5.0 ml
Na-acetate $\times 3 \text{H}_2\text{O}$ 0.1 g
Agar (if necessary) 12.0 g
 $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 10% (w/v) solution 3.0 ml
Distilled water 1000.0 ml
Adjust pH to 7.5 before autoclaving.

Trace element solution:

EDTA 0.2 g

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.7 g

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.01 g

$\text{MnSO}_4 \times 4 \text{ H}_2\text{O}$ 0.02 g

$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$ 5.0 μg

H_3BO_3 10.0 mg

$\text{Co}(\text{NO}_3)_2$ 1.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 1.0 mg

Distilled water 1000.0 ml

Basal medium and $\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ solution autoclave separately at 121°C 15 min.

270. TRYPTICASE SOY BROTH AGAR

Trypticase Soy Broth (BBL 11768, Oxoid CM129 or Merck 5459) 30.0 g or

Pancreatic digest of casein 18.0 g

Papaic digest of soybean 6.0 g

NaCl 6.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.3

Autoclave at 121°C for 15 min.

271. MARINE SPIROCHETE MEDIUM

Tryptone 2.0 g

Yeast extract 1.0 g

Na-Thioglycolate 1.0 g

Resazurin 0.5 mg

Charcoal-filtered, natural seawater 800.0 ml

Distilled water 200.0 ml

Dissolve ingredients (except thioglycolate), boil medium for 3 min, then cool to room temperature under N_2 gas atmosphere. Add thioglycolate and adjust pH of medium to 7.5 with 10 N KOH. Dispense under N_2 gas atmosphere in culture vessels and autoclave at 121°C for 15 min. Prepare 10% cellobiose solution (10.0 g in 100.0 ml distilled water) under nitrogen atmosphere, filter-sterilize and add 0.2 ml to 10.0 ml autoclaved medium.

272. ECTOTHIORHODOSPIRA MAGNA MEDIUM

KH_2PO_4 0.5 g

NaCl 30.0 g

NH_4Cl 0.5 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 g

Na-acetate $\times 3 \text{ H}_2\text{O}$ 1.0 g

$\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ 0.5 g

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.5 g

NaHCO_3 5.0 g

Na_2CO_3 5.0 g

Yeast extract 0.1 g

Vitamin B_{12} 10.0 μg

Trace elements solution (see below) 1.0 ml

Distilled water 1000.0 ml

pH 9.0-9.5

Trace elements solution:

EDTA 5.0 g

FeSO₄ × 7 H₂O 2.2 g
ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.03 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.03 g
NiCl₂ × 6 H₂O 0.03 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0-4.0

Prepare the medium without the NaHCO₃, Na₂S × 9 H₂O, Na₂CO₃ and vitamin B₁₂, under a nitrogen atmosphere.

Autoclave at 121°C for 15 min and add the vitamin B₁₂ from a filter-sterilized stock solution and the NaHCO₃ (autoclaved in sealed, half full vessels), Na₂S × 9 H₂O, Na₂CO₃ from autoclaved at 121°C for 15 min stock solutions. The final pH of the medium should be 9.0-9.5.

273. LAMPROBACTER MODESTOHALOPHILUS MEDIUM

KH₂PO₄ 0.5 g
NaCl 40.0 g
NH₄Cl 0.5 g
MgCl₂ × 6 H₂O 0.2 g
Na-acetate × 3 H₂O 0.5 g
Na₂S₂O₃ × 5 H₂O 0.5 g
Na₂S × 9 H₂O 0.5 g
NaHCO₃ 1.5 g
Yeast extract 0.1 g
Vitamin B₁₂ 20.0 µg
Trace elements solution (see below) 1.0 ml
Distilled water 1000.0 ml
pH 7.5

Trace elements solution:

EDTA 5.0 g
FeSO₄ × 7 H₂O 2.2 g
ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.03 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.03 g
NiCl₂ × 6 H₂O 0.03 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0-4.0

Prepare the medium without the NaHCO₃, Na₂S × 9 H₂O, Na₂CO₃ and vitamin B₁₂, under a nitrogen atmosphere. Autoclave at 121°C for 15 min and add the vitamin B₁₂ from a filter-sterilized stock solution and the NaHCO₃ (autoclaved in sealed, half full vessels), Na₂S × 9 H₂O, Na₂CO₃ from autoclaved at 121°C for 15 min stock solutions. The final pH of the medium should be 9.0-9.5.

274. GRANULICELLA PALUDICOLA MEDIUM

Fructose 0.5 g
Yeast extract 0.05 g
Casamino acids 0.05 g

MgSO₄ × 7 H₂O 0.04 g
CaCl₂ × 2 H₂O 0.02 g
KH₂PO₄ 0.1 g
(NH₄)₂SO₄ 0.1 g
Distilled water 1000.0 ml
The medium may be solidified with 15 g/l agar. Adjust to pH 4.0 – 5.0 with alginic acid.
Autoclave at 121°C for 15 min.

275. TELMATOCOLA MEDIUM

Mineral salts solution:

KH₂PO₄ 0.1 g
(NH₄)₂SO₄ 0.1 g
MgSO₄ × 7 H₂O 0.1 g
CaCl₂ × 2 H₂O 0.02 g
Hutner's basal salts medium 1.0 ml
Staley's vitamin solution 1.0 ml

Yeast extract 0.1 g

Glucose (1% v/v) 10.0 ml

Distilled water 1000.0 ml

Trace elements solution 44:

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g

MgSO₄ × 7 H₂O 29.7 g

CaCl₂ × 2 H₂O 3.335 g

(NH₄)₆MoO₇O₂₄ × 4 H₂O 9.25 mg

FeSO₄ × 7 H₂O 99.0 mg

"Metals 44" 50.0 ml

Distilled water 950.0 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.

"Metals 44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.2 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of the heavy metal ions.

Staley's vitamin solution:

Vitamin B₁₂ 0.1 mg

Biotin 2.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Ca-pantothenate 5.0 mg

Folic acid 2.0 mg

Riboflavin 5.0 mg

Nicotinamide 5.0 mg

p-Aminobenzoic acid 5.0 mg

Pyridoxine hydrochloride 10.0 mg

Distilled water 1000.0 ml

Prepare the liquid medium. Adjust to pH 4.8 - 5.5. Dispense the medium into serum bottles under gassing with CO₂ (5% v/v). Autoclave at 121°C for 15 min. Before inoculation add from filter-sterilized *Staley's* vitamin solution and autoclaving at 121°C for 15 min glucose.

276. PROTEINIVORAX MEDIUM

KH₂PO₄ 0.3 g
MgSO₄ × 7 H₂O 0.12 g
CaCl₂ × 2 H₂O 30.0 mg
NaCl 20.0 g
Tryptone 3.0 g
Trace element solution *SL-10* 1.0 ml
Na₂CO₃ 60.0 g
NaHCO₃ 50.0 g
Na-Thioglycolate 1.0 g
Distilled water 1000.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

The trace element solution preparation: FeCl₂ × 4 H₂O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Dissolve ingredients (except carbonates and thioglycolate), boil medium for 1 min, then cool to room temperature under N₂ gas atmosphere. Add carbonates and adjust pH to 8.5 – 9.0. Dispense under same gas atmosphere in culture vessels and autoclave. After autoclaving at 121°C for 15 min add thioglycolate from a sterile anoxic stock solution sterilized by filtration.

277. MS1 MEDIUM

NH₄Cl 2.0 g
MgSO₄ × 7 H₂O 0.2 g
K₂SO₄ 0.5 g
FeSO₄ × 7 H₂O 2.5 mg
CaCl₂ × 2 H₂O 10.0 mg
CuSO₄ × 5 H₂O 2.0 mg
H₃BO₃ 0.06 mg
ZnCl₂ 20.0 mg
MnSO₄ × H₂O 1.0 mg
NiCl₂ × 6 H₂O 0.05 mg
Na₂MoO₄ × 2 H₂O 0.3 mg
Na-glutamate 10.0 g
Distilled water 1000.0 ml
pH 7.0-7.5
Autoclave at 121°C for 15 min.

278. MODIFIED S MEDIUM

KH₂PO₄ 0.14 g

MgSO₄ × 7 H₂O 0.4 g
CaCl₂ × 2 H₂O 0.1 g
NH₄Cl 0.25 g
KCl 0.36 g
NaCl 6.0
Yeast extract 0.1 g
Trace elements solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O (Mohr's salt) 784.0 mg
HCl (concentrated) 5.0 ml
CoCl₂ × H₂O 238.0 mg
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg
Na₂MoO₄ × H₂O 24.0 mg
Na₂WO₄ × 2 H₂O 33.0 mg
ZnSO₄ × 7 H₂O 144.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Na₂SeO₄ 94.0 mg
HBO₃ 6.0 mg
MnCl₂ × 4 H₂O 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 100.0 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution and trace elements from sterile stock solution.

279. M3 MEDIUM

KH₂PO₄ 0.1 g
NH₄Cl 0.2 g
MgSO₄ × 7 H₂O 0.1 g
CaCl₂ × 2 H₂O 0.02 g
Yeast extract 0.1 g
Glucose (or malate) 0.5 g
Trace element solution 'SLA' (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution SLA:

FeCl₂ × 4 H₂O 1.8 mg
CoCl₂ × 6 H₂O 250.0 mg
NiCl₂ × 6 H₂O 10.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 10.0 mg
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 70.0 mg
 ZnCl_2 100.0 mg
 H_3BO_3 500.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 30.0 mg
 $\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$ 10.0 mg
Distilled water 1000.0 ml
Autoclave basal medium and trace element solution separately at 121°C 15 min.
pH 5.8-6.0

280. *PLANCTOMYCES* MEDIUM

KH_2PO_4 0.1 g
 $(\text{NH}_4)_2\text{SO}_4$ 0.2 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g
 $\text{CaCl}_2 \times 6 \text{H}_2\text{O}$ 0.02 g
NaCl 0.01 g
N-acetylglucosamine 1.0 g
Glucose 0.5 g
Yeast extract 0.2 g
Hutner's basal salts (see below) 1.0 ml
Staley's vitamin solution (see below) 1.0 ml
Phytigel (for solid medium) 8.0 g
Distilled water 1000.0 ml
Adjust pH to 5.8-6.5
Hutner's basal salts
Nitrilotriacetic acid (NTA) 10.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 29.7 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 3.34 g
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 12.67 mg
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 99.0 mg
Metal salt solution "44" (see below) 50.0 ml
Dissolve NTA first by neutralizing with KOH, then add other salts.
pH 7.2 (adjust with KOH or H_2SO_4).
Adjust volume to 1000.0 ml with distilled water.

Metal solution "44":

Na-EDTA 250.0 mg
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 1095.0 mg
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 500.0 mg
 $\text{MnSO}_4 \times 7 \text{H}_2\text{O}$ 154.0 mg
 $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 39.0 mg
 $\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$ 24.8 mg
 $\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$ 17.7 mg
Distilled water 1000.0 ml
Dissolve Na-EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of heavy metal ions.

Staley's vitamin solution:

Vitamin B₁₂ 0.1 mg
Biotin 2.0 mg
Thiamine-HCl $\times 2 \text{H}_2\text{O}$ 5.0 mg
Ca-pantothenate 5.0 mg
Folic acid 2.0 mg
Riboflavin 5.0 mg

Nicotinamide 5.0 mg
p-Aminobenzoic acid 5.0 mg
Pyridoxine-HCl 10.0 mg
Distilled water 1000.0 ml
Store in the dark and cold (5°C).
Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution.

281. AZOSPIRILLUM AMAZONENSE MEDIUM

K₂HPO₄ 0.2 g
KH₂PO₄ 0.6 g
CaCl₂ × 2 H₂O 0.02 g
MgSO₄ × 7 H₂O 0.2 g
Na₂MoO₄ × 2 H₂O 0.02 g
FeCl₂ × 4 H₂O 0.01 g
Bromothymol blue (0.5% in 0.2N KOH) 5.0 ml
Sucrose 5.0 g
Distilled water 1000.0 ml
Adjust pH to 6.0
For semisolid medium, add 0.5 g of agar; for solid medium, add 15 g of agar.
Autoclave at 111°C for 30 min.

282. AZOSPIRILLUM HALOPRAEFERENS MEDIUM

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
pH 7.4
Autoclave at 121°C 15 min.

283. AZOSPIRILLUM VM MEDIUM

Döbereiner's basic 10.0 ml
Fe₃ EDTA 0.66% (w/v) 10.0 ml
NH₄Cl 0.5 g
NaCl 1.0 g
Yeast extract 1.0 g
Peptone 3.0 g
Phosphate buffer pH 6.8 3.0 ml
Agar 15.0 g
Distilled water 1000.0 ml
Döbereiner's basic:
MgSO₄ 20.0 g
NaCl 10.0 g
CaCl₂ × 2 H₂O 2.64 g
Na₂MoO₄ × 2 H₂O 0.2 g
MnSO₄ × 2 H₂O 1.0 g
Distilled water 1000.0 ml
Phosphate buffer pH 6.8:
KH₂PO₄ 0.6 g
K₂HPO₄ 0.4 g
Distilled water 3.0 ml

Autoclave basal medium, Fe₃EDTA solution, Döbereiner's basic and phosphate buffer separately at 121°C 15 min.

284. METHYLOCYSTIS MEDIUM

KNO₃ 0.2 g
KH₂PO₄ 0.1 g
MgSO₄ × 7 H₂O 0.05 g
CaCl₂ × 2 H₂O 0.01 g
NaCl 0.01 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml
Final pH 5.8-6.2.

Trace element solution:

EDTA 5.0 g
CuCl₂ × 2 H₂O 0.1 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 0.1 g
NiCl₂ × 6 H₂O 0.02 g
CoCl₂ × 6 H₂O 0.2 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml

Dispense the medium into serum bottles with a medium to headspace ratio of about 1:4.

Autoclave at 121°C for 15 min. Add 20% methane to the gas phase. Incubate the cultures with shaking.

285. TELMATOBACTER BRADUS MEDIUM

Yeast extract 0.05 g
Proteose peptone 0.05 g
Casamino acids 0.05 g
Glucose 0.05 g
Soluble starch 0.05 g
MgSO₄ × 7 H₂O 0.05 g
Distilled water 1000.0 ml
Adjust pH to 4.5 - 5.0 with alginic acid (or MES), fill tubes for anaerobes (Hungate) half with medium, close tightly. Autoclave at 121°C for 15 min. Do not shake.

286. LARKINELLA ARBORICOLA MEDIUM

KH₂PO₄ 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 (see below) 20.0 ml
Distilled water 980.0 ml
Hutner's salts:
Nitrilotriacetic acid (NTA) 10.0 g
MgSO₄ × 7 H₂O 29.7 g
CaCl₂ × 2 H₂O 3.335 g
(NH₄)₆MoO₇O₂₄ × 4 H₂O 9.25 mg
FeSO₄ × 7 H₂O 99.0 mg
"Metals 44" 50.0 ml

Distilled water 950.0 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 H₂SO₄.

"Metals 44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.2 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated H₂SO₄ 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. Autoclave at 121°C for 15 min.

287. METHYLOCAPSA AUREA MEDIUM

NaNO₃ 200.0 mg

MgSO₄ × 6 H₂O 200.0 mg

CaCl₂ × 2 H₂O 40.0 mg

Fe-EDTA solution (see below) 3.0 ml

Phosphate buffer pH 5.8 100 mM (see below) 10.0 ml

Trace elements solution (see below) 1.0 ml

Distilled water 986.0 ml

Trace elements solution:

EDTA 5.0 g

CuCl₂ × 2 H₂O 0.1 g

FeSO₄ × 7 H₂O 2.0 g

ZnSO₄ × 7 H₂O 0.1 g

NiCl₂ × 6 H₂O 0.02 g

CoCl₂ × 6 H₂O 0.2 g

Na₂MoO₄ 0.03 g

Distilled water 1000.0 ml

Fe-EDTA solution:

FeSO₄ × 7 H₂O 1.54 g

Na₂EDTA 2.06 g

Distilled water 1000.0 ml

Phosphate buffer 100 mM:

K₂HPO₄ × 3 H₂O 1.71 g

KH₂PO₄ 12.58 g

Distilled water 1000.0 ml

Autoclave at 121°C for 15 min. Final pH 5.0-5.8. 20-30% methane is added to the gas phase.

The strain should be grown with shaking.

288. ACIDICAPSA BOREALIS MEDIUM

Yeast extract 0.05 g

Proteose peptone 0.05 g

Casamino acids 0.05 g

Glucose 0.2 g

Soluble starch 0.05 g

Na-pyruvate 0.03 g

K₂HPO₄ 0.03 g
MgSO₄ × 7 H₂O 0.05 g
Distilled water 1000.0 ml
Adjust pH to 5.0 – 5.5. Autoclave at 121°C for 15 min.

289. MEDIUM FOR *ECTOTHIORHODOSPIRA*

KH₂PO₄ 0.5 g
CaCl₂ × 2 H₂O 0.05 g
NH₄Cl 0.8 g
MgCl₂ × 6 H₂O 0.1 g
NaCl 30.0 g
Na₂SO₄ 20.0 g
Na₂CO₃ 6.0 g
Trace element solution (see below) 1.0 ml
Yeast extract 0.5 g
Na-succinate 1.0 g
Na₂S × 9 H₂O 1.0 g
Distilled water 800.0 ml
Adjust pH to 8.5.
Trace element solution:
FeCl₂ × 4 H₂O 1.8 g
CoCl₂ × 6 H₂O 250.0 mg
NiCl₂ × 6 H₂O 10.0 mg
CuCl₂ × 2 H₂O 10.0 mg
MnCl₂ × 4 H₂O 70.0 mg
ZnCl₂ 100.0 mg
H₃BO₃ 500.0 mg
Na₂MoO₄ × 2 H₂O 30.0 mg
Na₂SeO₃ × 5 H₂O 10.0 mg
Distilled water 1000.0 ml
For dissolving adjust pH to about 3 with 1 N HCl.

Vitamin solution:

Biotin 10.0 mg
Nicotinamide 35.0 mg
Thiamine-HCl × 2 H₂O 30.0 mg
p-Aminobenzoic acid 20.0 mg
Pyridoxal-HCl 10.0 mg
Ca-pantothenate 10.0 mg
Vitamin B₁₂ 5.0 mg
Distilled water 100.0 ml

Bicarbonate solution:

NaHCO₃ 14.0 g
H₂O 200.0 ml

Autoclave at 121°C for 15 minutes in screw-capped bottles. After sterilization add 1 ml/l filter-sterilized vitamin solution and 200 ml of filter-sterilized bicarbonate solution.

290. *MARINOSPIRILLUM CELERE* MEDIUM

NH₄Cl 0.5 g
KH₂PO₄ 0.5 g
NaCl 25.0 g
Na₂SO₄ 0.5 g
MgSO₄ × 7 H₂O 0.2 g

KCl 0.5 g
Yeast extract 1.0 g
Peptone 1.0 g
Lactate 1.0 g
Vitamin B₁₂ 15.0 µg
Na₂CO₃ 5.0 g
NaHCO₃ 5.0 g
Trace element solution *SL-10* (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution SL-10:

Distilled water 1000.0 ml
HCl (25%) 7.7 ml
FeSO₄ × 7 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg

Autoclave the Na₂CO₃, NaHCO₃, lactate and the medium separately at 121°C for 15 min and combine after cooling. Vitamin B₁₂ is added from a sterilized by filtration stock solution. The final pH of the medium should be 9.5. Add 15.0 g/l agar for solid media.

291. PROSTHECOMICROBIUM MEDIUM

NH₄H₂PO₄ 0.3 g
Na₂HPO₄ 0.71 g
KH₂PO₄ 0.36 g
MgSO₄ × 7 H₂O 0.3 g
Trace element solution (see below) 1.0 ml
Yeast extract 0.3 g
Glucose 0.3 g
Distilled water 1000.0 ml

Trace element solution:

Na-EDTA 0.25 g
ZnSO₄ × 7 H₂O 1.1 g
FeSO₄ × 7 H₂O 0.5 g
MnSO₄ × H₂O 0.154 g
CuSO₄ × 5 H₂O 0.04 g
Co(NO₃)₂ × 6 H₂O 0.025 g
Na₂B₄O₇ × 10 H₂O 0.018 g
Distilled water 100.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Adjust pH to 7.0. Autoclave at 121°C for 15 min.

292. R2A AGAR MEDIUM

Bacto yeast extract 0.5 g
Proteose peptone 0.5 g
Casamino acids 0.5 g
Glucose 0.5 g
Soluble starch 0.5 g

Na-pyruvate 0.3 g
KH₂PO₄ 0.3 g
MgSO₄ × 7 H₂O 50 mg
Agar 15.0 g
Distilled water 1000.0 ml. Autoclave at 121°C for 15 min.

293. TPT 18 MEDIUM

Glucose 0.5 g
Yeast extract 0.1 g
Casitone (pancreatic digest of casein) 0.1 g
MgSO₄ × 7 H₂O 50 mg
CaCl₂ × 2 H₂O 20 mg
Agar (for solid medium) 14.0 g
Distilled water 1000.0 ml
Adjust to pH 6.0. Autoclave at 121°C for 15 min.

294. METHYLOMICROBIUM JAPANENSE MEDIUM

NH₄NO₃ 0.1 g
KH₂PO₄ 0.01 g
Fe(III)-EDTA 2.5 mg
Vitamin solution (see below) 10.0 ml
Trace elements solution (see below) 10.0 ml
Methanol 2.0 ml
Seawater 980.0 ml
pH 8.1
Vitamin solution
Vitamin B₁₂ 0.275 mg
Biotin 0.25 mg
Thiamine-HCl 50 mg
Distilled water 1000.0 ml
Trace elements solution
Na-EDTA 37.2 mg
CuSO₄ × 5 H₂O 0.025 mg
ZnSO₄ × 7 H₂O 0.575 mg
MnCl₂ × 4 H₂O 0.455 mg
CoCl₂ × 6 H₂O 0.06 mg
(NH₄)₆Mo₇O₂₄ × 4 H₂O 0.027 mg
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min, add methanol and vitamin solution sterilized by filtration.

295. NAUTILIA MEDIUM

Synthetic seawater (2 × conc.) (see below) 500.0 ml
NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
Resazurin 0.5mg
Distilled water 400.0 ml
Synthetic seawater (2 × conc.):
NaCl 55.4 g
SrCl₂ × 6 H₂O 0.03 g
MgSO₄ × 7 H₂O 14.0 g
KI 0.1 mg
MgCl₂ × 6 H₂O 11.0 g

Na₃-citrate 20.0 mg
KCl 1.3 g
CaCl₂ × 2 H₂O 1.5 g
NaBr 0.2 g
H₃BO₃ 0.06 g
Distilled water 1000.0 ml
Sulfur, powdered 10.0 g
Na-formate (20% w/v) 15.0 ml
NaHCO₃ (5% w/v) 50.0 ml
Trace element solution (see below) 10.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O (3% w/v) 20.0

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 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 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3.0 mg
Na₂WO₄ × 2 H₂O 4.0 mg
Distilled water 1000.0 ml

Prepare medium anoxically under 80% N₂ + 20% CO₂ gas mixture. Vitamin solution are prepared under N₂ gas atmosphere and sterilized by filtration. Na-formate, NaHCO₃, trace element solution, selenite-tungstate solution and Na₂S × 9 H₂O solution sterilize separately under

100% N₂ gas at 121°C for 15 min. Place the sulfur in screw-capped tubes or bottles and autoclave at 112°C for 15 min. Before use, aseptically layer the sulfur onto the surface of autoclaved liquid basal medium.

296. *ACIDICAPSA LIGNI* MEDIUM

KH₂PO₄ 0.1 g
MgSO₄ × 7 H₂O 0.1 g
NaNO₃ 0.1 g
CaCl₂ × 2 H₂O 0.1 g
Yeast extract 0.1 g
Cellobiose 0.1 g
Distilled water 1000.0 ml
Adjust pH to 5.0 – 5.5. Autoclave at 121°C for 15 min.

297. *EDAPHOBACTER LICHENICOLA* MEDIUM

MgSO₄ × 7 H₂O 0.8 g
KNO₃ 0.2 g
CaCl₂ × 2 H₂O 0.02 g
Glucose 0.8 g
Yeast extract 0.1 g
Casamino acids 0.1 g
Phytigel 9.0g
Distilled water 1000.0 ml
Adjust to pH 5.0-5.6. Autoclave at 121°C for 15 min.

298. *METHYLOSULA POLARIS* MEDIUM

Mineral salts solution:

(NH₄)₂SO₄ 0.05 g
MgCl₂ × 6 H₂O 0.04 g
KH₂PO₄ 0.07 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution:

Na₂EDTA 500.0 mg
FeSO₄ × 7 H₂O 200.0 mg
ZnSO₄ × 7 H₂O 10.0 mg
MgCl₂ × 6 H₂O 3.0 mg
H₃BO₃ 30.0 mg
CoCl₂ × 6 H₂O 20.0 mg
CaCl₂ × 2 H₂O 1.0 mg
NiCl₂ × 6 H₂O 2.0 mg
Na₂MoO₄ × 2 H₂O 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. Autoclave at 121°C for 15 min. Before inoculation add filter sterilized CH₃OH to a final concentration of 0.5% v/v.

299. *SULFITOBACTER PONTIACUS* MEDIUM

Na-acetate 20.0 mM
HEPES 8.0 g
K₂HPO₄ 1.0 g
NH₄Cl 0.5 g

Yeast extract 1.0 g
Peptone 0.5 g
NaCl 15.0 g
MgSO₄ × 7 H₂O 1.0 g
CaCl₂ × 2 H₂O 0.05 g
Trace element solution (see below) 1.0 ml

Biotin 0.1 g
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Prepare the HEPES, NaCl, K₂HPO₄ and NH₄Cl as a basal salt solution, adjusting the pH to 7.5-7.8 with NaOH before sterilization. All other components are added from sterile stock solutions. Biotin solution is filter sterilized and all other solutions autoclave at 121°C for 15 min.

300. TELMATOSPIRILLUM MEDIUM

K₂HPO₄ 0.25 g
KH₂PO₄ 1.0 g
(NH₄)₂SO₄ 1.0 g
CaCl₂ 0.1 g
MgSO₄ 0.4 g
Na-EDTA 0.01 g
FeCl₃ × 6 H₂O 1.0 mg
KI 0.2 mg
CoCl₂ × 6 H₂O 0.2 mg
MnCl₂ × 4 H₂O 0.8 mg
ZnSO₄ 0.8 mg
H₃BO₃ 0.1 mg
Na₂MoO₄ × 2 H₂O 0.1 mg
CuCl₂ × 2 H₂O 0.1 mg
NiCl₂ × 6 H₂O 0.2 mg
Na-citrate 2.0 g
Distilled water 1000.0 ml

Prepare the medium anaerobically under nitrogen. Adjust to pH 5.5 – 6.5 with 1M H₃PO₄.

Dispense into serum bottles or Hungate tubes (under a nitrogen atmosphere) that can be sealed with rubber stoppers. Autoclave at 121°C for 15 min.

301. METHYLOFERULA STELLATA MEDIUM

KH₂PO₄ 100.0 mg
(NH₄)₂SO₄ 100.0 mg
MgSO₄ × 7 H₂O 50.0 mg
CaCl₂ × 2 H₂O 10.0 mg
NaCl 20.0 mg
Fe-EDTA solution (see below) 3.0 ml
Trace elements solution (see below) 1.0 ml
Methanol 10.0 ml
Distilled water 1000.0 ml

Trace elements solution:

EDTA 5.0 g
CuCl₂ × 2 H₂O 0.1 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 0.1 g
NiCl₂ × 6 H₂O 0.02 g
CoCl₂ × 6 H₂O 0.2 g
Na₂MoO₄ 0.03 g
Distilled water 1000.0 ml

Fe-EDTA solution:

FeSO₄ × 7 H₂O 1.54 g
Na₂EDTA 2.06 g
Distilled water 1000.0 ml
Final pH 5.0-5.8.

The medium is fairly weakly buffered so the pH should be checked before and after autoclaving at 121°C for 15 min. Methanol is filter sterilized. The pH should be adjusted with H₃PO₄ (sterilized if added to sterile medium). The strain should be grown with shaking.

302. PALUDIBACULUM FERMENTANS MEDIUM

KH₂PO₄ 0.1 g
(NH₄)₂SO₄ 0.1 g
MgSO₄ × 7 H₂O 0.05 g
CaCl₂ × 2 H₂O 0.01 g
Yeast extract 0.1 g
Glucose 0.5 g
Phytigel 8.0 g
Distilled water 1000.0 ml
pH 5.5-6.0
Autoclave at 121°C for 15 min.

303. AMMONIFEX MEDIUM

KH₂PO₄ 0.34 g
NH₄Cl 0.34 g
CaCl₂ × 2 H₂O 0.05 g
KCl 0.34 g
Trace element solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
NaHCO₃ 1.5 g
Na₂S × 9 H₂O 0.7 g
Na₂S₂O₃ × 5 H₂O 0.5 g
NaCl 25.0 g

MgCl₂ × 6 H₂O 0.33 g

Na-formate 2.0 g

pH 7.0

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O (*Mohr's salt*) 784.0 mg

CoCl₂ × H₂O 238.0 mg

(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg

Na₂MoO₄ × 2 H₂O 24.0 mg

Na₂WO₄ × 2 H₂O 33.0 mg

ZnSO₄ × 7 H₂O 144.0 mg

CuCl₂ × 2 H₂O 2.0 mg

Na₂SeO₄ 94.0 mg

H₃BO₃ 6.0 mg

MnCl₂ × 4 H₂O 99.0 mg

Vitamin solution:

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine 100.0 mg

Riboflavin 50.0 mg

Pantotenoic acid 50.0 mg

p-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B₁₂ 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

Dissolve ingredients except Na-formate, Na₂S₂O₃, vitamins and sulfide. Adjust pH to 3.5 with H₂SO₄ and sparge medium with 100% CO₂ gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., 10 ml medium in 25 ml Balch-type tubes) and autoclave at 121°C for 15 min. Add Na-formate, Na₂S₂O₃, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas.

304. HALONATRONUM SACCHAROPHILUM MEDIUM

KH₂PO₄ 0.2 g

MgCl₂ × 6 H₂O 0.1 g

NH₄Cl 0.5 g

KCl 0.2 g

NaCl 50.0 g

Na₂CO₃ 68.0 g

NaHCO₃ 38.0 g

Na₂S × 9 H₂O 0.7 g

Yeast extract 0.2 g

Sucrose 5.0 g

Trace elements solution (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Resazurin 0.01 g

Distilled water 1000.0 ml

Final pH 9.5-10.0

Trace element solution:

Nitrilotriacetic acid 1.5 g

MgSO₄ × 7 H₂O 3.0 g

MnSO₄ × H₂O 0.5 g

NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Prepare the medium without adding the vitamins, sucrose, yeast extract, NH₄Cl, Na₂CO₃, and NaHCO₃ using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH₄Cl, Na₂CO₃, and NaHCO₃ after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave at 121°C for 15 min, and to the cooled medium add the vitamins (filter sterilized under N₂), sucrose, yeast extract, and from anaerobic, sterile stock solutions.

305. GEOGLOBUS MEDIUM

NaCl 18.0 g
MgCl₂ × 6 H₂O 4.0 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.33 g
(NH₄)₂SO₄ 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
NaHCO₃ 2.5 g
Na-acetate × 3 H₂O 1.5 g
KH₂PO₄ 0.33 g
Yeast extract 0.2 g
Vitamin solution (see below) 1.0 ml
Amorphous Fe(OH)₃ sludge (see below) 200.0 ml
Distilled water 800.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3 mg
Na₂WO₄ × 2 H₂O 4 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, acetate, phosphate, yeast extract, vitamins and amorphous iron. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Thereafter, dispense suspension under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add acetate, phosphate, yeast extract and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The pH of the complete medium should be at 6.5 – 6.8. Amorphous Fe(OH)₃: Slowly titrate 320 ml of a FeCl₃ × 6 H₂O stock solution (60.0 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. Resuspend the pellet in medium as described above.

306. *FERVIDICOCCUS* MEDIUM

NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.44 g
MgCl₂ × 6 H₂O 0.7 g
NaCl 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Yeast extract 0.5 g
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 0.8 g
Trypticase peptone 2.0 g
Vitamin solution (see below) 10.0 ml

Na₂S × 9 H₂O 0.5 g
Distilled water 1000.0 ml
Trace element solution SL-10:
HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, trypticase, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ 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 Trypticase, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.0 - 6.1, if necessary.

307. *FERVIDOBACTERIUM* MEDIUM

NH₄Cl 0.9 g
NaCl 0.9 g
MgCl₂ × 6 H₂O 0.4 g
KH₂PO₄ 0.75 g
K₂HPO₄ 1.5 g
Trace element solution (see below) 9.0 ml
FeSO₄ × 7 H₂O solution (0.1% w/v in 0.1 N H₂SO₄) 3.0 ml
Yeast extract 3.0 g
Trypticase peptone 10.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Vitamin solution (see below) 5.0 ml
D-Glucose 5.0 g
Na₂S × 9 H₂O 1.0 g
Distilled water 1000.0 ml
Trace element solution:
Nitrilotriacetic acid (NTA) 12.8 g
FeCl₂ × 4 H₂O 0.2 g
MnCl₂ × 4 H₂O 0.1 g

CoCl₂ × 6 H₂O 0.17 g
CaCl₂ × 2 H₂O 0.1 g
ZnCl₂ 0.1 g
CuCl₂ × 2 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
NaCl 1.0 g
Na₂SeO₃ × 5 H₂O 0.03 g
Distilled water 1000.0 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 1000.0 ml.

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except vitamins, glucose and sulfide), sparge medium with 100% N₂ 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% N₂ gas. Adjust pH of complete medium to 7.2 - 7.4.

308. THERMOSIPHO AFFECTUS MEDIUM

Sea salts (SIGMA) 30.0 g
Yeast extract (OXOID) 0.1 g
Trace element solution *SL-10* (see below) 1.0 ml
Trypticase peptone 2.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
D-Glucose 2.0 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 0.25 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except glucose, vitamins and sulfide, then sparge medium with 100% N₂ 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% N₂ gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.5 – 7.0, if necessary.

309. MOORELLA MEDIUM

NH₄Cl 1.0 g
Na₂SO₄ 2.0 g
Na₂S₂O₃ $\times 5 \text{ H}_2\text{O}$ 1.0 g
MgSO₄ $\times 7 \text{ H}_2\text{O}$ 1.0 g
CaCl₂ $\times 2 \text{ H}_2\text{O}$ 0.1 g
KH₂PO₄ 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Yeast extract 1.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 2.0 g
Vitamin solution (see below) 10.0 ml
Na-DL-lactate 2.5 g
Na₂S $\times 9 \text{ H}_2\text{O}$ 0.1 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ $\times 4 \text{ H}_2\text{O}$ 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ $\times 4 \text{ H}_2\text{O}$ 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ $\times 6 \text{ H}_2\text{O}$ 190.0 mg
CuCl₂ $\times 2 \text{ H}_2\text{O}$ 2.0 mg
NiCl₂ $\times 6 \text{ H}_2\text{O}$ 24.0 mg
Na₂MoO₄ $\times 2 \text{ H}_2\text{O}$ 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ $\times 5 \text{ H}_2\text{O}$ 3 mg
Na₂WO₄ $\times 2 \text{ H}_2\text{O}$ 4 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg
Thiamine-HCl $\times$ 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins, lactate and sulfide), sparge medium with 100% N₂ 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% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

310. *CLOSTRIDIUM THERMOCELLUM* MEDIUM

(NH₄)₂SO₄ 1.3 g
MgCl₂ $\times$ 6 H₂O 2.6 g
KH₂PO₄ 1.43 g
K₂HPO₄ 5.5 g
CaCl₂ $\times$ 2 H₂O 0.13 g
Na₂- β -glycerol phosphate $\times$ 4 H₂O 6.0 g
FeSO₄ $\times$ 7 H₂O solution (0.1% w/v in 0.1 N H₂SO₄) 1.1 ml
L-Glutathione reduced 0.25 g
Yeast extract 4.5 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Cellobiose 5.0 g
Distilled water 1000.0 ml

Dissolve ingredients except cellobiose, sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas and sterilized by filtration.

311. *CLOSTRIDIUM* MEDIUM I

K₂HPO₄ 3.0 g
KH₂PO₄ 2.0 g
NH₄Cl 2.0 g
MgCl₂ $\times$ 6 H₂O 0.2 g
CaCl₂ $\times$ 2 H₂O 0.05 g
Yeast extract 5.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
D-Glucose 5.0 g
L-Cysteine-HCl $\times$ H₂O 0.5 g
Na₂S $\times$ 9 H₂O 0.5 g
Distilled water 1000.0 ml

Dissolve ingredients except glucose, cysteine and sulfide. Sparge medium with 100% N₂ 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% N₂ gas. Adjust pH of the complete medium to 7.0 - 7.2, if

necessary.

312. *THERMOTOGA NEAPOLITANA* MEDIUM

Starch, soluble 5.0 g
KH₂PO₄ 0.5 g
Trace element solution (see below) 15.0 ml
NiCl₂ × 6 H₂O solution (0.1% w/v) 2.0 ml
Sea water (see below) 250.0 ml
Yeast extract 2.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
L-Cysteine-HCl × H₂O 0.5 g
Na₂S × 9 H₂O 0.5 g
Distilled water 750.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

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

Artificial sea water:

NaCl 27.7 g
MgSO₄ × 7 H₂O 7.0 g
MgCl₂ × 6 H₂O 5.5 g
KCl 0.65 g
NaBr 0.1 g
H₃BO₃ 30.0 mg
SrCl₂ × 6 H₂O 15.0 mg
Citric acid 10.0 mg
KI 0.05 mg
CaCl₂ × 2 H₂O 2.25 g
Distilled water 1000.0 ml

Dissolve ingredients (except sulfide and cysteine) and adjust pH to 6.5. Boil medium for 1 min, then cool to room temperature under 100% N₂ gas atmosphere. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of volume and autoclave. Add sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 6.5, if necessary.

313. *PYROCOCCUS* MEDIUM

NaCl 13.85 g

MgSO₄ × 7 H₂O 3.5 g
MgCl₂ × 6 H₂O 2.75 g
KCl 0.33 g
NaBr 0.05 g
H₃BO₃ 15.0 mg
SrCl₂ × 6 H₂O solution (0.1% w/v) 7.0 ml
(NH₄)₂SO₄ 10.0 mg
Citric acid solution (0.1% w/v) 5.0 ml
KI solution (0.01% w/v) 0.5 ml
CaCl₂ × 2 H₂O 0.75 g
KH₂PO₄ 0.5 g
NiCl₂ × 6 H₂O (0.1% w/v) 2.0 ml
Trace element solution (see below) 10.0 ml
Peptone (BD Bacto) 5.0 g
Yeast extract (OXOID) 1.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Sulfur, powdered 30.0 g
Neutralized sulfide solution 16.7 ml
Distilled water 1000.0 ml
Neutralized sulfide solution:
Na₂S × 9 H₂O 3.0 g
Distilled water 100.0 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 N H₂SO₄ 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.

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except sulfur and sulfide, adjust the pH to 6.5, and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 1 - 2 hours on each of 3 successive days. Add neutralized sulfide stock solution.

314. NATRANAEROBACULUM MEDIUM

KH₂PO₄ 0.2 g
MgCl₂ × 6 H₂O 0.1 g
NH₄Cl 1.0 g
KCl 0.2 g
NaCl 16.0 g
Trace element solution *SL-11* (see below) 1.0 ml
Yeast extract 0.2 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Na₂CO₃ 68.0 g
NaHCO₃ 38.0 g
Ethanol 5.0 ml
Vitamin solution (see below) 10.0 ml

Na₂S × 9 H₂O 1.0 g
Distilled water 1000.0 ml
Trace element solution SL-11:

Na₂-EDTA × 2 H₂O 5.2 g
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 1000.0 ml

Dissolve EDTA in 800.0 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 1000.0 ml.

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except carbonate, bicarbonate, ethanol, vitamins and sulfide. Sparge medium with 100% N₂ 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% N₂ gas. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 9.5 – 10.0, if necessary.

315. *FUCHSIELLA* MEDIUM

KH₂PO₄ 0.2 g
MgCl₂ × 6 H₂O 0.1 g
NH₄Cl 0.5 g
KCl 0.2 g
NaCl 60.0 g
Na₂CO₃ 68.3 g
NaHCO₃ 38.3 g

Trace element solution *SL-4* (see below) 1.0 ml

Resazurin 0.5 mg

Vitamin solution (see below) 10.0 ml

Yeast extract 0.05 g

Ethanol (50% v/v) 10.0 ml

Na₂S × 9 H₂O 1.0 g

NaNO₃ 0.85 g

Distilled water 1000.0 ml

Trace element solution SL-4:

EDTA 0.5 g

FeSO₄ × 7 H₂O 0.2 g

Trace element solution *SL-6* (see below) 100.0 ml

Distilled water 900.0 ml

Trace element solution SL-6:

ZnSO₄ × 7 H₂O 0.1 g

MnCl₂ × 4 H₂O 0.03 g

H₃BO₃ 0.3 g

CoCl₂ × 6 H₂O 0.2 g

CuCl₂ × 2 H₂O 0.01 g

NiCl₂ × 6 H₂O 0.02 g

Na₂MoO₄ × 2 H₂O 0.03 g

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 8.8- 9.5

Dissolve ingredients except the sodium carbonate, sodium bicarbonate, ethanol, and sodium sulfide. Boil medium for a few minutes and cool to room temperature while flushing with N₂.

Add and dissolve sodium carbonate, sodium bicarbonate and sodium sulfide while gassing the head space only. Dispense and autoclave under N₂. Before use add 10 ml/l (v/v) of 50% (v/v) ethanol solution (flushed and autoclaved under N₂).

316. *MELIORIBACTER* MEDIUM

Solution A:

NaCl 1.0 g

MgCl₂ × 6 H₂O 0.4 g

KCl 0.5 g

CaCl₂ × 2 H₂O 0.1 g

NH₄Cl 0.25 g

KH₂PO₄ 0.2 g

Na₂SO₄ 4.0 g

Trace element solution (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Yeast extract 2.0 g

NaHCO₃ 0.1 g

Distilled water 1000.0 ml

pH 7.2 – 7.8

Solution B:

Na₂S × 9 H₂O 3.0 g

Distilled water 100.0 ml

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O 784.0 mg

ZnSO₄ × 7 H₂O 143.6 mg

MnCl₂ × 4 H₂O 99.0 mg

Na₂SeO₄ 94.6 mg

H₃BO₃ 6.0 mg

Na₂WO₄ × 2 H₂O 33.0 mg

CuCl₂ × 2 H₂O 1.8 mg

CoCl₂ × 6 H₂O 238.0 mg

NiSO₄(NH₄)₂SO₄ × 6 H₂O 395.0 mg

Na₂MoO₄ × 2 H₂O 24.0 mg

conc. HCl 10.0 ml

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Autoclave and add the following component after autoclaving from stock solutions: sterile solution B Na₂S (3%) end concentration 0.3% and filter-sterilized vitamin solution (see below) 0.1 ml /10 ml.

317. *ORNATILINEA* MEDIUM

KH₂PO₄ 0.14 g
MgCl₂ × 6 H₂O 0.2 g
CaCl₂ × 2 H₂O 0.15 g
NH₄Cl 0.54 g
Trace element solution *SL-11* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 2.5 g
Yeast extract 2.3 g
Vitamins solution (see below) 10.0 ml

D-Glucose 2.2 g
L-Cysteine-HCl × H₂O 0.25 g

Na₂S × 9 H₂O 0.25 g
Distilled water 1000.0 ml

Trace element solution SL-11:

Na₂-EDTA × 2 H₂O 5.2 g

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 1000.0 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 1000.0 ml.

Selenite-tungstate solution:

NaOH 0.5 g

Na₂SeO₃ × 5 H₂O 3 mg

Na₂WO₄ × 2 H₂O 4 mg

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, yeast extract, vitamins, glucose and reducing agents), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add solid bicarbonate and adjust pH to 7.0. Dispense medium under 80% N₂ and 20% CO₂ 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% N₂ gas. Adjust pH of complete medium to 7.0, if necessary.

318. *TEPIDIBACILLUS* MEDIUM

Sea salts (SIGMA) 30.0 g

NaNO₃ 1.0 g

Wolfe's mineral elixier (see below) 2.0 ml

VOSO₄ × 5 H₂O solution (0.01% w/v) 0.5 ml

Na-resazurin solution (0.1% w/v) 0.5 ml

FeSO₄ × 7 H₂O 0.1 g

NaHCO₃ 1.0 g

Yeast extract 0.5 g

D-glucose 2.5 g

Vitamins solution (see below) 20.0 ml

Distilled water 1000.0 ml

Wolfe's mineral elixier:

MgSO₄ × 7 H₂O 30.0 g

MnSO₄ × H₂O 5.0 g

NaCl 10.0 g

FeSO₄ × 7 H₂O 1.0 g

CoCl₂ × 6 H₂O 1.8 g

CaCl₂ × 2 H₂O 1.0 g

ZnSO₄ × 7 H₂O 1.8 g

CuSO₄ × 5 H₂O 0.1 g

KAl(SO₄)₂ × 12 H₂O 0.18 g

H₃BO₃ 0.1 g

Na₂MoO₄ × 2 H₂O 0.1 g

(NH₄)₂Ni(SO₄)₂ × 6 H₂O 2.8 g

Na₂WO₄ × 2 H₂O 0.1 g

Na₂SeO₄ 0.1 g

Distilled water 1000.0 ml First adjust pH to 1.0 with diluted H₂SO₄, then add and dissolve the salts.

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except ferrous sulfate, bicarbonate, yeast extract, D-glucose and vitamins), adjust pH to 7.0 and sparge medium with 100% N₂ 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% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Prepare the stock solution of ferrous sulfate by dissolving 1% (w/v) FeSO₄ × 7 H₂O in 0.1 N H₂SO₄. The stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.8-7.0. 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% N₂ and filter-sterilized), if the medium is not completely reduced after inoculation.

319. *DEFERRISOMA* MEDIUM

KH₂PO₄ 0.33 g
NH₄Cl 0.33 g
KCl 0.33 g
NaCl 18.0 g
CaCl₂ × 2 H₂O 0.33 g
MgCl₂ × 6 H₂O 4.0 g
Yeast extract 0.2 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Fe(III)-citrate (19% Fe) 2.5 g
NaHCO₃ 2.5 g
Vitamin solution (see below) 10.0 ml

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g

Na₂SeO₃ × 5 H₂O 3 mg

Na₂WO₄ × 2 H₂O 4 mg

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except ferric citrate, bicarbonate and vitamins) and sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Sterilize vitamins by filtration. Adjust pH of the complete medium to 6.5, if necessary.

320. *THERMOSULFURIMONAS* MEDIUM

NaCl 18.0 g
MgCl₂ × 6 H₂O 4.0 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.33 g
(NH₄)₂SO₄ 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
NaHCO₃ 2.5 g
KH₂PO₄ 0.33 g
Vitamin solution (see below) 1.0 ml

Na-acetate × 3 H₂O 1.5 g
Na₂S₂O₃ × 5 H₂O 3.5 g
Yeast extract 0.2 g
Distilled water 1000.0 ml
Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:
NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3 mg
Na₂WO₄ × 2 H₂O 4 mg
Distilled water 1000.0 ml

Vitamin solution:
Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, phosphate, vitamins, acetate, thiosulfate and yeast extract, then sparge medium with 80% H₂ and 20% CO₂ 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 phosphate, vitamins, acetate, thiosulfate and yeast extract from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. The pH of the complete medium should be at 6.5 – 6.8.

321. *BROCKIA LITHOTROPHICA* MEDIUM

NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.44 g
MgCl₂ × 6 H₂O 0.7 g
NaCl 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Sulfur, powdered 10.0 g
NaHCO₃ 0.8 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 0.5 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3 mg
Na₂WO₄ × 2 H₂O 4 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4, and dispense medium under 80% H₂ and 20% CO₂ 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 bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins (sterilized by filtration) and sulfide are added to the medium from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 6.5, if necessary.

322. CLOSTRIDIUM MEDIUM II

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 1000.0 ml, and then add:

Casitone 30.0 g

Yeast extract 5.0 g

K₂HPO₄ 5.0 g

Resazurin solution (0.1% w/v) 0.5 ml

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% N₂ gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under same gas atmosphere 7.0 ml medium into Hungate-type tubes.

323. PSYCHROPHYLIC CLOSTRIDIUM MEDIUM

Tryptone 10.0 g

Gelatin peptone 10.0 g

Yeast extract 5.0 g

D-glucose 1.0 g

Sodium chloride 5.0 g

L-Arginine 1.0 g

Sodium pyruvate 1.0 g

Menadione 0.5 mg

Haemin 5.0 mg

Distilled water 990 ml

Dissolve all components 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 80% N₂ and 20% CO₂ gas mixture. Add L-cysteine-HCl × H₂O (0.3 g/l), dispense the medium into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving supplement medium with 5.0 g/l D-glucose added from a sterile anoxic stock solution sterilized by filtration. Adjust pH of medium to 6.8 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

324. SPHAEROCHAETA MEDIUM

NaCl 1.0 g

KCl 0.5 g

MgCl₂ × 6 H₂O 0.4 g

CaCl₂ × 2 H₂O 0.1 g

NH₄Cl 0.3 g

KH₂PO₄ 0.2 g

Na₂SO₄ 0.15 g

Xylose 20 mmol

Yeast extract 1.0 g

NaHCO₃ 0.5 g

Trace element solution *SL-7* (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Distilled water 990.0 ml

Trace mineral solution:

FeCl₂ × 4 H₂O 1.99 g
CoCl₂ 0.13 g
MnCl₂ × 4 H₂O 0.198 g
ZnCl₂ 0.136 g
H₃BO₃ 0.062 g
NiCl₂ × 6 H₂O 0.013 g
AlCl₃ × 6 H₂O 0.0133 g
Na₂MoO₄ × 2 H₂O 0.0242 g
CuCl₂ × 2 H₂O 0.017 g
Distilled water 1000.0 ml

Seven vitamins solution:

Vitamin B₁₂ 100.0 µg
p-Aminobenzoic acid 80.0 mg
D(+)-Biotin 20.0 mg
Nicotinic acid 200.0 mg
Calcium pantothenate 100.0 mg
Pyridoxine hydrochloride 300.0 mg
Thiamine-HCl × 2 H₂O 200.0 mg
Distilled water 1000.0 ml

Dissolve all components 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 80% N₂ and 20% CO₂ gas mixture. Add L-cysteine-HCl × H₂O (0.3 g/l), dispense the medium into anoxic Hungate-type tubes or serum vials and autoclave. Sodium hydrogen carbonate, vitamins, xylose and antibiotics (when necessary) were added to the autoclaved medium from sterile anaerobic stock solutions. Adjust pH of medium to 7.2-7.3 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

325. COPROBACTER MEDIUM

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

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 1000.0 ml, and then add:

Casitone 30.0 g
Yeast extract 5.0 g
K₂HPO₄ 5.0 g
Resazurin 1.0 mg

To make medium anoxic bring it to a boil, cool under 100% N₂ gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under 100% N₂ gas atmosphere by filling 7 ml medium into anoxic Hungate-type tubes. Autoclave at 121°C for 15 min. Add to 1000.0 ml of medium after autoclaving: Haemin solution (see below) 10.0 ml Vitamin K₁ or Vitamin K₃ solution (see below) 0.2 ml.

Haemin solution: Dissolve 50 mg haemin in 1.0 ml 1 N NaOH; make up to 100.0 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K₁ solution: Dissolve 0.1 ml of vitamin K₁/K₃ in 20.0 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K₃ solution: Dissolve 5 mg/ml of vitamin K₃ in 10.0 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

326. METHANOCALCULUS NATRONOPHILUS MEDIUM

KH₂PO₄ 0.2 g
MgCl₂ × 6 H₂O 0.1 g
NH₄Cl 1.0 g
KCl 0.2 g
NaCl 60.0 g
Trace element solution *SL-11* (see below) 1.0 ml
Na-acetate × 3 H₂O 0.2 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Na₂CO₃ 68.0 g
NaHCO₃ 38.0 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 1.0 g
Distilled water 1000.0 ml

Trace element solution SL-11:

Na₂-EDTA × 2 H₂O 5.2 g
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 1000.0 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 1000.0 ml.

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except carbonate, bicarbonate, vitamins and sulfide, then sparge medium with 80% H₂ and 20% CO₂ gas mixture 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 vitamins from an anoxic stock solution sterilized by filtration. Adjust pH of the complete medium to 9.0.

327. THERMOANAEROBACTER SIDEROPHILUS MEDIUM

Tryptone 1.0 g
Peptone (meat) 1.0 g
Yeast extract 1.0 g
K₂HPO₄ 1.6 g
NaH₂PO₄ × 2 H₂O 1.0 g
NH₄Cl 0.5 g

MgSO₄ × 6 H₂O 0.16 g
Trace element solution *SL-11* (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
CaCl₂ × 2 H₂O 0.06 g
NaHCO₃ 1.0 g
Vitamin solution (see below) 10.0 ml

L-Cysteine-HCl × H₂O 0.3 g

Na₂S × 9 H₂O 0.3 g

Distilled water 1000.0 ml

Trace element solution SL-11:

Na₂-EDTA × 2 H₂O 5.2 g

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 1000.0 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 1000.0 ml.

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.0 and sparge medium with 100% N₂ 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% N₂ gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are prepared under 100% N₂ gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

328. MOORELLA GLYCERINI MEDIUM

KH₂PO₄ 0.33 g

NH₄Cl 0.33 g

KCl 0.33 g

MgCl₂ × 6 H₂O 0.33 g

CaCl₂ × 2 H₂O 0.33 g

Yeast extract 0.5 g

Glycerol (87%) 3.0 ml

Trace element solution *SL-10* (see below) 1.0 ml

Selenite-tungstate solution (see below) 1.0 ml

Na-resazurin solution (0.1% w/v) 0.5 ml

NaHCO₃ 10.0 g

Vitamin solution (see below) 10.0 ml

Na₂S × 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g

Na₂SeO₃ × 5 H₂O 3 mg

Na₂WO₄ × 2 H₂O 4 mg

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, vitamins and sulfide, then sparge medium with 100% CO₂ gas for 30 – 45 min to make it anoxic. Dissolve bicarbonate 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% N₂ gas. Vitamins should be sterilized by filtration

329. *TEPIDIBACTER* MEDIUM

NaCl 18.0 g

MgCl₂ × 6 H₂O 4.0 g

KCl 0.34 g

NH₄Cl 0.25 g

CaCl₂ × 2 H₂O 0.11 g

K₂HPO₄ 0.18 g

Fe(NH₄)₂(SO₄)₂ × 6 H₂O 20.0 mg

Trace element solution *SL-10* (see below) 1.0 ml

Selenite-tungstate solution (see below) 1.0 ml

Yeast extract 0.2 g

Proteose peptone 10.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 5.0 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 0.5 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3 mg
Na₂WO₄ × 2 H₂O 4 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ 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) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. The pH of the complete medium should be 6.5 - 7.0.

330. CLOSTRIDIUM CELLULOLYTICUM MEDIUM

(NH₄)₂SO₄ 1.3 g
KH₂PO₄ 1.5 g
K₂HPO₄ × 3 H₂O 2.9 g
FeSO₄ × 7 H₂O solution (0.1% w/v in 0.1 N H₂SO₄) 1.25 ml
Trace element solution SL-10 (see below) 1.0 ml
Yeast extract 2.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
MgCl₂ × 6 H₂O 0.2 g
CaCl₂ × 2 H₂O 75.0 mg
Cellobiose 6.0 g

L-Cysteine-HCl $\times$ H₂O 0.5 g

Na₂CO₃ 2.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ $\times$ 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ $\times$ 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ $\times$ 6 H₂O 190.0 mg

CuCl₂ $\times$ 2 H₂O 2.0 mg

NiCl₂ $\times$ 6 H₂O 24.0 mg

Na₂MoO₄ $\times$ 2 H₂O 36.0 mg

Distilled water 990.0 ml

Dissolve ingredients except magnesium chloride, calcium chloride, cellobiose, cysteine and carbonate, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. After autoclaving add magnesium chloride, calcium chloride and cellobiose from anoxic stock solutions prepared under 100% N₂ gas. Cellobiose has to be sterilized by filtration. Prior to inoculation add cysteine from a sterile anoxic stock solution prepared under 100% N₂ gas and adjust pH to 7.2 by adding a sterile anoxic stock solution of Na₂CO₃ (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

331. THERMOCOCCUS STETTERI MEDIUM

NH₄Cl 0.33 g

CaCl₂ $\times$ 2 H₂O 0.33 g

MgCl₂ $\times$ 6 H₂O 0.33 g

KCl 0.33 g

KH₂PO₄ 0.33 g

Casitone 5.0 g

Trace element solution *SL-10* (see below) 1.0 ml

Na-resazurin solution (0.1% w/v) 0.5 ml

Sulfur, powder 10.0 g

NaCl 2.5 g

Vitamin solution (see below) 10.0 ml

Na₂S $\times$ 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ $\times$ 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ $\times$ 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ $\times$ 6 H₂O 190.0 mg

CuCl₂ $\times$ 2 H₂O 2.0 mg

NiCl₂ $\times$ 6 H₂O 24.0 mg

Na₂MoO₄ $\times$ 2 H₂O 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl $\times$ 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except sulfur, vitamins and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 5.7, dispense under 80% N₂ and 20% CO₂ 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. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use adjust pH of complete medium to 6.5 with sterile, anaerobic NaHCO₃-solution.

332. *THERMOGUTTA* MEDIUM

KH₂PO₄ 0.33 g

MgCl₂ $\times$ 6 H₂O 0.33 g

CaCl₂ $\times$ 2 H₂O 0.33 g

NH₄Cl 0.33 g

KCl 0.33 g

NaCl 6.0

Yeast extract 0.2 g

Xanthan 5.0 g

KNO₃ 2.0 g

NaHCO₃ 2.0 g

Trace element solution (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Trace element solution:

(NH₄)₂Fe(SO₄)₂ $\times$ 6 H₂O (Mohr's salt) 784.0 mg

CoCl₂ $\times$ H₂O 238.0 mg

(NH₄)₂Ni(SO₄)₂ $\times$ 6 H₂O 395.0 mg

Na₂MoO₄ $\times$ 2 H₂O 24.0 mg

Na₂WO₄ $\times$ 2 H₂O 33.0 mg

ZnSO₄ $\times$ 7 H₂O 144.0 mg

CuCl₂ $\times$ 2 H₂O 2.0 mg

Na₂SeO₄ 94.0 mg

H₃BO₃ 6.0 mg

MnCl₂ $\times$ 4 H₂O 99.0 mg

Mohr's salt is dissolved firstly in concentrated HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine 10.0 mg

Riboflavin 50.0 mg

Pantotenoic acid 50.0 mg

p-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B₁₂ 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

The media was boiled and cooled under the flow of oxygen-free gases (CO₂ or N₂) to render the media anaerobic and finally heat-sterilized at 121°C for 60 min. No reducing agents were added.

333. MEDIUM FOR PSYCHROPHILIC *METHANOSARCINA*

K₂HPO₄ 0.35 g

KH₂PO₄ 0.23 g

NH₄Cl 0.5 g

MgSO₄ × 7 H₂O 0.5 g

CaCl₂ × 2 H₂O 0.25 g

NaCl 2.25 g

FeSO₄ × 7 H₂O solution (0.1% w/v in 0.1 H₂SO₄) 2.0 ml

Trace element solution *SL-10* (see below) 1.0 ml

Yeast extract 2.0 g

Casitone 2.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

NaHCO₃ 0.85 g

Vitamin solution (see below) 10.0 ml

Methanol 10.0 ml

L-Cysteine-HCl × H₂O 0.3 g

Na₂S × 9 H₂O 0.3 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Then add and dissolve bicarbonate, adjust pH to 6.8 and dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N₂ gas

atmosphere as concentrated solutions in tightly closed tubes. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Appropriate volumes of the solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.

334. *METHANOSPIRILLUM* MEDIUM

KH₂PO₄ 0.23 g
K₂HPO₄ 0.23 g
MgSO₄ × 7 H₂O 0.09 g
NaCl 0.46 g
NH₄Cl 0.4 g
CaCl₂ × 2 H₂O 60.0 mg
(NH₄)₂SO₄ × 7 H₂O 0.23 g
Trace element solution *SL-10* (see below) 10.0 ml
Vitamin solution (see below) 10.0 ml
Yeast extract 1.0 g
Na-acetate × 3 H₂O 1.0 g
Na-formate 2.0 g
Trypticase 0.5 g
Casamino acids 0.2 g
Na-resazurin solution (0.1% w/v) 0.5 ml

NaHCO₃ 4.0 g
L-Cysteine-HCl × H₂O 0.5 g
Na₂S × 9 H₂O 0.5 g

Distilled water 980.0 ml
Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, cysteine and sulfide. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes

and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

335. CARNOBACTERIUM MEDIUM

Trypticase soy broth 30.0 g
Yeast extract 3.0 g
NaCl 13.0 g
KCl 0.34 g
MgCl₂ × 6 H₂O 4.0 g
MgSO₄ × 7 H₂O 3.45 g
NH₄Cl 0.25 g
CaCl₂ × 2 H₂O 0.14 g
Distilled water 1000.0 ml
Adjust pH to 7.

336. ALKALIPHILIC SPIROCHAETE MEDIUM

Na₂CO₃ 10.0 g
NaHCO₃ 15.0 g
NaCl 10.0 g
K₂HPO₄ 0.2 g
NH₄Cl 1.0 g
KCl 0.2 g
Na₂S × 9 H₂O 1.0 g
Yeast extract 0.5 g
Sucrose 5.0 g
Vitamin solution (see below) 10.0 ml
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Prepare the medium under N₂, omitting Na₂CO₃, NaHCO₃, Na₂S × 9 H₂O, sucrose and vitamin solution. Boil the medium and cool under nitrogen, add the Na₂CO₃ and NaHCO₃ and adjust the pH to 9.7 with 6N NaOH (about 15 ml). Autoclave at 121°C for 15 min and add the Na₂S × 9 H₂O (neutralized), vitamins and sucrose from sterile stock solutions.

337. CUNICULIPLASMA MEDIUM

Beef extract 3 g
Betaine 0.6 g
(NH₄)₂SO₄ 1.3 g
KH₂PO₄ 0.28 g
MgSO₄ × 7 H₂O 0.25 g
CaCl₂ × 2 H₂O 0.07 g
FeCl₃ × 6 H₂O 0.02 g
Trace element solution *SL-10* (see below) 1.0 ml
Vitamin solution (see above) 10.0 ml
Distilled water 990.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

myo-Inositol 1.0 g
Thiamine-HCl 100.0 mg
Pyridoxine-HCl 100.0 mg
Folic acid 40.0 mg
D-Biotin 10.0 mg
L-Ascorbic acid 200.0 mg
D-Ca- pantothenate 100.0 mg
Choline chloride 100.0 mg
Nicotinamide 100.0 mg
Riboflavin 20.0 mg
Vitamin B₁₂ 20.0 µg
Vitamin A 10.0 mg
p-Aminobenzoic acid 20.0 mg
Distilled water 990.0 ml

The medium was adjusted to pH 1.0–1.2 with concentrated H₂SO₄. The cultures were incubated for about 5 days at 37°C with shaking.

338. MAGNETOSPIRILLUM MEDIUM

KH₂PO₄ 0.68 g
NaNO₃ 0.12 g
L(+)-Tartaric acid 0.37 g
Succinic acid 0.37 g
Na-acetate × 3 H₂O 0.05 g
Vitamin solution (see below) 10.0 ml
Trace element solution (see below) 5.0 ml
Fe(III) quinate solution (see below) 2.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Na-thioglycolate 0.05 g
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Ferric Quinate Solution, 0.01 M:

FeCl₃ × 6 H₂O 4.5 g
Quinic acid 1.9 g
Distilled water 1000.0 ml

Sterilize by filtration under 100% N₂ gas atmosphere.

Dissolve ingredients (except thioglycolate) and adjust pH to 6.75 with NaOH. Sparge medium with 100% N₂ gas for 30-45 min and dispense under the same gas atmosphere into anoxic Hungate – type tubes to 50% of their volume. Before inoculation add thioglycolate from a 0.5% (w/v) stock solution, freshly prepared under 100% N₂ gas and filter-sterilized. Then add sterile air to a concentration of 2.5 % O₂ in the vial.

339. ACETOBACTERIUM MEDIUM

NH₄Cl 1.0 g
KH₂PO₄ 0.33 g
K₂HPO₄ 0.45 g
MgSO₄ × 7 H₂O 0.1 g
Trace element solution (see below) 20.0 ml
Yeast extract 2.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 10.0 g
D-Fructose 10.0 g
Vitamin solution (see below) 10.0 ml
L-Cysteine-HCl × H₂O 0.5 g
Na₂S × 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine and sulfide, bring to the boil and cool to room temperature under 80% N₂ and 20% CO₂ 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 8.0 - 8.2 by adding a sterile anoxic stock solution of

sodium carbonate (5% w/v) prepared under 80% N₂ and 20% CO₂ gas mixture (0.25 ml per 10.0 ml medium) and add fructose, vitamins (sterilized by filtration), cysteine and sulfide from anoxic sterile stock solutions prepared under 100% N₂.

340. DISSULFURIMICROBIUM MEDIUM

KH₂PO₄ 0.33 g
NH₄Cl 0.33 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.33 g
MgCl₂ × 6 H₂O, 0.33 g
NaHCO₃ 2.0 g
Vitamin solution (see below) 10.0 ml
Trace element solution (see below) 1.0 ml
Ferrihydrite
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4

Trace element solution:

Na₂-EDTA × 2 H₂O 5.2 g
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 1000.0 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 1000.0 ml.

The pH of the autoclaved medium was 6.7–6.8.

Medium (10.0 ml) was dispensed into 17 ml Hungate tubes; the headspace was filled with CO₂ (100 %).

341. RUTHENIBACTERIUM MEDIUM

Trypticase peptone 5.0 g
Peptone 5.0 g
Yeast extract 10.0 g

Beef extract 5.0 g
Glucose 5.0 g
K₂HPO₄ 2.0 g
Tween 80 1.0 ml
L-Cysteine-HCl × H₂O 0.5 g
Resazurin 1.0 mg
Salt solution (see below) 40.0 ml
Distilled water 950.0 ml
Haemin solution (see below) 10.0 ml
Vitamin K₁ solution (see below) 0.2 ml

The vitamin K₁, haemin solution and the cysteine are added after the medium has been boiled and cooled under CO₂. Adjust pH to 7.2 using 8 N NaOH. Distribute under N₂ and autoclave.

Salt solution:

CaCl₂ × 2 H₂O 0.25 g
MgSO₄ × 7 H₂O 0.5 g
K₂HPO₄ 1.0 g
KH₂PO₄ 1.0 g
NaHCO₃ 10.0 g
NaCl 2.0 g

Distilled water 1000.0 ml

Haemin solution: Dissolve 50 mg haemin in 1.0 ml 1 N NaOH; make up to 100.0 ml with distilled water. Store refrigerated.

Vitamin K₁ solution: Dissolve 0.1 ml of vitamin K₁ in 20.0 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

342. RHODOVULUM MEDIUM

Yeast extract 0.3 g
Na₂-succinate 1.0 g
(NH₄)-acetate 0.5 g
Fe(III) citrate solution (0.1% in H₂O) 5.0 ml
KH₂PO₄ 0.5 g
MgSO₄ × 7 H₂O 0.4 g
NaCl 0.4 g
NH₄Cl 0.4 g
CaCl₂ × 2 H₂O 0.05 g
Vitamin B₁₂ solution (10 mg in 100 ml H₂O) 0.4 ml
Trace element solution SL-6 (see below) 1.0 ml
L-Cysteine chloride × H₂O 0.3 g
Resazurin (0.1%) 0.5 ml
Distilled water 1000.0 ml

Adjust pH to 6.8. Boil the medium for a few minutes. Bubble the medium with nitrogen gas and fill 10.0 ml in 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:

ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.3 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.02 g
Na₂MoO₄ × 2 H₂O 0.03 g

Distilled water 1000.0 ml

343. HALOMONAS MEDIUM

NaCl 80.0 g

Casamino acids 7.5 g

Proteose peptone 5.0 g

Yeast extract 1.0 g

Sodium citrate 3.0 g

MgSO₄ × 7 H₂O 20.0 g

K₂HPO₄ 0.5 g

Fe(NH₄)₂(SO₄)₂ × 6 H₂O 0.05 g

Distilled water 1000.0 ml

Adjust pH to 7.0 with KOH.

Autoclave at 121°C for 15 min.

344. COLUMBIA AGAR

Special mixture of peptones 23.0 g

Starch 1.0 g

NaCl 5.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.3±0.2.

Adjust pH to 7.3±0.2. Autoclave at 121°C for 15 min.

345. NATRONOSPIRILLUM SPERANDUS MEDIUM

NaCl 34.0 g

NaHCO₃ 20.0 g

K₂HPO₄ 0.3 g

MgCl₂ × 6 H₂O 0.12 g

CaCl₂ × 2 H₂O 0.03 g

Casamino acids 3.0 g

Yeast extract 0.1 g

Agar 20.0 g

Trace element solution (see below) 10.0 ml

Distilled water 1000.0 ml. Autoclave at 121°C for 15 min.

Final pH 9.0 – 9.5

Trace element solution:

FeSO₄ × 7 H₂O 0.6 mg

CoCl₂ × 6 H₂O 0.12 mg

Ni(NH₄)₂(SO₄)₂ × 6 H₂O 0.9 mg

MnCl₂ × 4 H₂O 0.1 mg

ZnSO₄ × 7 H₂O 0.14 mg

H₃BO₃ 0.03 mg

AlK(SO₄)₂ × 12 H₂O 0.24 mg

Na₂MoO₄ × 2 H₂O 0.02 mg

Na₂WO₄ × 2 H₂O 0.03 mg

Na₂SeO₃ 0.02 mg

CuCl₂ × 2 H₂O 0.02 mg

Distilled water 1000.0 ml

Autoclave the trace element solution and NaHCO₃ separately at 121°C for 15 min and add to the medium.

Final pH 8.7 – 8.9

346. THERMAEROBACTER BAIKALENSIS MEDIUM

Peptone 1.25 g
Yeast extract 0.25 g
Fe(III) citrate 0.025 g
NaCl 5.0 g
 $\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 1.0 g
 Na_2SO_4 0.8 g
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.45 g
KCl 0.1 g
 NaHCO_3 0.4 g
KBr 0.02 g
 $\text{SrCl}_2 \times 6 \text{ H}_2\text{O}$ 0.03 g
 H_3BO_3 0.02 g
Agar 20.0 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml. Autoclave at 121°C for 15 min.
Final pH 7.0

Trace element solution:

Na-silicate 4.0 g
NaF 2.4 g
 NH_4NO_3 1.6 g
 Na_2HPO_4 8.0 g
Distilled water 1000.0 ml. Autoclave the trace element solution separately at 121°C for 15 min and add to the medium.

347. SUCCINATE MINIMAL SALT MEDIUM (SMS)

EDTA 0.01 g
 KH_2PO_4 0.6 g
 K_2HPO_4 0.9 g
 NH_4Cl 1.0 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.2 g
 $\text{CaCl}_2 \times 6 \text{ H}_2\text{O}$ 0.075 g
Na-succinate 2.2 g
Yeast extract 0.1 g
Trace elements solution (see below) 2.0 ml
Vitamin solution (see below) 2.0 ml
Distilled water 1000.0 ml

Trace elements solution:

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 300.0 mg
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 5.0 mg
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 3.0 mg
 H_3BO_3 2.0 mg
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 5.0 mg
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 1.0 mg
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 2.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 3.0 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 80.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 400.0 mg
Nicotinic acid 400.0 mg

Vitamin B₁₂ 20.0 µg

Distilled water 1000.0 ml

Adjust pH to 6.8. Prepare the medium without the vitamin solution. Autoclave at 121°C for 15 min and add the vitamin solution from a filter-sterilized stock solution.

348. RO (RICH ORGANIC) MEDIUM

MgSO₄ × 7 H₂O 0.3 g

KH₂PO₄ 0.3 g

NH₄Cl 0.1 g

CaCl₂ × 2 H₂O 1.0 g

Na-acetate × 3 H₂O 1.0 g

Yeast Extract 0.5 g

Peptone 0.5 g

Casamino Acid 0.5 g

Trace elements solution (see below) 2.0 ml

Vitamin solution (see below) 2.0 ml

Distilled water 1000.0 ml

Trace elements solution:

FeSO₄ × 7 H₂O 300.0 mg

ZnSO₄ × 7 H₂O 5.0 mg

MnCl₂ × 4 H₂O 3.0 mg

H₃BO₃ 2.0 mg

CoCl₂ × 6 H₂O 5.0 mg

CuCl₂ × 2 H₂O 1.0 mg

NiCl₂ × 6 H₂O 2.0 mg

Na₂MoO₄ × 2 H₂O 3.0 mg

Distilled water 1000.0 ml

Vitamin solution:

Biotin 80.0 mg

Thiamine-HCl × 2 H₂O 400.0 mg

Nicotinic acid 400.0 mg

Vitamin B₁₂ 20.0 µg

Distilled water 1000.0 ml

Autoclave base medium, KH₂PO₄ (in 10.0 ml distilled water) and trace element solution at 121°C for 15 min. Vitamin solution sterilize by filtration.

349. TAUTONIA SOCIABILIS MEDIUM

KH₂PO₄ 0.33 g

MgCl₂ × 6 H₂O 0.33 g

CaCl₂ × 2 H₂O 0.33 g

NH₄Cl 0.33 g

KCl 0.33 g

Xylose or trehalose 1.0 g

Trace element solution (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Distilled water 1000.0 ml

Autoclave base medium, KH₂PO₄ (in 10.0 ml distilled water) and trace element solution at 121°C for 15 min. Vitamin solution sterilize by filtration. Adjust pH to 7.5-7.7.

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O (*Mohr's salt*) 784.0 mg

HCl (concentrated) 5.0 ml

CoCl₂ × H₂O 238.0 mg

$(\text{NH}_4)_2\text{SO}_4\text{NiSO}_4 \times 6 \text{ H}_2\text{O}$ 395.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 33.0 mg

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 144.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

Na_2SeO_4 94.0 mg

H_3BO_3 6.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 99.0 mg

Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with 1000.0 ml distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine 100.0 mg

Riboflavin 50.0 mg

Pantotenoic acid 50.0 mg

p-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B₁₂ 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

Adjust pH to 5.9.

350. THERMOGEMMATA POLYSACCHARIDOLYTICA MEDIUM

KH_2PO_4 0.165 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.165 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.165 g

NH_4Cl 0.165 g

KCl 0.165 g

Maltose or trehalose 1.0 g

Trace element solution (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Distilled water 1000.0 ml

Trace element solution:

$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \times 6 \text{ H}_2\text{O}$ (Mohr's salt) 784.0 mg

HCl (concentrated) 5.0 ml

$\text{CoCl}_2 \times \text{H}_2\text{O}$ 238.0 mg

$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \times 6 \text{ H}_2\text{O}$ 395.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 33.0 mg

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 144.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

Na_2SeO_4 94.0 mg

H_3BO_3 6.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 99.0 mg

Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg

Folic acid 20.0 mg
Pyridoxine 100.0 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml
Autoclave base medium, KH₂PO₄ (in 10.0 ml distilled water) and trace element solution at 121°C for 15 min. Vitamin solution sterilize by filtration. Adjust pH to 7.0-7.5.

351. *PSEUDOALTEROMONAS SPIRALIS* MEDIUM

MgSO₄ × 7 H₂O 0.5 g
KH₂PO₄ 0.3 g
NH₄Cl 0.3 g
CaCl₂ × 2 H₂O 0.05 g
Na-acetate × 3 H₂O 1.0 g
Yeast extract 0.5 g
Casamino acids 0.5 g
NaCl 15.0 g
Trace elements solution (see below) 2.0 ml
Vitamin solution (see below) 2.0 ml
Distilled water 1000.0 ml

Trace elements solution:

MnCl₂ × 4 H₂O 0.3 g
FeSO₄ × 7 H₂O 0.03 g
Distilled water 1000.0 ml

Vitamin solution:

Biotin 80 mg
Thiamine-HCl 400.0 mg
Nicotinic acid 400.0 mg
Vitamin B₁₂ 20.0 µg
Distilled water 1000.0 ml

After autoclaving at 121°C for 15 min adjust to pH 7.8 with NaHCO₃. Add the vitamin solution and trace elements solution from sterile stock solutions.

352. *THERMUS* MEDIUM

Bacto Yeast Extract (Difco) 1.0 g
Bacto Tryptone (Difco) 1.0 g
Na- glutamate × H₂O 1.0 g
CaSO₄ × 2 H₂O 0.06 g
MgSO₄ × 7 H₂O 0.1 g
NaCl 0.08 g
KNO₃ 0.1 g
NaNO₃ 0.69 g
Na₂HPO₄ 0.11 g
Trace element solution (see below) 10.0 ml
Agar, if necessary 20.0 g
Distilled water to 1000.0 ml
Adjust pH to 7.5 with NaOH. Autoclave at 121°C for 15 min.

Trace elements solution:

Nitrilotriacetic acid 12.8 g

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.35 g

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.1 g

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.024 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 g

ZnCl_2 0.1 g

CuCl_2 0.025 g

H_3BO_3 0.01 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.024 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.12 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ 0.04 g

Distilled water 1000.0 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with NaOH, and then add minerals. Final pH 7.0.

353. ALCALIPHILIC AMPHIBACILLUS MEDIUM

KH_2PO_4 0.2 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.1 g

NH_4Cl 0.5 g

KCl 0.2 g

Na_2CO_3 63.6 g

NaHCO_3 50.4 g

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.7 g

Yeast extract 0.2 g

Sucrose 5.0 g

Trace elements (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Resazurin 0.01 g

Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 3.0 g

$\text{MnSO}_4 \times \text{H}_2\text{O}$ 0.5 g

NaCl 1.0 g

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g

$\text{CoSO}_4 \times 7 \text{ H}_2\text{O}$ 0.18 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 g

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.18 g

$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$ 0.01 g

$\text{KAl}(\text{SO}_4)_2 \times 12 \text{ H}_2\text{O}$ 0.02 g

H_3BO_3 0.01 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.01 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.03 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ 0.3 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 0.4 mg

Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Final pH 9.5-10.0. Prepare the medium without adding the vitamins, sucrose, yeast extract, NH₄Cl, Na₂CO₃, and NaHCO₃ using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH₄Cl, Na₂CO₃, and NaHCO₃ 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, sucrose, yeast extract, and Na₂S $\times 9 \text{ H}_2\text{O}$ from anaerobic, sterile stock solutions.

354. BACTO MARINE BROTH (DIFCO 2216)

Bacto peptone 5.0 g
Bacto yeast extract 1.0 g
Fe(III) citrate 0.1 g
NaCl 80.0 g
MgCl₂ (anhydrous) 5.9 g
Na₂SO₄ 3.24 g
CaCl₂ $\times 2 \text{ H}_2\text{O}$ 1.8 g
KCl 0.55 g
NaHCO₃ 0.16 g
KBr 0.08 g
SrCl₂ 34.0 mg
H₃BO₃ 22.0 mg
Na-silicate 4.0 mg
NaF 2.4 mg
NH₄NO₃ 1.6 mg
Na₂HPO₄ 8.0 mg
Distilled water 1000.0 ml C.

Final pH should be 7.6 ± 0.2 at 25

If using the complete medium from Difco add 37.4 g per liter water + 6% NaCl.

355. CLOSTRIDIUM PYG MEDIUM

Trypticase peptone 5.0 g
Peptone from meat (pepsin-digested) 5.0 g
Yeast extract 10.0 g
Salt solution (see below) 40.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
L-Cysteine-HCl $\times \text{H}_2\text{O}$ 0.5 g
Na₂CO₃ 2.5 g
D-Glucose 5.0 g
Distilled water 1000.0 ml

Salt solution:

CaCl₂ $\times 2 \text{ H}_2\text{O}$ 0.25 g
MgSO₄ $\times 7 \text{ H}_2\text{O}$ 0.5 g
K₂HPO₄ 1.0 g
KH₂PO₄ 1.0 g

NaHCO₃ 10.0 g

NaCl 2.0 g

Distilled water 1000.0 ml

Dissolve ingredients (except cysteine, carbonate and glucose) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add cysteine, then dispense under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose from a sterile anoxic stock solution prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Adjust pH of complete medium to 7.0, if necessary.

356. ANOYXNATRONUM MEDIUM

Na₂CO₃ 25.0 g

NaHCO₃ 25.0 g

KCl 0.2 g

MgCl₂ × 6 H₂O 0.1 g

NH₄Cl 0.5 g

K₂HPO₄ 0.2 g

Yeast extract 0.2 g

Trace elements *SL-10* (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Na₂S × 9 H₂O 0.7 g

Glucose 5.0 g

Distilled water 1000.0 ml, pH 9.0

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Prepare the medium anaerobically without the vitamins, Na₂S × 9 H₂O, yeast extract, NaHCO₃, and Na₂CO₃. Boil the medium, cool under N₂, add the NaHCO₃, and Na₂CO₃, dispense under N₂ and autoclave the medium. Add the vitamin solution, yeast extract, Na₂S × 9 H₂O and glucose from sterile stock solution prepared under N₂ to the cooled, autoclaved medium.

357. THERMOLITHOBACTER MEDIUM

KCl 0.33 g
 MgCl₂ × 6 H₂O 0.52 g
 CaCl₂ × 2 H₂O 0.29 g
 NH₄Cl 0.33 g
 KH₂PO₄ 0.33 g
 Trace element solution *SL-11* (see below) 1.0 ml
 Na-resazurin solution (0.1% w/v) 0.5 ml
 NaHCO₃ 1.0 g
 Yeast extract 0.05 g
 Vitamin solution (see below) 10.0 ml
 Na₂S × 9 H₂O 0.7 g
 Distilled water 1000.0 ml
Trace element solution SL-11:
 Na₂-EDTA × 2 H₂O 5.2 g
 FeCl₂ × 4 H₂O 1.5 g
 ZnCl₂ 70.0 mg
 MnCl₂ × 4 H₂O 100.0 mg
 H₃BO₃ 6.0 mg
 CoCl₂ × 6 H₂O 190.0 mg
 CuCl₂ × 2 H₂O 2.0 mg
 NiCl₂ × 6 H₂O 24.0 mg
 Na₂MoO₄ × 2 H₂O 36.0 mg
 Distilled water 1000.0 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 1000.0 ml.
Vitamin solution:
 Biotin 2.0 mg
 Folic acid 2.0 mg
 Pyridoxine-HCl 10.0 mg
 Thiamine-HCl × 2 H₂O 5.0 mg
 Riboflavin 5.0 mg
 Nicotinic acid 5.0 mg
 D-Ca-pantothenate 5.0 mg
 Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
 Lipoic acid 5.0 mg
 Distilled water 1000.0 ml
 Dissolve ingredients except bicarbonate, yeast extract, vitamins and sulfide, then sparge medium with 100% N₂ 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 yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. After completing the medium adjust pH to 6.8-7.0. Inoculated vessels are pressurized with sterile carbon monoxide gas to 2 bar overpressure.

358. THERMOANAEROBACTERIUM MEDIUM

KH₂PO₄ 0.3 g
 Na₂HPO₄ × 12 H₂O 5.3 g
 NH₄Cl 1.0 g
 MgCl₂ × 6 H₂O 0.2 g
 Trace elements solution (see below) 10.0 ml

FeSO₄ × 7 H₂O solution (0.1% w/v in 0.1 N H₂SO₄) 1.5 ml

Yeast extract 1.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

D-Glucose 5.0 g

Vitamin solution (see below) 5.0 ml

Na₂S × 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid (NTA) 12.8 g

FeCl₂ × 4 H₂O 0.2 g

MnCl₂ × 4 H₂O 0.1 g

CoCl₂ × 6 H₂O 0.17 g

CaCl₂ × 2 H₂O 0.1 g

ZnCl₂ 0.1 g

CuCl₂ × 2 H₂O 0.02 g

H₃BO₃ 0.01 g

Na₂MoO₄ × 2 H₂O 0.01 g

NiCl₂ × 6 H₂O 0.03 g

NaCl 1.0 g

Na₂SeO₃ × 5 H₂O 0.03 g

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 1000.0 ml.

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except glucose, vitamins and sulfide), adjust pH to 6.0, sparge medium with 100% N₂ 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% N₂ gas and vitamins from a filter-sterilized anoxic stock solution prepared under 100% N₂. Adjust pH of complete medium to 6.0 – 6.5, if necessary.

359. DESULFOVERMICULUS MEDIUM

NaCl 100.0 g

MgSO₄ × 7 H₂O 10.0 g

KCl 6.0 g

CaCl₂ × 2 H₂O 0.4 g

NH₄Cl 1.0 g

KH₂PO₄ 0.1 g

Yeast extract 0.5 g

Trace element solution *SL-10* (see below) 1.0 ml

Selenite-tungstate solution (see below) 1.0 ml

NaHCO₃ 4.0 g

Na-(DL)-malate 1.0 g

Resazurin 0.5 mg

Na₂S × 9 H₂O 0.3 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g

Na₂SeO₃ × 5 H₂O 3 mg

Na₂WO₄ × 2 H₂O 4 mg

Distilled water 1000.0 ml

Dissolve ingredients (except lactate, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N₂ and 20% CO₂ gas mixture. Dispense under same gas atmosphere in culture vessels and autoclave at 121°C 15 min. Add sodium lactate and sulfide from sterile anoxic stock solutions prepared under N₂ and bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ (all solutions sterilize separately at 121°C 15 min).

Final pH of the medium 7.0-7.2.

360. METHANOSARCINA MEDIUM

NaCl 5.0 g

MgCl₂ × 6 H₂O 0.2 g

CaCl₂ × 2 H₂O 0.1 g

NH₄Cl 1.0 g

Casamino acids 1.0 g

Resazurin 0.01 g

Methanol 10% 10.0 ml

Trace element solution (see below) 10.0 ml

Vitamin solution (see below) 5.0 ml

L-Cysteine chloride × H₂O 0.5 g

Distilled water 965.0 ml

Buffer solutions:

a) K₂HPO₄ 29.0 g

Distilled water 100.0 ml

b) KH₂PO₄ 15.0 g

Distilled water 100.0 ml

Trace element solution:

Nitrilotriacetic acid 12.8 mg

FeSO₄ × 7 H₂O 0.1 mg

MnCl₂ × 4 H₂O 0.1 mg

CoCl₂ × 2 H₂O 0.17 mg

CaCl₂ × 2 H₂O 0.1 mg

ZnCl₂ 0.1 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 0.02 mg
 H_3BO_3 0.01 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 0.01 mg
NaCl 1.0 mg
 Na_2SeO_4 0.017 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N₂ without O₂ up to sterilization. Solutions of buffer (per 1 ml) add to base medium after separate sterilization. Base medium, trace element and buffer solutions autoclave at 121°C for 15 min. Vitamin solution is filter sterilized.

361. METHANOBACTERIUM ARCTICUM MEDIUM

Solution 1:

KCl 0.34 g
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 0.4 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.35 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.1 g
 NH_4Cl 0.25 g
 KH_2PO_4 0.14 g
NaCl 5.0 g
Resazurin 0.01 g
 NaHCO_3 5.0 g
Trace element solution (see below) 10.0 ml
Distilled water 965.0 ml

Solution 2 (reducing agents):

L-Cysteine chloride $\times \text{H}_2\text{O}$ 0.5 g
 $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 0.5 g
Distilled water 10.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 3.0 g
 $\text{MnSO}_4 \times 7 \text{H}_2\text{O}$ 0.5 g
NaCl 1.0 g
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g
 CoSO_4 0.18 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.1 g
 ZnSO_4 0.18 g
 CuSO_4 0.01 g
 $\text{KAl}(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$ 0.02 g

H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.025 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Distilled water 1000.0 ml
pH 7.0-7.4.

Prepare medium in anaerobic conditions, blowing through with N₂ without O₂ up to sterilization. Solutions of reducing agents (10.0 ml) and others solutions add to base medium after separate sterilization at 121°C for 15 min. Sterilize vitamin solution by filtration. Cultivate in a gas mixture of 80% H₂ and 20% CO₂.

362. *GEOALKALIBACTER* MEDIUM

NH₄Cl 0.5 g
KCl 0.2 g
MgCl₂ × 6 H₂O 0.1 g
KH₂PO₄ 0.2 g
NaCl 1.0 g
Yeast extract 0.1 g
Trace element solution *SL-10* (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Na₂CO₃ 3.0 g
NaHCO₃ 10.0 g
Sulfur, powdered 10.0 g
Na-acetate × 3 H₂O 2.5 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g
Na₂SeO₃ × 5 H₂O 3.0 mg
Na₂WO₄ × 2 H₂O 4.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except carbonates, sulfur and acetate), then sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Add solid carbonate and bicarbonate, adjust pH to 9.0 - 9.2, dispense under 100% N₂ 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 water bath to 90 – 100°C for 1 – 2 hours on each of 3 successive days. Add acetate from a sterile anoxic stock solution prepared under 100% N₂ gas.

363. *ANOXYBACILLUS* MEDIUM

KH₂PO₄ 0.2 g
MgCl₂ × 6 H₂O 0.1 g

KCl 0.2 g
NH₄Cl 1.0 g
NaCl 5.0 g
Trace element solution *SL-10* (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Na₂CO₃ 2.76 g
NaHCO₃ 10.0 g
Yeast extract 0.5 g
D-Glucose 5.0 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 0.5 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve all ingredients except carbonates, yeast extract, glucose, vitamins and sulfide, then sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Add carbonate and hydrogencarbonate, adjust pH to 9.0 - 9.5, dispense medium under 100% N₂ 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% N₂ gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 9.5 – 9.7.

364. CALDANAEROBACTER MEDIUM

KCl 0.33 g
MgCl₂ × 6 H₂O 0.52 g
CaCl₂ × 2 H₂O 0.29 g
NH₄Cl 0.33 g
KH₂PO₄ 0.33 g
Trace element solution *SL-4* (see below) 10.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 2.5 g
D-glucose 2.0 g

Yeast extract 0.05 g
Vitamin solution 1 (see below) 9.0 ml
Seven vitamins solution (see below) 1.0 ml
 $\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.3 g
Distilled water 1000.0 ml

Trace element solution SL-4:

$\text{Na}_2\text{-EDTA}$ 0.5 g
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.2 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.03 g
 H_3BO_3 0.3 g
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 g
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 0.01 g
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.02 g
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.03 g
Distilled water 1000.0 ml

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

Vitamin solution 1:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Seven vitamins solution:

Vitamin B₁₂ 100.0 mg
p-Aminobenzoic acid 80.0 mg
D(+)-Biotin 20.0 mg
Nicotinic acid 200.0 mg
D-Ca-pantothenate 100.0 mg
Pyridoxine-HCl 300.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 200.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, D-glucose, yeast extract, vitamins and sulfide, then sparge medium with 80% N₂ and 20% CO₂ 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 and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ 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 Na₂CO₃ (5% w/v), if necessary.

365. THERMOANAEROBACTER MEDIUM II

NH₄Cl 1.0 g
NaCl 0.1 g

MgCl₂ × 6 H₂O 0.1 g
CaCl₂ × 2 H₂O 0.05 g
K₂HPO₄ × 3 H₂O 0.4 g
Trace element solution (see below) 10.0 ml
Yeast extract 0.75 g
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 2.6 g
Cellobiose 4.0 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 0.25 g
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.3 mg
Na₂WO₄ × 2 H₂O 0.4 mg
Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, cellobiose, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of cellobiose and vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

366. *DESULFUROCOCCUS KAMCHATKENSIS* MEDIUM

NH₄Cl 0.33 g

KH₂PO₄ 0.33 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.44 g
MgCl₂ × 6 H₂O 0.7 g
NaCl 0.5 g
Trace element solution *SL-10* (see below) 1.0 ml
Yeast extract (OXOID) 0.2 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Sulfur, powdered 10.0 g
D-Glucose 2.5 g
Vitamin solution (see below) 10.0 ml
Na₂S × 9 H₂O 0.5 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients (except sulfur, glucose, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Adjust pH to 6.2 - 6.4 and dispense medium under 80% N₂ and 20% CO₂ 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% N₂ gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

367. ANAEROBRANCA MEDIUM

KH₂PO₄ 0.5 g
Na₂HPO₄ × 2 H₂O 3.9 g
KCl 0.5 g
Yeast extract 5.0 g
Trace element solution (see below) 5.0 ml
Na₂-fumarate 1.5 g

Vitamin solution (see below) 10.0 ml

L-Cysteine-HCl $\times$ H₂O 0.15 g

Na₂S $\times$ 9 H₂O 0.15 g

Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g

MgSO₄ $\times$ 7 H₂O 3.0 g

MnSO₄ $\times$ H₂O 0.5 g

NaCl 1.0 g

FeSO₄ $\times$ 7 H₂O 0.1 g

CoSO₄ $\times$ 7 H₂O 0.18 g

CaCl₂ $\times$ 2 H₂O 0.1 g

ZnSO₄ $\times$ 7 H₂O 0.18 g

CuSO₄ $\times$ 5 H₂O 0.01 g

KAl(SO₄)₂ $\times$ 12 H₂O 0.02 g

H₃BO₃ 0.01 g

Na₂MoO₄ $\times$ 2 H₂O 0.01 g

NiCl₂ $\times$ 6 H₂O 0.03 g

Na₂SeO₃ $\times$ 5 H₂O 0.3 mg

Na₂WO₄ $\times$ 2 H₂O 0.4 mg

Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl $\times$ 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except fumarate, vitamins, cysteine and sulfide), adjust pH to 8.5 and sparge medium with 100% N₂ 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% N₂ gas. Stock solutions of fumarate and vitamins are sterilized by filtration. Adjust pH of complete medium to 8.5, if necessary.

368. METHANOBACTERIUM VETERUM MEDIUM

Solution 1:

Na-acetate $\times$ 3 H₂O 0.5 g

(NH₄)₂SO₄ 0.45 g

K₂HPO₄ 0.29 g

KH₂PO₄ 0.18 g

MgSO₄ $\times$ 7 H₂O 0.12g

CaCl₂ $\times$ 2 H₂O 0.06 g

NaCl 0.05

Resazurin 0.01 g

Na₂CO₃ 5.0 g
Trace element solution (see below) 10.0 ml
Vitamin solution (see below) 10.0 ml
Distilled water 965.0 ml

Solution 2 (reducing agents):

L-Cysteine-HCl × H₂O 0.5 g
Na₂S × 9 H₂O 0.5 g
Distilled water 10.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
MgSO₄ × 7 H₂O 3.0 g
MnSO₄ × 7 H₂O 0.5 g
NaCl 1.0 g
FeSO₄ × 7 H₂O 0.1 g
CoSO₄ 0.18 g
CaCl₂ × 2 H₂O 0.1 g
ZnSO₄ 0.18 g
CuSO₄ 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ 0.025 g
Na₂SeO₃ 0.3 mg
Distilled water 1000.0 ml
pH 7.0-7.4

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic (tioctoic) acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N₂ without O₂ up to sterilization. Solutions of reducing agents (10.0 ml) and others solutions add to base medium after separate sterilization at 121°C for 15 min. Sterilize vitamin solution by filtration. Cultivate in a gas mixture of 80% H₂ and 20% CO₂.

369. CLOSTRIDIUM TEPIDIPROFUNDI MEDIUM

NaCl 18.0 g
MgCl₂ × 6 H₂O 4.0 g
KCl 0.34 g
NH₄Cl 0.25 g
CaCl₂ 0.11 g
K₂HPO₄ 0.18 g
Fe(NH₄)₂(SO₄)₂ × 6 H₂O 20.0 mg
Trace element solution *SL-10* (see below) 1.0 ml

Selenite-tungstate solution (see below) 1.0 ml

Yeast extract 0.2 g

Proteose peptone 10.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

NaHCO₃ 5.0 g

Vitamin solution (see below) 10.0 ml

Na₂S × 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g

Na₂SeO₃ × 5 H₂O 3 mg

Na₂WO₄ × 2 H₂O 4 mg

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ 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) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. The pH of the complete medium should be 6.5 - 7.0.

370. ACIDILOBUS MEDIUM

NH₄Cl 0.33 g

KCl 0.33 g

KH₂PO₄ 0.33 g

MgCl₂ × 6 H₂O 0.33 g

CaCl₂ × 2 H₂O 0.33 g

Trace mineral solution *SL-10* (see below) 1.0 ml

Na-resazurin solution (0.1% w/v) 0.5 ml

Sulfur, powdered 10.0 g

Yeast extract 0.1 g
D-glucose 2.0 g
Vitamin solution (see below) 10.0 ml
 $\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.45 g
Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
 $\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g
 ZnCl_2 70.0 mg
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg
 H_3BO_3 6.0 mg
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except D-glucose, 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 successive days. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The pH of the complete medium should be at 3.5 - 3.8.

371. MINERAL MEDIUM “P” FOR METHANOTROPHIC BACTERIA

$\text{Na}_2\text{HPO}_4 \times 12 \text{ H}_2\text{O}$ 1.5 g
 KH_2PO_4 0.7 g
 KNO_3 1.0 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.2 g
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.02 g
NaCl 30.0 g
 NaHCO_3 4.2 g
 Na_2CO_3 0.05 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml
Trace elements solution:
EDTA 5.0 g
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 g
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 2.0 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.02 g

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 g

Na_2MoO_4 0.03 g

Distilled water 1000.0 ml

Prepare the medium without the NaHCO_3 and Na_2CO_3 . Basal medium, trace element solution, NaHCO_3 and Na_2CO_3 sterilize separately at 121°C for 15 min. When preparing liquid media cool the mineral salts solution, NaHCO_3 and Na_2CO_3 to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool the NaHCO_3 and Na_2CO_3 stock solution and agar to $50\text{-}55^\circ\text{C}$ before mixing.

The pH of the complete medium should be at 9.0.

372. PLATE COUNT AGAR WITH 1% NaCl

Tryptone 5.0 g

Yeast extract 2.5 g

Glucose 1.0 g

NaCl 10.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.0 ± 0.2

Autoclave at 121°C for 15 min.

373. MARINE AMMONIUM MINERAL SALTS

Solution A:

NaCl 20.0 g

$(\text{NH}_4)_2\text{SO}_4$ 1.0 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.2 g

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 1.0 g

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.02 g

$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 0.03 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.02 g

Methanol 5.0 ml

Trace element solution SL-10 (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Distilled water 900.0 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 100.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Autoclave solution A, B and trace element solution separately at 121°C for 15 min and combine after cooling. Add filter sterilize methanol and vitamin solution.

374. AMS MEDIUM

Phosphate buffer:

KH₂PO₄ 0.54 g
K₂HPO₄ 0.7 g
Agar (if needed) 15.0 g
Distilled water 600.0 ml

Salt solution:

NH₄Cl 0.5 g
CaCl₂ × 2 H₂O 0.2 g
MgSO₄ × 7 H₂O 1.0 g
FeSO₄ solution (see below) 80 µl
Trace element solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Distilled water 400.0 ml

FeSO₄ solution:

FeSO₄ × 7 H₂O 0.5 g
HCl (5 M) few drops
Distilled water 10.0 ml

Trace element solution

ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ × 2 H₂O 10.0 mg
NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 60.0 mg
Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Distilled water 100.0 ml

Autoclave phosphate buffer, salt solution and trace element solution separately at 121°C for 15 min and mix before use. Add filter sterilize FeSO₄ solution and vitamin solution.
pH 6.8.

375. METHYLOMICROBIUM BURYATENSE MEDIUM

KH₂PO₄ × 12 H₂O 0.5 g
KNO₃ 0.5 g
MgSO₄ × 7 H₂O 0.4 g
NaCl 5.0 g
NaHCO₃ (1M) 25.0 ml
Na₂CO₃ (1M) 5.0 ml
Distilled water 1000.0 ml
pH 9.0–9.5
Trace element solution (see below) 1 ml

Trace elements solution:

EDTA 5.0 g
CuCl₂ × 2 H₂O 0.1 g
FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 0.1 g
NiCl₂ × 6 H₂O 0.02 g
CoCl₂ × 6 H₂O 0.2 g
Na₂MoO₄ 0.03 g
Distilled water 1000.0 ml

Prepare the medium without the NaHCO₃ and Na₂CO₃. Basal medium, trace element, NaHCO₃ (1M) and Na₂CO₃ (1M) sterilize separately at 121°C 15 min. When preparing liquid media cool the mineral salts solution, NaHCO₃ and Na₂CO₃ solutions to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool NaHCO₃ and Na₂CO₃ stock solutions and agar to 50-55°C before mixing.

376. MINERAL MEDIUM WITH GELATIN

NaCl 10.0 g
KH₂PO₄ 0.14 g
KCl 0.36 g
MgCl₂ × 6 H₂O 0.4 g
CaCl₂ × 2 H₂O 0.05 g
NH₄Cl 0.25 g
3-(N-Morpholino) propanesulfonic acid (MOPS) buffer 2.09 g
Gelatin 1.0 g
Distilled water 1000.0 ml
Adjust the pH to 7.5 if needed with NaOH/HCl. Autoclave at 121°C for 15 min.

377. CHLOROFLEXUS MEDIUM

KH₂PO₄ 0.5 g
NH₄Cl 0.5 g
MgCl₂ 0.3 g
KCl 0.5 g
NaCl 5.0 g
Na₂S₂O₃ × 5 H₂O 0.1 g
Yeast extract 1.0 g
Na-acetate × 3 H₂O 0.5 g
Na-malate 0.5 g
HEPES 3.0 g
Trace element solution according to *Pfennig* (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Distilled water 1000.0 ml
Trace element solution according to Pfennig:
EDTA 1.5 g

Trace element solution according to *Hogland* (see below) 6.0 ml

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.2 g

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.02 g

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g

Distilled water 1000.0 ml

Trace element solution according to Hogland:

EDTA 5.0 g

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 2.0 g

$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 100.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 30.0 mg

H_3BO_3 300.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 200.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 10.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 20.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 20.0 mg

Distilled water 1000.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B₁₂ 0.5 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 100.0 ml

After autoclaving at 121°C for 15 min, add 1 ml of 5% $\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ solution (autoclaved), 1 ml of 5% $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ solution (autoclaved) and 10 ml of 3% NaHCO_3 solution (filter-sterilized).

Vitamin solution is filter sterilized. Adjust pH to 7.5.

378. POLYMORPHOSOMA TUNDRAE MEDIUM

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.8 g

NH_4NO_3 0.1 g

KH_2PO_4 0.04 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.02 g

Fructose 0.5 g

Yeast extract 0.01 g

Pectin 0.05 g

Phytigel 9.0 g

Distilled water 1000.0 ml

Adjust to pH 4.9 – 5.5. Autoclave at 121°C for 15 min.

379. GRANULICELLA SIBIRICA MEDIUM

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.05 g

NH_4NO_3 0.1 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.05 g

Glucose 0.5 g

Yeast extract 0.25 g

Staley's vitamin solution (see below) 1.0 ml

Distilled water 1000.0 ml

Adjust pH to 5.0-5.5

Staley's Vitamin Solution:

Vitamin B₁₂ 0.1 mg

Biotin 2.0 mg

Thiamine-HCl × 2 H₂O 5.0 mg

Ca-pantothenate 5.0 mg

Folic acid 2.0 mg

Riboflavin 5.0 mg

Nicotinamide 5.0 mg

p-Aminobenzoic acid 5.0 mg

Pyridoxine hydrochloride 10.0 mg

Distilled water 1000.0 ml.

Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution.

380. LIMNOGLOBUS ROSEUS MEDIUM

Peptone 0.1 g

Yeast extract 0.25 g

NH₄NO₃ 0.1 g

Glucose 0.5 g

Hutner's basal salts 20.0 ml

Staley's vitamin solution, double concentration (see below) 1.0 ml

Phytigel 10.0 g

Distilled water 1000.0 ml

Adjust pH to 6.5.

Hutner's basal salts

Nitrilotriacetic acid (NTA) 10.0 g

MgSO₄ × 7 H₂O 29.7 g

CaCl₂ × 2 H₂O 3.34 g

Na₂MoO₄ × 2 H₂O 12.67 mg

FeSO₄ × 7 H₂O 99.0 mg

Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H₂SO₄).

Adjust volume to 1000.0 ml with distilled water.

Metal solution "44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × 7 H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.0 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Staley's vitamin solution, double concentration:

Vitamin B₁₂ 0.2 mg

Biotin 4.0 mg

Thiamine-HCl × 2 H₂O 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.
Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution.

381. ALKALICAULIS SATELLES MEDIUM

NaCl 42.0 g
NaHCO₃ 24.0 g
KH₂PO₄ 0.3 g
MgSO₄ × 7 H₂O 0.12 g
CaCl₂ × 2 H₂O 0.03 g
Trypton 2.0 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution:

FeSO₄ × 7 H₂O 0.6 mg
CoCl₂ × 6 H₂O 0.12 mg
Ni (NH₄)₂(SO₄)₂ × 6 H₂O 0.9 mg
MnCl₂ × 4 H₂O 0.1 mg
ZnSO₄ × 7 H₂O 0.14 mg
H₃BO₃ 0.03 mg
AlK(SO₄)₂ × 12 H₂O 0.24 mg
Na₂MoO₄ × 2 H₂O 0.02 mg
Na₂WO₄ × 2 H₂O 0.03 mg
Na₂SeO₃ × 5 H₂O 0.02 mg
CuCl₂ × 2 H₂O 0.02 mg
Distilled water 1000.0 ml

Prepare the medium without NaHCO₃. Basal medium, trace element solution and NaHCO₃ sterilize separately at 121°C for 15 min. When preparing liquid media cool the mineral salts solution and NaHCO₃ to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salt solution and autoclave. Cool the NaHCO₃ stock solution and agar to 50-55°C before mixing.

pH 8.0-9.0

382. VIBRIO HARVEYI MEDIUM

NaCl 30.0 g
MgSO₄ × 7 H₂O 0.2 g
KH₂PO₄ 1.0 g
Na₂HPO₄ 6.0 g
(NH₄)₂HPO₄ 0.5 g
Peptone 5.0 g
Glycerol 3.0 ml
Distilled water 1000.0 ml
Autoclave at 121°C 15 min.

383. MEDIUM FOR MARINE PLANCTOMYCETES

Hutner's basal salts medium (see below) 20.0 ml
Peptone Bacto 1.0 g
Yeast extract Bacto 1.0 g
HEPES 2.38 g
Artificial seawater (see below) 250.0 ml

Distilled water 690.0 ml (less for plates)

Adjust pH to 7.5 with 5 M KOH

After autoclaving and cooling (please see below for plates) add to the medium:

Glucose solution (25%, sterile-filtered) 4.0 ml

Vitamin solution 5.0 ml

N-Acetylglucosamine 20.0 ml of 50.0 g/l stock solution

Trace element solution 1.0 ml

Artificial sea water:

NaCl 46.94 g

Na₂SO₄ 7.84 g

MgCl₂ × 6 H₂O 21.28 g

CaCl₂ × 2 H₂O 2.86 g

NaHCO₃ 0.384 g

KCl 1.384 g

KBr 0.192 g

H₃BO₃ 0.052 g

SrCl₂ × 6 H₂O 0.08 g

NaF 0.06 g

Distilled water 1000.0 ml

Do not autoclave. Due to high salt concentration, no filtration. Sometimes precipitation after a while or fungal growth observed, thus better prepare fresh. Store at room temperature.

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g

MgSO₄ × 7 H₂O 29.7 g

CaCl₂ × 2 H₂O 3.34 g

Na₂MoO₄ × 2 H₂O 0.01267 g

FeSO₄ × 7 H₂O 0.099 g

Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H₂SO₄).

Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

Metal solution "44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × 7 H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.0 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

Vitamin solution:

Biotin 4.0 mg

Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg

Riboflavine 10.0 mg

Thiamine-HCl × 2 H₂O 10.0 mg

Nicotinamide 10.0 mg

D-Ca-pantothenate 10.0 mg

Vitamin B₁₂ 0.2 mg

p-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

Trace element solution:

Na-Nitrilotriacetat 1.5 g

MnSO₄ × H₂O 500.0 mg

FeSO₄ × 7 H₂O 100.0 mg

Co(NO₃)₂ × 6 H₂O 100.0 mg

ZnCl₂ 100.0 mg

NiCl₂ × 6 H₂O 50.0 mg

H₂SeO₃ 50 mg

CuSO₄ × 5 H₂O 10.0 mg

AlK(SO₄)₂ × 12 H₂O 10.0 mg

H₃BO₃ 10.0 mg

NaMoO₄ × 2 H₂O 10.0 mg

Na₂WO₄ × 2 H₂O 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

15.0 g/l Agar (Bacto) 3x washed with distilled water, autoclave separately and take volume of distilled water into account.

or

8.0 g/l Phytagel in 200.0 ml distilled water (mix before autoclaving), autoclave separately.

Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

384. MEDIUM FOR LIMNIC PLANCTOMYCETES

NH₄Cl 0.53 mg

KH₂PO₄ 1.4 mg

KNO₃ 10 mg

MgSO₄ × 7 H₂O 49.3 mg

CaCl₂ × 2 H₂O 14.7 mg

CaCO₃ 25 mg

NaHCO₃ 25 mg

Peptone Bacto 1.0 g

Yeast extract Bacto 1.0 g

HEPES 2.38 g

Hutner's basal salts medium (see below) 20.0 ml

Distilled water 950 ml (less for plates)

Adjust pH to 7.0 with 5 M KOH

After autoclaving and cooling (please see below for plates) add to the medium:

Glucose solution (25%, sterile-filtered) 4.0 ml

Vitamin solution 5.0 ml

N-Acetylglucosamine 20.0 ml of 50.0 g/l stock solution

Trace element solution 1.0 ml

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g

MgSO₄ × 7 H₂O 29.7 g

CaCl₂ × 2 H₂O 3.34 g

Na₂MoO₄ × 2 H₂O 0.01267 g

FeSO₄ × 7 H₂O 0.099 g

Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H₂SO₄).

Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

Metal solution "44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × 7 H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.0 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

Vitamin solution:

Biotin 4.0 mg

Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg

Riboflavine 10.0 mg

Thiamine-HCl × 2 H₂O 10.0 mg

Nicotinamide 10.0 mg

D-Ca-pantothenate 10.0 mg

Vitamin B₁₂ 0.2 mg

p-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

Trace element solution:

Na-Nitrilotriacetate 1.5 g

MnSO₄ × H₂O 500.0 mg

FeSO₄ × 7 H₂O 100.0 mg

Co(NO₃)₂ × 6 H₂O 100.0 mg

ZnCl₂ 100.0 mg

NiCl₂ × 6 H₂O 50.0 mg

H₂SeO₃ 50 mg

CuSO₄ × 5 H₂O 10.0 mg

AlK(SO₄)₂ × 12 H₂O 10.0 mg

H₃BO₃ 10.0 mg

NaMoO₄ × 2 H₂O 10.0 mg

Na₂WO₄ × 2 H₂O 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

15.0 g/l Agar (Bacto) 3x washed with distilled water, autoclave separately and take volume of distilled water into account.

or

8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately.

Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

385. MEDIUM SM

KH₂PO₄ 0.14 g

MgCl₂ × 6 H₂O 0.4 g
CaCl₂ × 2 H₂O 0.05 g
NH₄Cl 0.25 g
KCl 0.36 g
HEPES 2.38 g
Yeast extract 0.5 g
Trace elements solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O (Mohr's salt) 784.0 mg
HCl (concentrated) 5.0 ml
CoCl₂ × H₂O 238.0 mg
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
Na₂WO₄ × 2 H₂O 33.0 mg
ZnSO₄ × 7 H₂O 144.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Na₂SeO₄ 94.0 mg
H₃BO₃ 6.0 mg
MnCl₂ × 4 H₂O 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with water and other salts are dissolved in the sequence indicated.

Vitamin solution according to Wolin et al.:

Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 100.0 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

Autoclave at 121°C 15 min. Add filter-sterilized vitamin solution and trace elements from sterile stock solution.

386. SALINICOLA SALARIUS MEDIUM

Tryptone 5.0 g
Yeast extract 2.5 g
D-Glucose 1.0 g
NaCl 130.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

387. NATRANAEROBIUS MEDIUM

KH₂PO₄ 0.2 g
MgCl₂ × 6 H₂O 0.1 g
KCL 0.2 g

NH₄Cl 0.5 g
NaCl 100 g
Yeast extract (Difco) 6.0 g
Tryptone (Difco) 6.0 g
Trace element solution SL-10 (see below) 1.0 ml
Vitamin solution (see below) 10 ml
Na₂CO₃ 68.0 g
NaHCO₃ 38.0 g
L-Cysteine-HCl × H₂O 0.7 g
Glucose 5.0 g
Distilled water to 1000.0 ml
pH 10.5 (measured at 60°C)

Dissolve ingredients (except carbonates, cysteine, vitamins and sucrose), then sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Add carbonate, bicarbonate and cysteine and adjust pH to 10.5. Dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins and glucose from sterile anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration.

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 0.1 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2-7.4.

388. THERMODESULFOBIUM ACIDIPHILUM MEDIUM

NH₄Cl 0.33 g
KCl 0.33 g
KH₂PO₄ 0.33 g
MgCl₂ × 6 H₂O 0.33 g
CaCl₂ × 2 H₂O 0.33 g
Na₂SO₄ 2.8 g
Trace mineral solution SL-10 (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Yeast extract 3.0 g

Vitamin solution (see below) 10.0 ml

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.45 g

Distilled water 1000.0 ml

Dissolve ingredients except yeast extract, vitamins and sulfide. Adjust pH to 4.5 with H_2SO_4 and sparge medium with 100% CO_2 gas for 10 – 15 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes and autoclave. 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. The pH of the complete medium should be at 4.5.

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 0.1 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

p-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

Vitamin B_{12} 0.1 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.2-7.4

389. *TRICHOCOCCUS* MEDIUM

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.12 g

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.19 g

KH_2PO_4 0.45 g

K_2HPO_4 0.45 g

NaCl 0.9 g

$(\text{NH}_4)_2\text{SO}_4$ 0.9 g

Yeast extract 2.0 g

Trypticase peptone 2.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

L-Cysteine-HCl $\times \text{H}_2\text{O}$ 1.0 g

Na_2CO_3 2.5 g

D-Glucose 2.0 g

Distilled water 1000.0 ml

Dissolve ingredients (except cysteine, carbonate and glucose), adjust pH to 7.0 and sparge medium with 100% CO_2 gas for 10 – 15 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% N₂ gas atmosphere and sterilized by filtration.

390. *SULFURIMONAS* MEDIUM

NH₄Cl 0.33 g
KCl 0.33 g
CaCl₂ × 2H₂O 0.03 g
KH₂PO₄ 0.16 g
MgCl₂ × 6H₂O 0.33 g
NaCl 10.0 g
NaHCO₃ 2.0 g
Elemental sulfur 5.0 g
KNO₃ 1.0 g
Trace element solution (see below) 1 ml
Vitamin solution (see below) 1 ml
pH of 7.5–8.0 (measured at 25 °C)

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 0.1 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 7.2–7.4.

The anoxic medium is prepared by boiling and cooling it under N₂ flow. No reducing agents are added. Elemental sulfur as electron donor is added to the Hungate tubes prior sterilization; potassium nitrate from anoxic sterile stock solution was added as electron acceptor just before inoculation.

391. *ALKALIBACULUM* MEDIUM

NH₄Cl 0.33 g
KCl 0.33 g
CaCl₂ × 2 H₂O 0.033 g
KH₂PO₄ 0.33 g

MgCl₂ × 6 H₂O 0.33 g
NaOH 10.0 g
NaHCO₃ 2.0 g
Glucose 2.0 g
Yeast extract 50 mg
Na₂S × 9 H₂O 0.5 g
Resazurin 0.2 mg
Trace element solution (see below) 1 ml
Vitamin solution (see below) 1 ml
pH of 8.0-8.5 (measured at 25 °C)

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O (*Mohr's salt*) 784.0 mg
HCl (concentrated) 5.0 ml
CoCl₂ × H₂O 238.0 mg
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
Na₂WO₄ × 2 H₂O 33.0 mg
ZnSO₄ × 7 H₂O 144.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Na₂SeO₄ 94.0 mg
H₃BO₃ 6.0 mg
MnCl₂ × 4 H₂O 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 0.1 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

The medium is prepared anaerobically. NaHCO₃, NaOH and vitamins, Na₂S × 9 H₂O are added after boiling and cooling of the medium under 100% N₂ in gas phase. Adjust the pH between 6.0 and 6.5 with sterile HCl or NaOH after autoclaving. Yeast extract must be added to the final solution before inoculation.

392. CALORIBACTERIUM MEDIUM

NH₄Cl 0.33 g
KCl 0.33 g
CaCl₂ × 6 H₂O 0.033 g
KH₂PO₄ 0.33 g
MgCl₂ × 6 H₂O 0.33 g
NaOH 10.0 g
NaHCO₃ 2.0 g
Glucose 2.0 g
Yeast extract 50 mg

Na₂S × 9 H₂O 0.5 g
Resazurin 0.2 mg
Trace element solution (see below) 1 ml
Vitamin solution (see below) 1 ml
pH of 7.5–8.0 (measured at 25°C)
Trace element solution:
(NH₄)₂Fe(SO₄)₂ × 6 H₂O (*Mohr's salt*) 784.0 mg
HCl (concentrated) 5.0 ml
CoCl₂ × H₂O 238.0 mg
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
Na₂WO₄ × 2 H₂O 33.0 mg
ZnSO₄ × 7 H₂O 144.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Na₂SeO₄ 94.0 mg
H₃BO₃ 6.0 mg
MnCl₂ × 4 H₂O 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:
Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 0.1 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

The medium is prepared anaerobically. NaHCO₃, NaOH and vitamins, Na₂S × 9H₂O are added after boiling and cooling of the medium under 100% CO₂ in gas phase. Adjust the pH between 6.0 and 6.5 with sterile HCl or NaOH after autoclaving. Yeast extract must be added to the final solution before inoculation.

393. PLATE COUNT AGAR WITH 0.5% NaCl

Tryptone 5.0 g
Yeast extract 2.5 g
Glucose 1.0 g
NaCl 5.0 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 7.0±0.2
Autoclave at 121°C for 15 min.

394. *LICHENIBACTERIUM MINOR* MEDIUM

Glucose 1.0 g
KH₂PO₄ 0.2 g
NaCl 0.2 g
NH₄NO₃ 0.2 g

MgSO₄ × 5 H₂O 0.04 g
Yeast extract(without sodium chloride) 0.2 g
Vitamin solution (see below) 1 ml
Distilled water 1000.0 ml
pH 5.0-6.5

Vitamin solution:

p-Aminobenzoate 1.0 mg
Biotin 0.2 mg
Nicotinic acid 2.0 mg
Thiamine-HCl 1.0 mg
Ca-pantothenate 0.5 mg
Pyridoxamine 5.0 mg
Vitamin B₁₂ 2.0 mg
Distilled water 1000.0 ml

Prepare the medium without the vitamin solution. Autoclave at 121°C for 15 min and add the vitamin solution from a filter-sterilized stock solution.

395. *FRIGORIGLOBUS TUNDRICOLA* MEDIUM

Glucose 0.5 g
Yeast extract 0.25 g
Peptone 0.25 g
N-acetylglucosamine 0.5 g
KH₂PO₄ 0.1 g
MgSO₄ × 7 H₂O 0.1 g
NH₄NO₃ 0.1 g
CaCl₂ × 6 H₂O 0.02 g
Distilled water 1000.0 ml (800.0 ml for solid medium)
pH 5.8-6.0

Autoclave at 121°C for 15 min

For solid medium 8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

396. *LACIPIRELLULA PARVULA* MEDIUM

Glucose 0.5 g
Yeast extract 0.25 g
Peptone 0.25 g
N-acetylglucosamine 0.5 g
KH₂PO₄ 0.1 g
MgSO₄ × 7 H₂O 0.1 g
NH₄NO₃ 0.1 g
CaCl₂ × 6 H₂O 0.02 g
Distilled water 1000.0 ml (800.0 ml for solid medium)
pH 6.8-7.5

Autoclave at 121°C for 15 min

For solid medium 8.0 g/l Phytigel in 200.0 ml distilled water (mix before autoclaving), autoclave separately. Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

397. *JANTHINOBACTERIUM LIVIDUM* MEDIUM

Peptone 30.0 g
Na₂HPO₄ 2.0 g

NaCl 3.0 g
Agar 20.0 g
Distilled water 1000.0 ml
Autoclave at 121°C for 15 min

398. RHODOPSEUDOMONAS PARAPALUSTRIS MEDIUM

Tryptone 5.0 g
Yeast extract 2.5 g
Glucose 1.0 g
Distilled water 1000.0 ml
pH 6.8±0.2

399. GEMMATA PALUSTRIS MEDIUM

Hutner's basal salts medium (see below) 20.0 ml

Peptone 5.0 g
Yeast extract 0.25 g
Distilled water 965.0 ml (less for plates)
Adjust pH to 6.8-7.5 with 5 M KOH
After autoclaving and cooling (see below for plates) add to the medium
Vitamin solution 5.0 ml

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 29.7 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 3.34 g
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 0.0127 g
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 0.099 g
Metal salt solution "44" (see below) 50.0 ml
Dissolve NTA first by neutralizing with KOH, then add other salts.
pH 7.2 (adjust with KOH or H_2SO_4).
Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

Metal solution "44":

Na-EDTA 250.0 mg
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 1095.0 mg
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 500.0 mg
 $\text{MnSO}_4 \times 7 \text{H}_2\text{O}$ 154.0 mg
 $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 39.0 mg
 $\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$ 24.8 mg
 $\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$ 17.7 mg
Distilled water 1000.0 ml
Dissolve Na-EDTA and add a few drops of concentrated H_2SO_4 to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

Vitamin solution:

Biotin 4.0 mg
Folic acid 4.0 mg
Pyridoxine-HCl 20.0 mg
Riboflavin 10.0 mg
Thiamine-HCl $\times 2 \text{H}_2\text{O}$ 10.0 mg
Nicotinamide 10.0 mg
D-Ca-pantothenate 10.0 mg
Vitamin B₁₂ 0.2 mg
p-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

8.0 g/l Phytagel in 200.0 ml distilled water (mix before autoclaving), autoclave separately.

Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

400. THIOTHRIX MEDIUM

NH₄Cl 0.3 g

CaCl₂ 0.03 g

KH₂PO₄ 0.01 g

K₂HPO₄ 0.022 g

Na₂HPO₄ × 7 H₂O 0.035 g

MgSO₄ × 7 H₂O 0.05 g

FeCl₃ × 6H₂O 0.002 g

NaNO₃ 0.30 g

Peptone 0.2 g

Na-acetate (10%) 2.5 ml

Na-lactate (10%) 2.5 ml

NaHCO₃ (10%) 5.0 ml

Na₂S₂O₃ × 5 H₂O (10%) 10.0 ml

Vitamin solution (see below) 1.0 ml

Trace element solution according to *Hogland*: (see below) 1.0 ml

Distilled water 1000.0 ml

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 B₁₂ 1.0 mg

p-Aminobenzoic acid 50.0 mg

Distilled water 1000.0 ml

Trace element solution according to Hogland:

EDTA 5.0 g

FeSO₄ × 7 H₂O 2.0 g

ZnSO₄ × 7 H₂O 100.0 mg

MnCl₂ × 4 H₂O 30.0 mg

H₃BO₃ 300.0 mg

CoCl₂ × 6 H₂O 200.0 mg

CuCl₂ 10.0 mg

NiCl₂ × 6 H₂O 20.0 mg

Na₂MoO₄ × 2 H₂O 20.0 mg

Distilled water 1000.0 ml

pH 7.5 (adjust with 1% HCl)

To prepare the trace element solution, preliminarily acidify water to pH 3.0-4.0 with HCl.

Sterilize thiosulfate, acetate, lactate, bicarbonate, trace elements and vitamins separately and add to the main medium prior to inoculation. Sterilize the vitamin solution by filtration, others solutions and base medium by autoclaving at 121°C for 15 min.

401. *LICHENICOCCUS ROSEUS* MEDIUM (LRM)

Glucose 1.0 g
KH₂PO₄ 0.2 g
NaCl 0.2 g
NH₄NO₃ 0.2 g
MgSO₄ × 5 H₂O 0.04 g
Yeast extract(without sodium chloride) 0.2 g
Vitamin solution (see below) 1 ml
Distilled water 1000.0 ml
pH 4.5

Vitamin solution:

p-Aminobenzoic acid 1.0 mg
Biotin 0.2 mg
Nicotinic acid 2.0 mg
Thiamine-HCl 1.0 mg
D-Ca-pantothenate 0.5 mg
Pyridoxamine 5.0 mg
Vitamin B₁₂ 2.0 mg
Distilled water 100.0 ml

Prepare the medium without the vitamin solution. Autoclave at 121°C for 15 min and add the vitamin solution from a filter-sterilized stock solution.

402. *PROSTHECODIMORPA STALEYI* MEDIUM (PSM)

Glucose 0.3 g
Na₂HPO₄ 0.071 g
(NH₄)₂SO₄ 0.25 g
Yeast extract 0.05 g
Peptone 0.1 g
Vitamin solution (see below) 10.0 ml
Hutner's salt solution (see below) 20.0 ml
Distilled water 970.0 ml

Vitamin solution :

Biotin 2.0 mg
Folic acid 2.0 mg
Thiamine-HCl 5.0 mg
D-Ca- Panthothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
Riboflavin 5.0 mg
Distilled water 1000.0 ml

Hutner's Salt Solution:

Nitrilotriacetic acid 10.0 g
MgSO₄ × 7 H₂O 29.7 g
CaCl₂ × 2 H₂O 3.335 g
FeSO₄ × 7 H₂O 99.0 mg
(NH₄)₆Mo₇O₂₄ × 4 H₂O 9.25 mg
"Metals 44" 50.0 ml
Distilled water 1000.0 ml

"Metals 44":

ZnSO₄ × 7 H₂O 1.095 g
FeSO₄ × 7 H₂O 0.5 g
EDTA-Na 0.25 g
MnSO₄ × H₂O 0.154 g

CuSO₄ × 5 H₂O 39.2 mg
Co(NO₃)₂ × 6 H₂O 24.8 mg
Na₂B₄O₇ × 10 H₂O 17.7 mg
Distilled water 100.0 ml

403. ALKALISPIRILLUM MOBILE MEDIUM (AMM)

NaCl 20.0 g
Na₂S₂O₃ 1.0 g
KH₂PO₄ 0.8 g
Na-acetate 2.0 g
Yeast extract 1.0 g
Trace elements (see below) 1.0 ml
MgCl₂ × 7 H₂O 0.1 g
CaCl₂ × 2 H₂O 0.05 g
NH₄Cl 0.8 g
Na₂CO₃ 21.2 g
NaHCO₃ (8 %) 168.0 ml
Distilled water 832.0 ml

Trace element solution (A):

FeCl₂ × 4 H₂O 1.8 g
CoCl₂ × 6 H₂O 250.0 mg
NiCl₂ × 6 H₂O 10.0 mg
CuCl₂ × 2 H₂O 10.0 mg
MnCl₂ × 4 H₂O 70.0 mg
ZnCl₂ 100.0 mg
H₃BO₃ 500.0 mg
Na₂MoO₄ × 2 H₂O 30.0 mg
Na₂SeO₃ × 5 H₂O 10.0 mg
Distilled water 1000.0 ml
For dissolving adjust pH to about 3 with 1 N HCl.

We recommend that liquid and solid media be made by autoclaving the Na₂CO₃ 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 Na₂CO₃ 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 NaHCO₃ (8 %) to pH 9.0. Please note that the NaHCO₃ solution should be filter sterilized

404. PEPTONE MEAT AGAR WITH MANGANESE (PMA-Mn)

Peptone 10.0 g
NaCl 5.0 g
Beef extract 3.0 g
MnSO₄ × H₂O 100 mg
Agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Autoclave at 121°C for 15 min.

405. MAGNETOSPIRILLUM KUZNETSOVII MEDIUM (MKM)

KH₂PO₄ 0.68 g
NaNO₃ 0.12 g
L(+)-Tartaric acid 0.37 g

Succinic acid 0.37 g
Na-acetate $\times 3 \text{ H}_2\text{O}$ 0.05 g
Vitamin solution (see below) 10.0 ml
Trace element solution (see below) 5.0 ml
Fe(III) quinate solution (see below) 2.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Na-thioglycolate 0.05 g
Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.5 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 3.0 g
 $\text{MnSO}_4 \times \text{H}_2\text{O}$ 0.5 g
NaCl 1.0 g
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
 $\text{CoSO}_4 \times 7 \text{ H}_2\text{O}$ 0.18 g
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.1 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.18 g
 $\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$ 0.01 g
 $\text{KAl}(\text{SO}_4)_2 \times 12 \text{ H}_2\text{O}$ 0.02 g
 H_3BO_3 0.01 g
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.01 g
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.03 g
 $\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ 0.3 mg
 $\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 0.4 mg
Distilled water 1000.0 ml

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

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl $\times 2 \text{ H}_2\text{O}$ 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Ferric Quinate Solution, 0.01 M:

$\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$ 4.5 g
Quinic acid 1.9 g
Distilled water 1000.0 ml

Sterilize by filtration under 100% N₂ gas atmosphere.

Dissolve ingredients (except thioglycolate) and adjust pH to 6.75 with NaOH. Sparge medium with 100% N₂ gas for 30-45 min and dispense under the same gas atmosphere into anoxic Hungate – type tubes to 50% of their volume. Before inoculation add thioglycolate from a 0.5% (w/v) stock solution, freshly prepared under 100% N₂ gas and filter-sterilized. Then add sterile air to a concentration of 1.0 % O₂ in the vial.

406. COLUMBIA AGAR WITH BLOOD (CA-B)

Special mixture of peptones 23.0 g

Defibrinated blood 50.0 g
Starch 1.0 g
NaCl 5.0 g
Agar 15.0 g
Distilled water 1000.0 ml
pH 7.3±0.2.
Adjust pH to 7.3±0.2. Autoclave at 121°C for 15 min.

407. *PSEUDOALTEROMONAS TELLURITIREducens* MEDIUM (PTM)

MgSO₄ × 7 H₂O 0.5 g
KH₂PO₄ 0.3 g
NH₄Cl 0.3 g
CaCl₂ × 2 H₂O 0.05 g
Na-acetate × 3 H₂O 10.0 g
Yeast extract 0.5 g
Casamino acids 0.5 g
NaCl 15.0 g
Trace elements solution (see below) 2.0 ml
Vitamin solution (see below) 2.0 ml
Distilled water 1000.0 ml

Trace elements solution:

MnCl₂ × 4 H₂O 0.3 g
FeSO₄ × 7 H₂O 0.03 g
Distilled water 1000.0 ml

Vitamin solution:

Biotin 80 mg
Thiamine-HCl 400.0 mg
Nicotinic acid 400.0 mg
Vitamin B₁₂ 20.0 µg
Distilled water 1000.0 ml

After autoclaving at 121°C for 15 min adjust to pH 7.8 with NaHCO₃. Add the vitamin solution and trace elements solution from sterile stock solutions.

408. *PLANCTOMYCETES* SP. MEDIUM (PspM)

Hutner's basal salts medium (see below) 20.0 ml

Peptone Bacto 1.0 g
Yeast extract Bacto 1.0 g
HEPES 2.38 g
Artificial seawater (see below) 250.0 ml
Distilled water 690.0 ml (less for plates)
Adjust pH to 7.5 with 5 M KOH

After autoclaving and cooling (please see below for plates) add to the medium:

Vitamin solution 5.0 ml
N-Acetylglucosamine 20.0 ml of 50.0 g/l stock solution
Trace element solution 1.0 ml

Artificial sea water:

NaCl 46.94 g
Na₂SO₄ 7.84 g
MgCl₂ × 6 H₂O 21.28 g
CaCl₂ × 2 H₂O 2.86 g
NaHCO₃ 0.384 g
KCl 1.384 g

KBr 0.192 g

H₃BO₃ 0.052 g

SrCl₂ × 6 H₂O 0.08 g

NaF 0.06 g

Distilled water 1000.0 ml

Do not autoclave. Due to high salt concentration, no filtration. Sometimes precipitation after a while or fungal growth observed, thus better prepare fresh. Store at room temperature.

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g

MgSO₄ × 7 H₂O 29.7 g

CaCl₂ × 2 H₂O 3.34 g

Na₂MoO₄ × 2 H₂O 0.01267 g

FeSO₄ × 7 H₂O 0.099 g

Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H₂SO₄).

Adjust volume to 1000.0 ml with distilled water. Sterilize by filtration, store at +4°C.

Metal solution "44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × 7 H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.0 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions.

Sterilize by filtration, store at +4°C.

Vitamin solution:

Biotin 4.0 mg

Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg

Riboflavine 10.0 mg

Thiamine-HCl × 2 H₂O 10.0 mg

Nicotinamide 10.0 mg

D-Ca-pantothenate 10.0 mg

Vitamin B₁₂ 0.2 mg

p-Aminobenzoic acid 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

Trace element solution:

Na-Nitrilotriacetat 1.5 g

MnSO₄ × H₂O 500.0 mg

FeSO₄ × 7 H₂O 100.0 mg

Co(NO₃)₂ × 6 H₂O 100.0 mg

ZnCl₂ 100.0 mg

NiCl₂ × 6 H₂O 50.0 mg

H₂SeO₃ 50 mg

CuSO₄ × 5 H₂O 10.0 mg

AlK(SO₄)₂ × 12 H₂O 10.0 mg

H₃BO₃ 10.0 mg

NaMoO₄ × 2 H₂O 10.0 mg

Na₂WO₄ × 2 H₂O 10.0 mg

Distilled water 1000.0 ml

Sterilize by filtration, store in the dark and cold (+4°C)

For plates:

15.0 g/l Agar (Bacto) 3x washed with distilled water, autoclave separately and take volume of distilled water into account.

or

8.0 g/l Phytagel in 200.0 ml distilled water (mix before autoclaving), autoclave separately.

Gelrite gets solid below 80°C after autoclaving and in general polymerization starts fast after mixing with media.

409. SEA WATER MEDIUM FOR *HALOMONAS* (SWM-H)

Sea salt 37.9 g

Yeast extract 3.0 g

Peptone 10.0 g

NaCl 60 g

Agar 20.0 g

Distilled water to 1000.0 ml

pH 7.2-7.4

Autoclave at 121°C for 15 min.

410. *SHINELLA* SP. MEDIUM (SspM)

Tryptone 5.0 g

Yeast extract 2.5 g

Glucose 1.0 g

NaCl 5.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.0±0.2

Autoclave at 121°C for 15 min.

411. MEDIUM FOR *METHYLOLIGELLA HALOTOLERANS* (MMH)

KH₂PO₄ 2.0 g

(NH₄)₂SO₄ 2.0 g

MgSO₄ × 7 H₂O 0.025 g

NaCl 0.5 g

FeSO₄ × 7 H₂O 0.02 g

Methanol 20.0 ml

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min. Methanol is filter sterilized.

412. MEDIUM FOR *PARACOCCLUS SIMPLEX* (MPS)

KH₂PO₄ 2.0 g

(NH₄)₂SO₄ 2.0 g

MgSO₄ × 7 H₂O 0.025 g

NaCl 0.5 g

FeSO₄ × 7 H₂O 0.02 g

Methylamine solution 30.0 ml

Distilled water 1000.0 ml

pH 7.0

Autoclave at 121°C for 15 min. 10 % solution of methylamine autoclave at 111°C for 30 min.

413. GYM STREPTOMYCES MEDIUM (GSM)

Glucose 4.0 g

Yeast extract 4.0 g

Malt extract 10.0 g

CaCO₃ 2.0 g

Agar 12.0 g

Distilled water 1000.0 ml

Adjust pH to 7.2 before adding agar. Delete CaCO₃ if liquid medium is used.

414. METHYLOCAPSA ACIDIPHILA MEDIUM (MAM)

KH₂PO₄ 100.0 mg

MgSO₄ × 7 H₂O 50.0 mg

CaCl₂ × 2 H₂O 10.0 mg

Trace element solution (see below) 1.0 ml

Distilled water 1000.0 ml

Trace element solution:

EDTA 5.0 g

CuCl₂ × 5 H₂O 0.10 g

FeSO₄ × 7 H₂O 2.0 g

ZnSO₄ × 7 H₂O 0.1 g

NiCl₂ × 6 H₂O 0.02 g

CoCl₂ × 6 H₂O 0.2 g

Na₂MoO₄ 0.03 g

Distilled water 1000.0 ml

Final pH 4.0-4.5.

The medium is fairly weakly buffered so the pH should be checked before and after autoclaving.

In the early stage of growth the strain may form aggregates which break as the culture ages. 10-30% methane is added to the gas phase. Adding 100 mg/litre KNO₃ enhances growth.

The strain should be grown with shaking.

415. MEDIUM FOR ECTOTHIORHODOSYNUS (MERh)

KH₂PO₄ 0.5 g

NH₄Cl 0.5 g

NaCl 20.0 g

MgCl₂ × 6 H₂O 0.2 g

CaCl₂ 0.1 g

Na₂CO₃ 5.0 g

NaHCO₃ 5.0 g

Sodium acetate 1.0 g

Yeast extract 0.1 g

Na₂S₂O₃ × 5 H₂O 0.5 g

Na₂S × 9 H₂O 0.1 g

Trace element solution SL6 (see below) 1.0 ml

Vitamin B₁₂ 20.0 µg

Distilled water 1000.0 ml

Trace element solution SL-6:

ZnSO₄ × 7 H₂O 0.1 g

MnCl₂ × 4 H₂O 0.03 g

H₃BO₃ 0.3 g

CoCl₂ × 6 H₂O 0.2 g

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 0.01 g

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$ 0.02 g

Na_2MoO_4 0.03 g

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.

416. MEDIUM FOR *HELIOBACTERIUM* (MHB)

KH_2PO_4 0.5 g

NH_4Cl 0.5 g

NaCl 0.5 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 0.8 g

CaCl_2 0.1 g

NaHCO_3 1.5 g

Sodium acetate 1 g

Yeast extract 0.1 g

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 0.3 g

Vitamin B_{12} 20 μg

Biotin 20 μg

Trace element solution SL-6 (see below) 1 ml

Distilled water 998.0 ml.

Trace element solution SL-6:

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 0.03 g

H_3BO_3 0.3 g

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 0.2 g

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 0.01 g

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$ 0.02 g

Na_2MoO_4 0.03 g

Autoclave at 121 C° for 15 min. Adjust pH to 7.6. Boil the medium for few minutes under nitrogen gas. Add resazurin to a final concentration of 0.002% (2% solution, 1 ml per 1 L). Fill immediately into 50 ml screw-capped bottles with rubber septum and screw tighten or into Hungate tubes. Incubate in low light under anaerobic conditions.

417. MEDIUM FOR HALOPHILIC ANAEROBES (MHLAnb)

KH_2PO_4 0.2 g

MgCl_2 0.1 g

NH_4Cl 0.5 g

KCl 0.2 g

Na_2CO_3 9.3 g

NaHCO_3 44.1 g

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 0.7 g

Yeast extract 0.2 g

Glucose 5.0 g

Trace element solution (see below) 1 ml

Vitamin solution (see below) 10 ml

Resazurin 0.01 g

Distilled water 990.0 ml

Trace element solution:

Nitrilotriacetic acid 1.50 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 3.0 g

$\text{MnSO}_4 \times \text{H}_2\text{O}$ 0.50 g

NaCl 1.0 g
FeSO₄ × 7 H₂O 0.10 g
CoSO₄ × 7 H₂O 0.18 g
CaCl₂ × 2 H₂O 0.10 g
ZnSO₄ × 7 H₂O 0.18 g
CuSO₄ × 5 H₂O 0.01 g
KAl(SO₄)₂ × 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.03 g
Na₂SeO₃ × 5 H₂O 0.30 mg
Na₂WO₄ × 2 H₂O 0.40 mg
Distilled water 1000.0 ml

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

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
Ca-D-pantothenate 5.0 mg
Vitamin B₁₂ 0.10 mg
p-Aminobenzoic acid 5.0 mg
(±)-α-Lipoic acid 5.0 mg

pH 9.05. Gas phase was N₂. Anaerobic conditions required.

418. NUTRIENT AGAR (NAGAR)

Peptone- 5.0 g
Meat extract 3.0 g
Agar 18.0 g
Distilled water 1000.0 ml.
Adjust pH to 7.2. Autoclave at 121 C° for 15 min.

419. THIOHALOSPIRA HALOPHILA MEDIUM (T HM)

NaCl 233.0 g
K₂HPO₄ 1.50 g
NH₄Cl 0.50 g
Trace element solution (see below) 1.0 ml
CaCl₂ × 2 H₂O 0.05 g
MgCl₂ × 6 H₂O 0.4 g
Na₂S₂O₃ × 5 H₂O 5.0 g
NaHCO₃ 2.50 g
Seven vitamins solution (see below) 1.0 ml
Distilled water 1000.0 ml
Trace elements solution (Pfennig & Lippert, 1966)
EDTA 5.0 g
FeSO₄ × 7 H₂O 2.2 g
ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g

H₃BO₃ 0.03 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.03 g
NiCl₂ × 6H₂O 0.03 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0-4.0

Seven vitamins solution:

Vitamin B₁₂ 100.0 mg
p-Aminobenzoic acid 80.0 mg
D(+)-Biotin 20.0 mg
Nicotinic acid 200.0 mg
Calcium pantothenate 100.0 mg
Pyridoxine HCl 300.0 mg
Thiamine-HCl × 2 H₂O 200.0 mg
Distilled water 1000.0 ml

Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30–45 min to remove dissolved oxygen and to saturate the solution with CO₂. Dispense solution under air atmosphere in Erlenmeyer flasks to 10% of volume, close with screw caps and autoclave. Add trace elements, calcium chloride, magnesium chloride, thiosulfate, bicarbonate and vitamins from sterile stock solutions and adjust pH of the medium to 7.0-7.2, if necessary. Incubate with shaking.

420 THIOHALOPHILUS MEDIUM (THM)

NaCl 120.0 g
K₂HPO₄ 1.50 g
NH₄Cl 0.50 g
Trace element solution (see below) 1.0 ml
CaCl₂ × 2 H₂O 0.05 g
MgSO₄ × 7 H₂O 0.50 g
Na₂S₂O₃ × 5 H₂O 5.0 g
NaHCO₃ 5.0 g
Seven vitamins solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace elements solution (Pfennig & Lippert, 1966)

EDTA 5.0 g
FeSO₄ × 7 H₂O 2.2 g
ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.03 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.03 g
NiCl₂ × 6H₂O 0.03 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0-4.0

Seven vitamins solution:

Vitamin B₁₂ 100.0 mg
p-Aminobenzoic acid 80.0 mg
D(+)-Biotin 20.0 mg
Nicotinic acid 200.0 mg
Calcium pantothenate 100.0 mg

Pyridoxine HCl 300.0 mg
Thiamine-HCl $\times$ 2 H₂O 200.0 mg
Distilled water 1000.0 ml

Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30 – 45 min to remove dissolved oxygen and to saturate the solution with CO₂. 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).

421. THIOHALOMONAS DENITRIFICANS MEDIUM (TMDM)

NaCl 120.0 g
K₂HPO₄ 1.50 g
NH₄Cl 0.50 g
KNO₃ 1.0 g
Trace element solution (see below) 1.0 ml
CaCl₂ $\times$ 2 H₂O 0.05 g
MgSO₄ $\times$ 7 H₂O 0.50 g
Na₂S₂O₃ $\times$ 5 H₂O 5.0 g
NaHCO₃ 5.0 g
Seven vitamins solution (see below) 1.0 ml

Trace element solution (Pfennig & Lippert, 1966)

EDTA 5.0 g
FeSO₄ $\times$ 7 H₂O 2.2 g
ZnSO₄ $\times$ 7 H₂O 0.1 g
MnCl₂ $\times$ 4 H₂O 0.03 g
H₃BO₃ 0.03 g
CoCl₂ $\times$ 6 H₂O 0.2 g
CuCl₂ $\times$ 2 H₂O 0.03 g
NiCl₂ $\times$ 6H₂O 0.03 g
Na₂MoO₄ $\times$ 2 H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0-4.0

Seven vitamins solution:

Vitamin B₁₂ 100.0 mg
p-Aminobenzoic acid 80.0 mg
D(+)-Biotin 20.0 mg
Nicotinic acid 200.0 mg
Calcium pantothenate 100.0 mg
Pyridoxine HCl 300.0 mg
Thiamine-HCl $\times$ 2 H₂O 200.0 mg
Distilled water 1000.0 ml

Dissolve sodium chloride, potassium hydrogenphosphate, ammonium chloride and potassium nitrate, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30 – 45 min to remove dissolved oxygen. Dispense solution under same gas atmosphere in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 50% volume, close vials with butyl rubber septa and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.5 - 7.8 using a sterile anoxic

stock solution of sodium carbonate (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

422. THIOHALORHABDUS DENITRIFICANS MEDIUM (TRDM)

NaCl 180.0 g
K₂HPO₄ 1.50 g
NH₄Cl 0.50 g
Trace element solution (see below) 1.0 ml
CaCl₂ × 2 H₂O 0.05 g
MgSO₄ × 7 H₂O 0.50 g
Na₂S₂O₃ × 5 H₂O 5.0 g
NaHCO₃ 5.0 g
Seven vitamins solution (see below) 1.0 ml
Trace elements solution (Pfennig & Lippert, 1966)
EDTA 5.0 g
FeSO₄ × 7 H₂O 2.2 g
ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.03 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.03 g
NiCl₂ × 6H₂O 0.03 g
Na₂MoO₄ × 2 H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0-4.0

Seven vitamins solution:

Vitamin B₁₂ 100.0 mg
p-Aminobenzoic acid 80.0 mg
D(+)-Biotin 20.0 mg
Nicotinic acid 200.0 mg
Calcium pantothenate 100.0 mg
Pyridoxine HCl 300.0 mg
Thiamine-HCl × 2 H₂O 200.0 mg
Distilled water 1000.0 ml

Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30 – 45 min to remove dissolved oxygen and to saturate the solution with CO₂. Dispense solution in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 30% volume under 80% N₂ and 20% CO₂ gas atmosphere. Close vials with butyl rubber septa 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). After inoculation, add sterile air (with hypodermic needle through the rubber closure) to a concentration of ca. 4% (v/v) O₂ (e.g., add 7 ml of air to a Balch-type tube with a total volume of 27 ml).

423. METHYLOSARCINA QUISQUILLARUM AND M. FIBRATA MEDIUM (MQ M)

Solution 1 (10x NMS salts):

KNO₃ 10.0 g
MgSO₄ × 6 H₂O 10.0 g
CaCl₂ × 2 H₂O 2.0 g
Distilled water 1000.0 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 1000.0 ml

Solution 3 (Sodium molybdate):

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 0.26 g

Distilled water 1000.0 ml

Trace element solution:

EDTA Na_2 25.0 mg

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 100.0 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 50.0 mg

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 40.0 mg

H_3BO_3 1.50 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 5.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$ 1.0 mg

Distilled water 100.0 ml

May be stored at 4°C in the dark

Phosphate buffer:

$\text{Na}_2\text{HPO}_4 \times 12 \text{H}_2\text{O}$ 71.60 g

KH_2PO_4 26.0 g

Distilled water 1000.0 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 ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.

424. PALUDISPHERA BOREALIS MEDIUM PX4 (PBM PX4)

N-acetylglucosamine 1.0 g

KH_2PO_4 0.10 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.05 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.01 g

Hutners basal salts (see below) 20.0 ml

Na-ampicillin 0.20 g

Peptone 0.10 g

Yeast extract 0.10 g

Glucose 0.10 g

Agar-agar (Difco) 18.0 g

Distilled water 1000.0 ml

Adjust pH to 5.8 – 6.5.

Hutners basal salts:

Nitrilotriacetic acid (NTA) 10.0 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 29.70 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 3.34 g

$(\text{NH}_4)\text{MoO}_7\text{O}_{24} \times 4 \text{H}_2\text{O}$ 9.25 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 99.0 mg

“Metals 44” (see below) 50.0 ml

Distilled water 950.0 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 H₂SO₄.

"Metals 44":

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.2 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of the heavy metal ions.

425. *PROTEINIVORAX* MEDIUM (PHM)

KH₂PO₄ 0.30 g

MgSO₄ × 7 H₂O 0.12 g

CaCl₂ × 2 H₂O 0.03 g

NaCl 20.0 g

Tryptone (BD Bacto) 3.0 g

Trace element solution SL-10 (see below) 1.0 ml

Na₂CO₃ 60.0 g

NaHCO₃ 50.0 g

Na-Thioglycolate 1.0 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Dissolve ingredients (except carbonates and thioglycolate), then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Add carbonate and hydrogencarbonate, adjust pH to 8.5 - 9.0, dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add thioglycolate from an anoxic stock solution prepared under 100% N₂ gas and sterilized by filtration.

426. *SCHLESNERIA PALUDICOLA* MEDIUM (SPM)

N-acetylglucosamine 1.0 g

KH₂PO₄ 0.10 g

MgSO₄ × 7 H₂O 0.05 g

CaCl₂ × 2 H₂O 0.01 g

Hutners basal salts (see below) 20.0 ml

Na-ampicillin 0.20 g

Peptone 0.10 g

Yeast extract 0.10 g

Agar-agar (Difco) 18.0 g

Distilled water 1000.0 ml

Adjust pH to 5.5 – 5.6.

Hutners basal salts:

Nitrilotriacetic acid (NTA) 10.0 g

MgSO₄ × 7 H₂O 29.70 g

CaCl₂ × 2 H₂O 3.34 g

(NH₄)MoO₇O₂₄ × 4 H₂O 9.25 mg

FeSO₄ × 7 H₂O 99.0 mg

“Metals 44” (see below) 50.0 ml

Distilled water 950.0 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 H₂SO₄.

“Metals 44”:

Na-EDTA 250.0 mg

ZnSO₄ × 7 H₂O 1095.0 mg

FeSO₄ × 7 H₂O 500.0 mg

MnSO₄ × H₂O 154.0 mg

CuSO₄ × 5 H₂O 39.2 mg

Co(NO₃)₂ × 6 H₂O 24.8 mg

Na₂B₄O₇ × 10 H₂O 17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of the heavy metal ions.

427. TINDALLIA MEDIUM (TM)

Na₂CO₃ 8.0 g

KCl 0.2 g

NaCl 10.0 g

NH₄Cl 0.5 g

K₂HPO₄ 0.2 g

Yeast extract 4.0 g

Trace elements SL-10 (see below) 1.0 ml

Vitamin solution (see below) 1.0 ml

Na₂S × 9 H₂O 0.5 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Wolin's 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
Ca-D-pantothenate 5.0 mg
Vitamin B₁₂ 0.10 mg
p-Aminobenzoic acid 5.0 mg
α-Lipoic acid 5.0 mg
Distilled water 1000.0 ml
pH 9.0

Prepare the medium anaerobically without the vitamins. Boil the medium, cool under N₂ and dispense under N₂ and autoclave the medium. Add the vitamin solution to the cooled, autoclaved medium.

428. BLUE LAKE MEDIUM (BL-M)

NaCl 12.0 g
MgSO₄ × 7 H₂O 0.5 g
KH₂PO₄ 0.3 g
NH₄Cl 0.3 g
KCl 0.3 g
CaCl₂ × 6 H₂O 0.05 g
NaHCO₃ 0.5 g
Malic acid 1.0 g
Acetate Na 1.0 g
Yeast extract 1.0 g
Bactopeptone 0.5 g
Trace element solution (see below) 2 ml
Vitamin solution (see below) 2 ml
Distilled water 1000.0 ml
Adjust to 5.9 pH and autoclave. Adjust to 7.0 pH with 0.5 N NaOH

Trace element solution

FeSO₄ × 7 H₂O 0.3 g
MnCl₂ × 4 H₂O 0.003 g
CoCl₂ × 6 H₂O 0.005 g
CuCl₂ × 5 H₂O 0.001 g
NiCl₂ × 6 H₂O 0.002 g
Na₂MoO₄ × 2 H₂O 0.003 g
ZnSO₄ × 7 H₂O 0.005 g
H₃BO₃ 0.002 g

Dissolve in 0.5 l of distilled water and adjust pH to 3.0. Fill to 1 l and refrigerate

Vitamin solution:

Nicotinic Acid 0.4 g
Thiamin 0.4 g
Biotin 0.08 g
Vitamin B₁₂ 0.02 g
Filter sterilize and refrigerate

429. RICH ORGANIC MEDIUM WITH 2% NaCl (ROMNa)

NaCl 20.0 g
MgSO₄ × 7 H₂O 0.5 g
KH₂PO₄ 0.3 g
NH₄Cl 0.3 g
KCl 0.3 g
CaCl₂ × 6 H₂O 0.05 g

Acetate Na 1.0 g
Yeast extract 1.0 g
Bactopeptone 0.5 g
Casamino acids 0.5 g
Trace element solution (see below) 2 ml
Vitamin solution (see below) 2 ml
Distilled water 1000.0 ml
Adjust pH to 5.9 and autoclave. Adjust pH to 6.8-7.0 with 0.5N NaOH
Trace element solution
 $\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$ 0.3 g
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 0.003 g
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.005 g
 $\text{CuCl}_2 \times 5 \text{ H}_2\text{O}$ 0.001 g
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.002 g
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 0.003 g
 $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$ 0.005 g
 H_3BO_3 0.002 g
Dissolve in 0.5 l of distilled water and adjust pH to 3.0. Fill to 1 l and refrigerate
Vitamin solution:
Nicotinic acid 0.4 g
Thiamin 0.4 g
Biotin 0.08 g
Vitamin B₁₂ 0.02 g
Filter sterilize and refrigerate

430. THIOTHRUX THIOSULFATIREDUCTENS MEDIUM

$(\text{NH}_4)_2\text{SO}_4$ 0.5 g
 $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.03 g
 KH_2PO_4 0.11 g
 K_2HPO_4 0.085 g
 $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ 0.1 g
 NaNO_3 0.3 g
 NaHCO_3 (10%) 5.0 ml
 $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ (10%) 10.0 ml
Na-acetate (10%) 2.0 ml
Vitamin solution (see below) 1.0 ml
Trace element solution (see below): 1.0 ml
Agar 0.5 g
Distilled water 980 ml
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 B₁₂ 5.0 mg
p-Aminobenzoic acid 50.0 mg
Distilled water 1000.0 ml
Trace element solution:
EDTA 5.0 g

FeSO₄ × 7 H₂O 2.0 g
ZnSO₄ × 7 H₂O 100.0 mg
MnCl₂ × 4 H₂O 30.0 mg
H₃BO₃ 300.0 mg
CoCl₂ × 6 H₂O 200.0 mg
CuCl₂ 10.0 mg
NiCl₂ × 6 H₂O 20.0 mg
Na₂MoO₄ × 2 H₂O 20.0 mg
Distilled water 1000.0 ml

To prepare the trace element solution, preliminarily acidify water to pH 3.0-4.0 with HCl. Sterilize thiosulfate, acetate, bicarbonate, trace elements and vitamins separately and add to the main medium prior to inoculation. Sterilize the vitamin solution by filtration, others solutions and base medium by autoclaving at 121°C for 15 min.

431. METHANOSAETA PELAGICA MEDIUM (MPM)

MgCl₂ × 6 H₂O 3.05 g
CaCl₂ × 2 H₂O 0.15 g
NH₄Cl 0.54 g
NaCl 20.00 g
Na-acetate 6.56 g
Trace elements solution (see below) 2.00 ml
Yeast extract (OXOID) 1.50 g
Trypticase peptone (BD BBL) 0.40 g
Na-resazurin solution (0.1% w/v) 0.50 ml
KH₂PO₄ 1.19 g
K₂HPO₄ 0.21 g
Na₂CO₃ 1.50 g
2-Mercaptoethanesulfonic acid (coenzyme M) 0.14 g
Wolin's vitamin solution (see below) 10.00 ml
Na₂S × 9 H₂O 0.36 g
Distilled water 1000.00 ml

Wolin's 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
Nicotinic acid 5.00 mg
Ca-D-pantothenate 5.00 mg
Vitamin B₁₂ 0.10 mg
p-Aminobenzoic acid 5.00 mg
(±)-α-Lipoic acid 5.00 mg
Distilled water 1000.00 ml

Trace element solution:

Nitrilotriacetic acid (NTA) 12.80 g
FeCl₂ × 4 H₂O 1.00 g
MnCl₂ × 4 H₂O 0.10 g
CoCl₂ × 6 H₂O 0.03 g
CaCl₂ × 2 H₂O 0.10 g
ZnCl₂ 0.10 g
CuCl₂ 0.02 g
H₃BO₃ 0.01 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 0.03 g

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$ 0.10 g

NaCl 1.00 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$ 0.03 g

$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$ 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.

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.

432. *PYROBACULUM FERRIREDUCTENS* MEDIUM (PFM)

NH_4Cl 0.33 g

KCl 0.33 g

KH_2PO_4 0.33 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 0.33 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.33 g

Trace element solution *SL-10* (see below) 1.00 ml

Selenite-tungstate solution (see below) 1.00 ml

Yeast extract 1.00 g

KNO_3 1.00 g

NaHCO_3 2.00 g

Wolin's vitamin solution (see below) 10.00 ml

Distilled water 1000.00 ml

Trace element solution SL-10

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution:

NaOH 0.5 g

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$ 3.0 mg

$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$ 4.0 mg

Distilled water 1000.0 ml

Wolin's 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

Nicotinic acid 5.00 mg
Ca-D-pantothenate 5.00 mg
Vitamin B₁₂ 0.10 mg
p-Aminobenzoic acid 5.00 mg
(±)-α-Lipoic acid 5.00 mg
Distilled water 1000.00 ml

Dissolve ingredients (except yeast extract, nitrate, bicarbonate and vitamins) and sparge medium with 100% CO₂ 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% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 6.5 – 6.8, if necessary.

433. *HALODESULFOVIBRIO* MEDIUM (HdvM)

Solution A:

NaCl 25.0 g
K₂HPO₄ 0.5 g
NH₄Cl 1.0 g
Na₂SO₄ 1.0 g
CaCl₂ x 2 H₂O 0.1 g
MgSO₄ x 7 H₂O 2.0 g
Na-DL-lactate 2.0 g
Yeast extract 1.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Distilled water 980.0 ml

Solution B:

FeSO₄ x 7 H₂O 0.5 g
Distilled water 10.0 ml

Solution C:

Na-thioglycolate 0.1 g
Ascorbic acid 0.1 g
Distilled water 10.0 ml

Bring solution A to the boil, then cool to room temperature while sparging with 100% N₂ gas. Add solutions B and C, adjust pH to 7.8 with NaOH, and distribute under N₂ 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.

434. *TENIUFILUM THALASSICUM* MEDIUM (TTM)

KH₂PO₄ 0.33 g
NH₄Cl 0.33 g
KCl 0.33 g
NaCl 10.00 g
MgCl₂ x 6 H₂O 0.33 g
CaCl₂ x 6 H₂O 0.033 g
NaHCO₃ 2.00 g
Yeast extract 0.05 g
Tryptone 2.00 g
Fructose 2.00 g
Resazurin 1.00 mg
Trace element solution (see below) 10.0 ml

Vitamin solution (see below) 10.00 ml

Na₂S x 9 H₂O 0.50 g

Distilled water 1000.0 ml

Trace element solution:

Nitrilotriacetic acid 1.50 g

MgSO₄ x 7 H₂O 3.00 g

MnSO₄ x H₂O 0.50 g

NaCl 1.00 g

FeSO₄ x 7 H₂O 0.10 g

CoSO₄ x 7 H₂O 0.18 g

CaCl₂ x 2 H₂O 0.10 g

ZnSO₄ x 7 H₂O 0.18 g

CuSO₄ x 5 H₂O 0.01 g

KAl(SO₄)₂ x 12 H₂O 0.02 g

H₃BO₃ 0.01 g

Na₂MoO₄ x 2 H₂O 0.01 g

NiCl₂ x 6 H₂O 0.03 g

Na₂SeO₃ x 5 H₂O 0.30 mg

Na₂WO₄ x 2 H₂O 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.

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

Nicotinic acid 5.00 mg

Ca-D-pantothenate 5.00 mg

Vitamin B₁₂ 0.10 mg

p-Aminobenzoic acid 5.00 mg

(±)-α-Lipoic acid 5.00 mg

Distilled water 1000.00 ml

Dissolve ingredients (except carbonate, and sulfide), adjust pH to 8.0, boil for 10 min. and sparge medium with 100% N₂ 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% N₂ gas and carbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ 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.

435. BHI MEDIUM (BHIM)

Brain Heart Infusion (Difco) 37.0 g

Bovine serum 100 g

Maltose 10 g

Hemin 0.1 g

Distilled water 1000.0 ml

436. MODIFIED PYGV AGAR (MPYGV)

Mineral salt solution (see below) 20.0 ml

Peptone 0.25 g

Yeast extract 0.25 g
NH₄NO₃ 0.1 g
Agar (Bacto) 15.0 g
Distilled water 965.0 ml
Sterilize 20 min./121°C. After cooling to 60°C add to the medium:
Glucose solution (2.5%, sterile-filtered) 10.0 ml
Vitamin solution (see below) 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).

Mineral salt solution:

Nitrilotriacetic acid (NTA) 10.0 g
MgSO₄ × 7 H₂O 29.70 g
CaCl₂ × 2 H₂O 3.34 g
Na₂MoO₄ × 2 H₂O 12.67 mg
FeSO₄ × 7 H₂O 99.0 mg
Metall salt sol. "44" (see medium 590) 50.0 ml
Distilled water 900.0 ml
Dissolve NTA first by neutralizing with KOH, then add other salts. Adjust pH to 7.2 with KOH or H₂SO₄. Adjust volume to 1000.0 ml with distilled water.

Vitamin solution:

Biotin 4.0 mg
Folic acid 4.0 mg
Pyridoxine-HCl 20.0 mg
Riboflavin 10.0 mg
Thiamine-HCl × 2 H₂O 10.0 mg
Nicotinamide 10.0 mg
D-Ca-pantothenate 10.0 mg
Vitamin B₁₂ 0.20 mg
p-Aminobenzoic acid 10.0 mg
Distilled water 1000.0 ml
Store in the dark and cold (5°C).

"Metals 44":

Na-EDTA 250.0 mg
ZnSO₄ × 7 H₂O 1095.0 mg
FeSO₄ × 7 H₂O 500.0 mg
MnSO₄ × H₂O 154.0 mg
CuSO₄ × 5 H₂O 39.200 mg
Co(NO₃)₂ × 6 H₂O 24.800 mg
Na₂B₄O₇ × 10 H₂O 17.700 mg
Distilled water 1000.0 ml

437. MODIFIED MARINE MEDIUM (MMM)

NaCl 20.0 g
K₂HPO₄ 0.15 g
CaCl₂ × 2H₂O 0.15 g
NH₄Cl 0.25 g
MgSO₄ × 7H₂O 3.45 g
MgCl₂ × 6H₂O 2.75 g
KCl 0.33 g
NiCl₂ × 6H₂O 0.5 mg
Na₂SeO₄ × 5H₂O 0.5 mg
Fe(NH₄)₂(SO₄)₂ × 6H₂O 0.01 g

Trace mineral solution (see below) 10.0 ml
 Vitamin solution (see below) 5.0 ml
 Yeast extract 0.10 g
 Trypticase peptone 1.0 g
 Distilled water 1000.0 ml
Trace mineral solution (Kevbrin & Zavarzin, 1992):
 $(\text{NH}_4)_2\text{SO}_4\text{FeSO}_4 \times 6\text{H}_2\text{O}$ 784.0 mg
 $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ 99.0 mg
 $\text{CoCl}_2 \times 6\text{H}_2\text{O}$ 238.0 mg
 $(\text{NH}_4)_2\text{SO}_4\text{NiSO}_4 \times 6\text{H}_2\text{O}$ 395.0 mg
 $\text{Na}_2\text{MoO}_4 \times \text{H}_2\text{O}$ 24.0 mg
 $\text{Na}_2\text{WO}_4 \times 2\text{H}_2\text{O}$ 33.0 mg
 $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ 144.0 mg
 $\text{CuCl}_2 \times 6\text{H}_2\text{O}$ 238.0 mg
 $\text{Na}_2\text{SeO}_4 \times 5\text{H}_2\text{O}$ 94.0 mg
 H_3BO_3 6.0 mg
 Distilled water 1000.0 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
 Ca-D-pantothenate 5.0 mg
 Vitamin B₁₂ 0.10 mg
 p-Aminobenzoic acid 5.0 mg
 (±)-α-Lipoic acid 5.0 mg
 Distilled water 1000.0 ml

438. *DESULFOMICROBIUM* MEDIUM (DsmM)

Solution 1:

Na_2SO_4 4.0 g
 KH_2PO_4 0.2 g
 NH_4Cl 0.25 g
 NaCl 1.0 g
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 0.4 g
 KCl 0.5 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.11 g
 Resazurin 1.0 mg
 Distilled water 930.0 ml

Solution 2:

Trace element solution SL-10 (see below) 1.0 ml

Solution 3:

Vitamin solution (see below) 1.0 ml

Solution 4:

NaHCO_3 2.5 g
 Distilled water 50.0 ml

Solution 5:

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$ (3 mg in 1000.0 ml 0.01 M NaOH) 1.0 ml

Solution 6:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 0.4 g

Distilled water 10.0 ml

Solution 7:

Na-lactate 7.3 mM

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl_2 70.0 mg

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg

H_3BO_3 6.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg

$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg

Distilled water 990.0 ml

Vitamin solution:

p-Aminobenzoic acid 4.0 mg

D(+)-Biotin 1.0 mg

Thiamine-HCl 10.0 mg

Distilled water 100.0 ml

pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically under 80% N_2 + 20% CO_2 at 121°C for 15 min. Solutions 2, 4, 5 and 6 are gassed with N_2 and autoclaved separately at 121°C for 15 min.

Solution 3 (gassed with N_2 + CO_2) and solution 7 (gassed with N_2) are filter-sterilized.

439. *DESULFOTOMACULUM* MEDIUM (DstM)

Solution 1:

Na_2SO_4 4.0 g

KH_2PO_4 0.2 g

NH_4Cl 0.25 g

NaCl 1.0 g

$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.4 g

KCl 0.5 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ 0.11 g

Resazurin 1.0 mg

Distilled water 930.0 ml

Solution 2:

Trace element solution SL-10 (see below) 1.0 ml

Solution 3:

Vitamin solution (see below) 1.0 ml

Solution 4:

NaHCO_3 2.5 g

Distilled water 50.0 ml

Solution 5:

$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ (3 mg in 1000.0 ml 0.01 M NaOH) 1.0 ml

Solution 6:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.4 g

Distilled water 10.0 ml

Solution 7:

Glycerol 11 mM

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g

ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

p-Aminobenzoic acid 4.0 mg
D(+)-Biotin 1.0 mg
Thiamine-HCl 10.0 mg
Distilled water 100.0 ml
pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically under 80% N₂ + 20% CO₂ at 121°C for 15 min. Solutions 2, 4, 5 and 6 are gassed with N₂ and autoclaved separately at 121°C for 15 min. Solution 3 (gassed with N₂ + CO₂) and solution 7 (gassed with N₂) are filter-sterilized.

440. RHODOSPIRILLACEAE MEDIUM (RsM)

Yeast extract 0.30 g
Na₂-succinate 1.0 g
(NH₄)-acetate 0.50 g
Fe(III) citrate solution (0.1% in H₂O) 5.0 ml
KH₂PO₄ 0.50 g
MgSO₄ × 7 H₂O 0.40 g
NaCl 0.40 g
NH₄Cl 0.40 g
CaCl₂ × 2 H₂O 0.05 g
Vitamin B₁₂ solution (10 mg in 100 ml H₂O) 0.40 ml
Trace element solution SL-6 (see below) 1.0 ml
L-Cysteine HCl 0.30 g
Resazurin (0,1%) 0.50 ml
Distilled water 1000.0 ml

Trace element solution SL-6:

ZnSO₄ × 7 H₂O 0.1 g
MnCl₂ × 4 H₂O 0.03 g
H₃BO₃ 0.3 g
CoCl₂ × 6 H₂O 0.2 g
CuCl₂ × 2 H₂O 0.01 g
NiCl₂ × 6 H₂O 0.02 g
Na₂MoO₄ 0.03 g
Distilled water 1000.0 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.

441. BHI MEDIUM II (BHIM II)

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

442. DESULFOTHERMOBACTER MEDIUM (DstbM)

NH₄Cl 0.33 g
KCl 0.33 g
MgCl₂ × 6 H₂O 0.33 g
CaCl₂ × 2 H₂O 0.33 g
KH₂PO₄ 0.33 g
Na₂SO₄ 2.80 g
Trace element solution SL-10 (see below) 1.0 ml
Selenite-tungstate solution (see below) 1.0 ml
Wolin's vitamin solution (see below) 1.0 ml

Na₂S × 9 H₂O 0.50 g

Distilled water 1000.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 ml

Selenite-tungstate solution

NaOH 0.5 g

Na₂SeO₃ × 5 H₂O 3.0 mg

Na₂WO₄ × 2 H₂O 4.0 mg

Distilled water 1000.0 ml

Wolin's 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

Ca-D-pantothenate 5.0 mg

Vitamin B₁₂ 0.10 mg

p-Aminobenzoic acid 5.0 mg

(±)-α-Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Dissolve all ingredients except vitamins and sulfide, then sparge medium with 80% H₂ and 20% CO₂ 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% N₂ gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 4.5 - 4.8, if necessary.

443. THERMODESULFOBIUM ACIDIPHILUM MEDIUM (TAM)

NH₄Cl 0.33 g

KCl 0.33 g

KH₂PO₄ 0.33 g

MgCl₂ × 6 H₂O 0.33 g

CaCl₂ × 2 H₂O 0.33 g

Na₂SO₄ 2.80 g

Trace mineral solution SL-10 (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.50 ml
Yeast extract 3.0 g
Wolin's vitamin solution (see below) 10.0 ml

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ 0.45 g
Distilled water 1000.0 ml
Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
 $\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ 1.5 g
 ZnCl_2 70.0 mg
 $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ 100.0 mg
 H_3BO_3 6.0 mg
 $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 190.0 mg
 $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ 2.0 mg
 $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 24.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ 36.0 mg
Distilled water 990.0 ml

Wolin's 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
Ca-D-pantothenate 5.0 mg
Vitamin B₁₂ 0.10 mg
p-Aminobenzoic acid 5.0 mg
(±)-α-Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except yeast extract, vitamins and sulfide. Adjust pH to 4.5 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 and autoclave. 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. The pH of the complete medium should be at 4.5.

444. ISACHENKONIA MEDIUM

NH_4Cl 0.5 g
 NaCl 20.0 g
 $\text{CaCl}_2 \times 6 \text{ H}_2\text{O}$ 0.033 g
 KH_2PO_4 0.2 g
 $\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$ 0.1 g
 Na_2CO_3 50.0 g
 NaHCO_3 60.0 g
Glucose 3.0 g
Yeast extract 0.05 g
Trace element solution (see below) 1 ml
Vitamin solution (see below) 10 ml
 $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ 3.0 g
pH 9.2

Trace element solution:

$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \times 6 \text{ H}_2\text{O}$ (Mohr's salt) 784.0 mg

HCl (concentrated) 5.0 ml
CoCl₂ × H₂O 238.0 mg
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
Na₂WO₄ × 2 H₂O 33.0 mg
ZnSO₄ × 7 H₂O 144.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Na₂SeO₄ 94.0 mg
H₃BO₃ 6.0 mg
MnCl₂ × 4 H₂O 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl. Then it is mixed with distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 100 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

The medium is prepared anaerobically. NaHCO₃, Na CO₃ and vitamins, Na₂S × 9H₂O are added after boiling and cooling of the medium under 100% CO₂ in gas phase. Adjust the pH between 9.0 and 9.2 with sterile NaOH after autoclaving. Yeast extract must be added to the final solution before inoculation.

445. METHANOTHRIX MEDIUM (MtM)

KH₂PO₄ 0.3 g
NaCl 0.6 g
MgCl₂ × 6H₂O 0.1 g
CaCl₂ × 2H₂O 0.08 g
NH₄Cl 1.0g
Trace element solution (see below) 10.0 ml
Vitamin solution (see below) 10.0 ml
Sodium acetate 6.8 g
NaHCO₃ 4.0 g
L-Cysteine·HCl·H₂O 0.5 g
Na₂S × 9H₂O 0.5g
Resazurin 1.0 mg
Distilled water 1000 mL

Mix ingredients, except vitamin solution, NaHCO₃, L-cysteine·HCl·H₂O and Na₂S × 9H₂O, and adjust pH to 7.0. Bring to a boil for 5-10 sec, then, cool down under a stream of N₂-CO₂ (80:20) and add NaHCO₃. Dispense the medium into suitable culture vessels (e.g., 50 ml of the medium in 120 ml serum bottles) under a stream of N₂-CO₂ (80:20) and seal with butyl rubber stoppers. After autoclaving, stand the medium overnight. Separately autoclave cysteine-HCl and Na₂S × 9H₂O as 5% solutions, respectively, under a N₂ atmosphere. Filter-sterilize vitamin solution under a N₂ atmosphere. Prior to inoculation, aseptically and anaerobically add vitamin solution,

L-cysteine-HCl and $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ solutions. Pressurize the inoculated bottles to 100 kPa $\text{N}_2\text{-CO}_2$ (80:20).

Trace element solution:

Nitrilotriacetic acid 12.8 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 0.1 mg

$\text{CoCl}_2 \times 2 \text{H}_2\text{O}$ 0.17 mg

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.1 mg

ZnCl_2 0.1 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 0.02 mg

H_3BO_3 0.01 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 0.01 mg

NaCl 1.0 mg

Na_2SeO_4 0.017 mg

Distilled water 1000.0 ml

Dissolve nitrilotriacetic acid first in 200 ml of distilled water, and adjust pH to 6.5 with KOH.

Then add the remaining salts and adjust volume to 1000 mL. Readjust pH to 7.0 with KOH.

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 10.0 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

p-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

Vitamin B_{12} 0.1 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.2-7.4

446. MALT AGAR 4 BALLING (MA4B)

Malt extract Balling 4 degrees 1000.0 ml

Agar 20.0 g

Adjust pH to 5.8

Autoclave at 121°C (1 bar) for 30 min.

447. OCEANISPIROCHAETA MEDIUM (OsM)

Trypticase peptone (BD BBL) 2.0 g

Yeast extract 1.0 g

Na-resazurin solution (0.1% w/v) 0.5 ml

Na-thioglycolate 1.0 g

D-xylose 5.0 g

Charcoal-filtered, natural seawater 800.0 ml

Distilled water 200.0 ml

Dissolve ingredients except thioglycolate, 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 D-xylose from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration.

448. GEOTOGA MEDIUM (GM)

Bacto peptone 5.0 g
 Bacto yeast extract 1.0 g
 Fe(III) citrate 0.1 g
 NaCl 80.0 g
 MgCl₂ (anhydrous) 5.9 g
 Na₂SO₄ 3.24 g
 CaCl₂ × 2 H₂O 1.8 g
 KCl 0.55 g
 NaHCO₃ 0.16 g
 KBr 0.08 g
 SrCl₂ 34.0 mg
 H₃BO₃ 22.0 mg
 Na-silicate 4.0 mg
 NaF 2.4 mg
 NH₄NO₃ 1.6 mg
 Na₂HPO₄ 8.0 mg
 D-Glucose 5.0 g
 Distilled water 1000.0 ml C.
 Final pH should be 6.5
 If using the complete medium from Difco 2216 add 37.4 g per liter water + D-Glucose, 5 g per liter.

449. *PROSTHECOCHLORIS MARINA* MEDIUM (PMM)

KH₂PO₄ 0.5 g
 NH₄Cl 0.5 g
 NaCl 15.0 g
 MgCl₂ × 6 H₂O 0.2 g
 CaCl₂ 0.1 g
 NaHCO₃ 3.0 g
 Sodium acetate 0.5 g
 Yeast extract 0.1 g
 Na₂S₂O₃ × 5 H₂O 1.0 g
 Na₂S × 9 H₂O 0.7 g
 Trace element solution SL6 (see below) 1.0 ml
 Vitamin solution 1.0 ml (see below)
 Distilled water 1000.0 ml
Trace element solution SL-6:
 ZnSO₄ × 7 H₂O 0.1 g
 MnCl₂ × 4 H₂O 0.03 g
 H₃BO₃ 0.3 g
 CoCl₂ × 6 H₂O 0.2 g
 CuCl₂ × 2 H₂O 0.01 g
 NiCl₂ × 6 H₂O 0.02 g
 Na₂MoO₄ 0.03 g
 Distilled water 1000.0 ml
Vitamin solution:
 Biotin 20.0 mg
 Folic acid 20.0 mg
 Pyridoxine 100 mg
 Riboflavin 50.0 mg
 Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Vitamin B₁₂ 1.0 mg

Lipoic acid 50.0 mg

Distilled water 1000.0 ml

Prepare sulfide as a single sterile stock solution and add to the sterile medium after autoclaving.

The pH should be adjust to 7.0-7.2.

450. *DESULFOSPOROSINUS* MEDIUM (DssM)

Solution 1:

Na₂SO₄ 4.0 g

KH₂PO₄ 0.2 g

NH₄Cl 0.25 g

NaCl 1.0 g

MgCl₂ × 6 H₂O 0.4 g

KCl 0.5 g

CaCl₂ × 2 H₂O 0.15 g

Sodium lactate 2.0 g

Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g

Distilled water 100.0 ml

Solution 4:

Vitamin solution (see below) 10.0 ml

Solution 5:

Na₂S × 9 H₂O 0.5 g

Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml

FeCl₂ × 4 H₂O 1.5 g

ZnCl₂ 70.0 mg

MnCl₂ × 4 H₂O 100.0 mg

H₃BO₃ 6.0 mg

CoCl₂ × 6 H₂O 190.0 mg

CuCl₂ × 2 H₂O 2.0 mg

NiCl₂ × 6 H₂O 24.0 mg

Na₂MoO₄ × 2 H₂O 36.0 mg

Distilled water 990.0 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

Ca DL-pantothenate 5.0 mg

Vitamin B₁₂ 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized. Others solutions autoclave at 121°C for 15 min and add to base medium aseptically.
Final pH of the complete medium 7.1-7.4

451. *DESULFOTOMACULUM* MEDIUM (DtM)

Solution 1:

Na₂SO₄ 4.0 g
KH₂PO₄ 0.2 g
NH₄Cl 0.25 g
NaCl 1.0 g
MgCl₂ × 6 H₂O 0.4 g
KCl 0.5 g
CaCl₂ × 2 H₂O 0.15 g
Sodium pyruvate 1.0 g
Distilled water 870.0 ml

Solution 2:

Trace element solution *SL-10* (see below) 1.0 ml

Solution 3:

NaHCO₃ 5.0 g
Distilled water 100.0 ml

Solution 4:

Vitamin solution (see below) 10.0 ml

Solution 5:

Na₂S × 9 H₂O 0.5 g
Distilled water 10.0 ml

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 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
Ca DL-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized. Others solutions autoclave at 121°C for 15 min and add to base medium aseptically. Final pH of the complete medium 7.1-7.4

452. BIOMAI BACTER MEDIUM (BbM)

Solution 1:

NaCl 0.3 g
MgCl₂ × 6 H₂O 0.1 g
CaCl₂ × 2 H₂O 0.11 g
NH₄Cl 0.3 g
KH₂PO₄ 0.41 g
Na₂HPO₄ 0.53 g
NaHCO₃ 4.0 g
D-Glucose 1.0 g
Yeast extract 0.5 g
Vitamin solution (see below) 1.0 ml
Trace element solution (see below) 1.0 ml
Distilled water 940.0 ml

Solution 2:

Na₂S × 9 H₂O 0.48 g
Distilled water 10.0 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
Ca DL-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Trace element solution:

FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 68.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 62.0 mg
CoCl₂ × 6 H₂O 120.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
HCl (0.05 M) 1000.0 ml
pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N₂ and 20% CO₂ with sodium bicarbonate added until an equilibrium pH of 6.9 - 7.1. Solution 1 is autoclaved under this gas mixture at 121°C for 15 min. Solution 2 is autoclaved under 100% N₂ at 121°C for 15 min. Vitamin solution is filter sterilized.

453. SULFURIMONAS MEDIUM (SmM)

KH₂PO₄ 0.33 g

NH₄Cl 0.33 g
KCl 0.33 g
MgCl₂ × 6H₂O 0.33 g
CaCl₂ × 6H₂O, 0.16 g
NaCl 10.0 g
NaHCO₃ 2.0 g
Trace element solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Elemental sulfur 5.0 g
KNO₃ 10 mM

Trace element solution:

(NH₄)₂Fe(SO₄)₂ × 6 H₂O (*Mohr's salt*) 784.0 mg
HCl (concentrated) 5.0 ml
CoCl₂ × H₂O 238.0 mg
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 395.0 mg
Na₂MoO₄ × 2 H₂O 24.0 mg
Na₂WO₄ × 2 H₂O 33.0 mg
ZnSO₄ × 7 H₂O 144.0 mg
CuCl₂ × 2 H₂O 2.0 mg
Na₂SeO₄ 94.0 mg
H₃BO₃ 6.0 mg
MnCl₂ × 4 H₂O 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:

Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 0.1 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

The anoxic medium is prepared by boiling and cooling it under N₂ flow. No reducing agents were added. The isolation medium is pH of 7.5–8.0 (measured at 25 °C). Elemental sulfur as electron donor is added to the Hungate's tubes prior sterilization; potassium nitrate from anoxic sterile stock solution is added as electron acceptor just before inoculation.

454. SOEHNGENIA MEDIUM (SM)

KH₂PO₄ 0.2 g
NH₄Cl 0.25 g
KCl 0.33 g
MgCl₂ × 6H₂O 0.4 g
CaCl₂ × 6H₂O, 0.1 g
NaCl 15.0 g
Trace element solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.50 ml

Peptone 2.0 g
Yeast extract 0.2 g
 $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ 0.2 g
Trace element solution:
 $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$ (Mohr's salt) 784.0 mg
HCl (concentrated) 5.0 ml
 $\text{CoCl}_2 \times \text{H}_2\text{O}$ 238.0 mg
 $(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$ 395.0 mg
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$ 24.0 mg
 $\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$ 33.0 mg
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 144.0 mg
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 2.0 mg
 Na_2SeO_4 94.0 mg
 H_3BO_3 6.0 mg
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 99.0 mg
Distilled water 995.0 ml

Mohr's salt is dissolved firstly in concentrated HCl, then is mixed with distilled water and other salts are dissolved in the sequence indicated.

Vitamin solution:
Biotin 20.0 mg
Folic acid 20.0 mg
Pyridoxine 0.1 mg
Riboflavin 50.0 mg
Pantotenoic acid 50.0 mg
p-Aminobenzoic acid 50.0 mg
Thiamine-HCl 50.0 mg
Nicotinic acid 50.0 mg
Vitamin B₁₂ 1.0 mg
Lipoic acid 50.0 mg
Distilled water 1000.0 ml

The medium is prepared anaerobically. Vitamins and $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ are added after boiling and cooling of the medium under 100% argon in gas phase. Adjust the pH between 6.5 and 7.0 with sterile HCl or NaOH after autoclaving.

455. ALKALIBACTER MEDIUM (AbM)

KH_2PO_4 0.33 g
 NH_4Cl 0.33 g
KCl 0.33 g
 $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ 0.33 g
 $\text{CaCl}_2 \times 6\text{H}_2\text{O}$, 0.033 g
NaCl 10.0 g
 NaHCO_3 2.0 g
Trace element solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.50 ml
D-Glucose 2.0 g
Yeast extract 0.05 g
 $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ 0.5 g
Trace element solution:
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 30.0 g
 $\text{MnSO}_4 \times \text{H}_2\text{O}$ 5.0 g
NaCl 10.0 g

FeSO₄ × 7 H₂O 1.0 g
CoCl₂ × 6 H₂O 1.8 g
CaCl₂ × 2 H₂O 1.0 g
ZnSO₄ × 7 H₂O 1.8 g
CuSO₄ × 5 H₂O 0.1 g
KAl(SO₄)₂ × 12 H₂O 0.18 g
H₃BO₃ 0.1 g
Na₂MoO₄ × 2 H₂O 0.1 g
(NH₄)₂Ni(SO₄)₂ × 6 H₂O 2.8 g
Na₂WO₄ × 2 H₂O 0.1 g
Na₂SeO₄ 0.1 g

Distilled water 1000.0 ml

First adjust pH to 1.0 with diluted H₂SO₄, then add and dissolve the salts.

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine 10.0 mg
Riboflavin 5.0 mg
Pantotenoic acid 5.0 mg
p-Aminobenzoic acid 5.0 mg
Thiamine-HCl 5.0 mg
Nicotinic acid 5.0 mg
Vitamin B₁₂ 0.1 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

NaHCO₃ and vitamins, Na₂S × 9H₂O are added after boiling and cooling of the medium under 100% N₂. Adjust the pH between 8.0 and 8.5 with sterile HCl or NaOH after autoclaving.

456. INFIRMIFILUM MEDIUM (IM)

KH₂PO₄ 0.165 g
NH₄Cl 0.165 g
KCl 0.165 g
MgCl₂ × 6H₂O 0.165 g
CaCl₂ × 6H₂O, 0.06 g
Trace element solution (see below) 1.0 ml
Vitamin solution (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.50 ml
Yeast extract 0.05 g
Na₂S × 9H₂O 0.3 g
D-Glucose 1.0 g

Trace element solution:

MgSO₄ × 7 H₂O 30.0 g
MnSO₄ × H₂O 5.0 g
NaCl 10.0 g
FeSO₄ × 7 H₂O 1.0 g
CoCl₂ × 6 H₂O 1.8 g
CaCl₂ × 2 H₂O 1.0 g
ZnSO₄ × 7 H₂O 1.8 g
CuSO₄ × 5 H₂O 0.1 g
KAl(SO₄)₂ × 12 H₂O 0.18 g
H₃BO₃ 0.1 g
Na₂MoO₄ × 2 H₂O 0.1 g

$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \times 6 \text{ H}_2\text{O}$ 2.8 g

$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ 0.1 g

Na_2SeO_4 0.1 g

Distilled water 1000.0 ml

First adjust pH to 1.0 with diluted H_2SO_4 , then add and dissolve the salts.

Vitamin solution:

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine 10.0 mg

Riboflavin 5.0 mg

Pantotenoic acid 5.0 mg

p-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg

Vitamin B₁₂ 0.1 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

The medium is prepared anaerobically. Vitamins, $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ are added after boiling and cooling of the medium under 100% CO_2 in gas phase. Adjust the pH between 6.0 and 6.5 with sterile HCl or NaOH after autoclaving. Yeast extract must be added to the final solution before inoculation.

457. SULFURISPAERA TOKODAI MEDIUM (STM)

KH_2PO_4 0.28 g

$(\text{NH}_4)_2\text{SO}_4$ 1.3 g

$\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 0.25 g

$\text{CaCl}_2 \times 6\text{H}_2\text{O}$ 0.07 g

$\text{FeCl}_3 \times 6\text{H}_2\text{O}$ 2.0 mg

$\text{MnCl}_2 \times 4\text{H}_2\text{O}$ 1.8 mg

$\text{Na}_2\text{B}_4\text{O}_7 \times 10\text{H}_2\text{O}$ 4.5 mg

$\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ 0.22 mg

$\text{CuCl}_2 \times 2\text{H}_2\text{O}$ 0.05 mg

$\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$ 0.03 mg

$\text{VOSO}_4 \times x\text{H}_2\text{O}$ 0.03 mg

$\text{CoSO}_4 \times 7\text{H}_2\text{O}$ 0.01 mg

Yeast extract (BD-Difco) 1.0 g

Distilled water 1000.0 ml

Adjust pH to 2.0 with 10 N H_2SO_4

458. LICHENIFUSTIS MEDIUM (LfM)

Mannitol 0.5 g

Arabinose 0.5 g

Mannose 0.5 g

$\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 0.1 g

KH_2PO_4 0.2 g

Yeast extract 0.2 g

Soya tripton 0.2 g

Casamino acids 0.1 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ 0.2 mg

$\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$ 0.3 mg

$\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ 1.0 mg

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ 0.1 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ 0.15 mg
Distilled water 1000.0 ml
pH 5.0

459. ACIDITHIOBACILLUS FERROOXIDANS MEDIUM (AFM)

Solution A:

$(\text{NH}_4)_2\text{SO}_4$ 3.0 g
KCl 0.1 g
 K_2HPO_4 0.5 g
 $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 0.5 g
 $\text{Ca}(\text{NO}_3)_2$ 0.01 g
Distilled water 700 ml

Solution B:

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 44.22 g
10 N H_2SO_4 4.5 ml
Distilled water 295.5 ml
Autoclave separately at 111°C for 30 min, cool to room temperature and mix.

460. MEDIUM FOR METHYLOMONAS SP. (MMsp)

KNO_3 0.5 g
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ 0.5 g
 CaCl_2 0.1 g
 $\text{Na}_2\text{HPO}_4 \times 12 \text{H}_2\text{O}$ 1.5 g
 KH_2PO_4 0.7 g
Trace element solution (see below) 1.0 ml
Distilled water 1000.0 ml

Trace elements solution:

EDTA 5.0 g
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ 2.0 g
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 0.1 g
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ 0.03 g
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$ 0.2 g
 $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ 0.1 g
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$ 0.02 g
 Na_2MoO_4 0.03 g
Distilled water 1000.0 ml
pH 6.7-7.1
Autoclave at 121°C 15 min.
Cultivation under mixture of methane and air (30:70).

461. ACIDILOBUS ACETICUS MEDIUM (MAcAc)

NH_4Cl 0.33 g
KCl 0.33 g
 KH_2PO_4 0.33 g
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 0.33 g
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.33 g
Trace mineral solution SL-10 (see below) 1.0 ml
Na-resazurin solution (0.1% w/v) 0.5 ml
Sulfur, powdered 10.0 g
Yeast extract 3.0 g
Vitamin solution (see below) 10.0 ml
 $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ 0.45 g

Distilled water 1000.0 ml.

Trace element solution SL-10:

HCl (25%; 7.7 M) 10.0 ml
FeCl₂ × 4 H₂O 1.5 g
ZnCl₂ 70.0 mg
MnCl₂ × 4 H₂O 100.0 mg
H₃BO₃ 6.0 mg
CoCl₂ × 6 H₂O 190.0 mg
CuCl₂ × 2 H₂O 2.0 mg
NiCl₂ × 6 H₂O 24.0 mg
Na₂MoO₄ × 2 H₂O 36.0 mg
Distilled water 990.0 ml

Vitamin solution:

Biotin 2.0 mg
Folic acid 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid 5.0 mg
D-Ca-pantothenate 5.0 mg
Vitamin B₁₂ 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1000.0 ml

Dissolve ingredients except sulfur, yeast extract, vitamins and sulfide.

Adjust pH to 3.5 with H₂SO₄ and sparge medium with 100% CO₂ 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 successive days.

Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas.

The pH of the complete medium should be at 3.5 - 3.8.

462. SULFOLOBUS MEDIUM (MSUL)

(NH₄)₂SO₄ 1.30 g
KH₂PO₄ 0.28 g
MgSO₄ × 7 H₂O 0.25 g
CaCl₂ × 2 H₂O 0.07 g
FeCl₃ × 6 H₂O 0.02 g
Trace element solution 10.0 ml
Sulfur (powder) 10.0 g
Yeast extract (OXOID) 0.50 g
Na₂S × 9 H₂O 0.50 g
Distilled water 1000.0 ml

Trace element solution:

MnCl₂ × 4 H₂O 180.0 mg
Na₂B₄O₇ × 10 H₂O 450.0 mg

ZnSO₄ × 7 H₂O 22.0 mg
CuCl₂ × 2 H₂O 5.0 mg
Na₂MoO₄ × 2 H₂O 3.0 mg
VOSO₄ × 2 H₂O 3.0 mg
CoSO₄ × 7 H₂O 1.0 mg
Distilled water 1000.0 ml.

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

Dissolve ingredients (except sulfur, yeast extract and sulfide), adjust pH of the salt solution at room temperature to 4.0 using 1 N H₂SO₄ and sparge medium with 100% N₂ gas 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.

Sterilize medium by heating cultivation vessels in a boiling water bath for 1-2 hours on each of 3 successive days.

Add yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas.

Prior to inoculation check pH and adjust to 4.0, if necessary.

463. MEDIUM FOR DENITRIFYING BACTERIA GROUP 2 (MDENgr2)

NaNO₂ 0.5 g
CaCO₃ 0.007 g
NaCl 0.5 g
MgCl₂ × 6H₂O 0.05 g
KH₂PO₄ 0.15 g
MnSO₄ × H₂O 33.8 µg
H₃BO₃ 49.4 µg
ZnSO₄ × 7H₂O 43.1 µg
(NH₄)₆ Mo₇O₂₄ × 4H₂O 37.1 µg
CuSO₄ × 5H₂O 25.0 µg

FeSO₄ × 7H₂O 973.0 µg
Distilled water 1000.0 ml
Adjust pH to 8.6

464. MN MEDIUM FOR ACIDOPHILES (MAcid)

KH₂PO₄ 0.1 g
(NH₄)₂SO₄ 0.2 g
MgSO₄ × 7H₂O 0.1 g
CaCl₂ × 2H₂O 0.02 g
Yeast extract 0.2 g
Glucose 0.5 g
Distilled water 1000 ml.
Adjust the pH to 5.8–6.0.
Reduced oxygen tension is desirable for the best growth.

465. MEDIUM FOR RHODOBACTER CLAVIFORME (MRHCL)

NH₄Cl 0.4 g
KH₂PO₄ 0.5 g
MgCl₂ 0.2 g
Na₂SO₄ 0.5 g
KCl 0.5 g
NaCl 20.0 g
NaHCO₃ 5.0 g
Yeast extract 1.0 g
Na acetate 1.0 g
Na pyruvate 1.0 g
Bacto pepton 1.0 g
Vitamin B₁₂ 20.0 µg
Trace element solution (see below) 1 ml
Distilled water 1000.0 ml.

Trace element solution:

EDTA-Na 5.0 g
FeSO₄ × 6H₂O 2.0 g
ZnSO₄ × H₂O 0.1 g
MnCl₂ × 4H₂O 0.03 g
H₃BO₃ 0.3 g
CoCl₂ × 2H₂O 0.2 g
CuCl₂ × 2H₂O 0.03 g
NiCl₂ × 6H₂O 0.02 g
Na₂MoO₄ × 2H₂O 0.03 g
Distilled water 1000.0 ml
pH 3.0–4.0

Prepare solutions of trace elements and NaHCO₃ (10%) separately in 50–100 ml screw capped vials and sterilize at 0.5 atm.

Add to the medium prior to inoculation. Sterilize the basal medium at 1 atm in 0.5 h. The final pH 8.0 - 8.5.

Maintain pure cultures in liquid media in the light under anaerobic conditions.

Cultures can also be grown in Petri dishes (2% agar) and under aerobic conditions in the dark in 50–100 ml vials.

466. ACIDITHIOBACILLUS CALDUS MEDIUM (MACCA)

(NH₄)₂SO₄ 3.0 g
K₂HPO₄ 3 H₂O 0.5 g
MgSO₄ × 7 H₂O 0.5 g
KCl 0.1 g
Ca(NO₃)₂ × 4 H₂O 0.02 g
Trace element solution (see below) 10.0 ml
Sulfur (powdered) 5.0 g
Distilled water 1000.0 ml

Trace element solution:

FeCl₃ × 6 H₂O 1.10 g
CuSO₄ × 5 H₂O 0.05 g
H₃BO₃ 0.20 g
MnSO₄ × H₂O 0.2
Na₂MoO₄ × 2 H₂O 0.08 g
CoCl₂ × 6 H₂O 0.06 g
ZnSO₄ × 7 H₂O 0.09 g
Distilled water 1000.0 ml

Dissolve ingredients except trace elements and sulfur.
Adjust pH with 6 N H₂SO₄ to 2.5, dispense into suitable cultivation vessels and autoclave.
After autoclaving add filter-sterilized trace elements solution and sterile sulfur powder (sterilized by steaming for 3 hours on each of 3 successive days).

467. YPG MEDIUM

Glucose 1.0 g
Peptone 1.0 g
Yeast extract 1.0 g
Distilled water 1000.0 ml
pH 5.6-6.0
Autoclave at 111°C for 30 min.
Agar may be added (15 g/l) to solidify the medium

468. *BRYOCELLA ELONGATA* MEDIUM (MBREL)

Glucose (or fructose) 0.5 g
Yeast extract 0.05 g
MgSO₄ × 7 H₂O 0.05 g
CaCl₂ × 2 H₂O 0.02 g
KH₂PO₄ 0.1 g
(NH₄)₂SO₄ 0.1 g
Distilled water 1000.0 ml

The medium may be solidified with 15 g/l agar.
Adjust to pH 5.0-5.8

469. *VERRUCOMICROBIUM* MEDIUM (MVER)

Peptone 0.25 g
Yeast extract 0.25 g
Glucose 0.25 g
Vitamin solution (see below) 10.0 ml
Hutner's basal salts (see below) 20.0 ml
0.1M Tris/HCl at pH 7.5 50.0 ml

Distilled water 670.0 ml
Artificial sea water (see below) 250.0 ml

Artificial sea water:

NaCl 23.477 g
Na₂SO₄ 3.917 g
MgCl₂ × 6 H₂O 4.981 g
CaCl₂ × 2 H₂O 1.1 g
NaHCO₃ 192.0 mg
KCl 664.0 mg
KBr 6.0 mg
H₃BO₃ 26.0 mg
SrCl₂ 24.0 g
NaF 3.0 mg
Distilled water 1000.0 ml

Hutner's basal salts medium:

Nitrilotriacetic acid (NTA) 10.0 g
MgSO₄ × 7 H₂O 29.7 g
CaCl₂ × 2 H₂O 3.34 g
Na₂MoO₄ × 2 H₂O 12.67 mg
FeSO₄ × 7 H₂O 99.0 mg
Metal salt solution "44" (see below) 50.0 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.
pH 7.2 (adjust with KOH or H₂SO₄).
Adjust volume to 1000.0 ml with distilled water

Metal solution "44":

Na-EDTA 250.0 mg
ZnSO₄ × 7 H₂O 1095.0 mg
FeSO₄ × 7 H₂O 500.0 mg
MnSO₄ × 7 H₂O 154.0 mg
CuSO₄ × 5 H₂O 39.0 mg
Co(NO₃)₂ × 6 H₂O 24.8 mg
Na₂B₄O₇ × 10 H₂O 17.7 mg
Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of heavy metal ions

Vitamin solution:

Vitamin B₁₂ 0.1 mg
Biotin 2.0 mg
Thiamine-HCl × 2 H₂O 5.0 mg
Ca-pantothenate 5.0 mg
Folic acid 2.0 mg
Riboflavin 5.0 mg
Nicotinamide 5.0 mg
Distilled water 1000.000 ml

Autoclave medium (except vitamin solution) and Artificial sea water separately at 121°C 15 min. After cooling to 60°C aseptically add to the medium vitamin solution (sterilize by filtration). Store vitamin solution in the dark and cold (5°C). Add Base medium to Artificial sea water and mix thoroughly.

470. THIOALKALIBACTER HALOPHILUS MEDIUM (MTHHA)

Na₂CO₃ 95.0 g

NaHCO₃ 15.0 g

NaCl 16.0 g

K₂HPO₄ 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 below) 1.0 ml/l

MgCl₂ × 6 H₂O (200.0 g/l) 1.0 ml/l (a white colloid will form which will rapidly dissolve after mixing)

Na₂S₂O₃ × 5 H₂O (1.0 M - 248.0 g/l) 20.0 ml/l

NH₄Cl - (1 M - 53.5 g/l) 4.0 ml/l

Trace element solution:

EDTA 5.0 mg

FeSO₄ × 7 H₂O 2.0 mg

ZnSO₄ × 7 H₂O 100.0 mg

MnCl₂ × 4 H₂O 30.0 mg

CoCl₂ × 6 H₂O 200.0 mg

NiCl₂ × 6 H₂O 20.0 mg

Na₂MoO₄ × 2 H₂O 30.0 mg

CuCl₂ × 2 H₂O 10.0 mg

H₃BO₃ 300.0 mg

Distilled water 1000.0 ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

471. MEDIUM FOR PALUDISPHAERA MUCOSA (PMuM)

KH₂PO₄ 0.1 g

N- acetylglucosamine 0.5 g

Glucose 0.5 g

Peptone 0.05 g

Yeast extract 0.05 g

Hunter salt solution 20 ml

Vitamin Solution No. 6 (Staley, 1968) 10 ml

Biotin 4.0 mg

Pyridoxine hydrochloride 20.0 mg

Thiamine hydrochloride 10.0 mg

Ca pantothenate 10.0 mg

p-Aminobenzoic acid 10.0 mg

Folic acid 4.0 mg

Riboflavin 10.0 mg

Nicotinamide or Nicotinic acid 10.0 mg

Vitamin B12 0.2 mg

Double-distilled water 1000 мл
(*Stirring of the mixture improves dissolution. Sterilize by filtration only. Store dark and cold (5° C)*)
pH 6.8-7.5
Distilled water 1000 мл

472. MEDIUM FOR *BDELLOVIBRIO* SP. (BV_{sp})

Yeast extract 0.75 g
Peptone 0.15 г
Distilled water 1000 мл
pH 7.2
Autoclave at 111° C 30 мин.

473. MEDIUM FOR *ALKALISALIBACTERIUM LIMINCOLA* (ALM)

R2A AGAR MEDIUM (№ 292)
Bacto yeast extract 0.5 g
Proteose peptone 0.5 g
Casamino acids 0.5 g
Glucose 0.5 g
Soluble starch 0.5 g
Na-pyruvate 0.3 g
KH₂PO₄ 0.3 g
MgSO₄ × 7 H₂O 50 mg
Agar 15.0 g
Distilled water 1000.0 ml. Autoclave at 121°C for 15 min

Sea salt 50 mg
Distilled water 1000.0 ml.
After autoclaving at 121°C for 15 min, adjust pH values up to 9.0-9.5 with separately sterilized 0.5% (w/v) Na₂CO₃

474. MEDIUM FOR *HALAMONAS DECHROMATICANS* (HDM)

Solution 1

Na₂CO₃ 76.0 g
NaHCO₃ 12.82 g
NaCl 69.0 g
K₂HPO₄ 1.0 g
Distilled water 900.0 ml.

Solution 2

Na acetate 2.0 g
NaNO₃ 1.0 g
Distilled water 100.0 ml.
MgSO₄ 10% 1 ml
NH₄Cl 10% 1 ml
Yeast extract 10% 1 ml
Trace element solution according to Pfennig (see below) 2.0 ml.

Trace element solution according to Pfennig:

EDTA 1.5 g
Trace element solution according to Hogland (see below) 6.0 ml.
FeSO₄ × 7 H₂O 0.2 g
MnCl₂ × 4 H₂O 0.02 g

ZnSO₄ × 7 H₂O 0.1 g
Distilled water 1000.0 ml.

Trace element solution according to Hogland:

EDTA 5.0 g,
FeSO₄ × 7 H₂O 2.0 g,
ZnSO₄ × 7 H₂O 100.0 mg,
MnCl₂ × 4 H₂O 30.0 mg,
H₃BO₃ 300.0 mg,
CoCl₂ × 6 H₂O 200.0 mg,
CuCl₂ × 2 H₂O 10.0 mg,
NiCl₂ × 6 H₂O 20.0 mg,
Na₂MoO₄ × 2 H₂O 20.0 mg,
Distilled water 1000.0 ml.

Autoclave solutions 1, 2, MgSO₄, NH₄Cl, yeast extract and trace element solution separately at 121 C for 15 min and mix aseptically.

475. RHODOCOCCUS MEDIUM WITH n-HEXADECANE (RHODO)

Composition per liter:

KNO₃ 1.0 g
KH₂PO₄ 1.0 g
K₂HPO₄ 1.0 g
NaCl 1.0 g
MgSO₄ 0.2 g
CaCl₂ 0.02 g
FeCl₃ 0.001 g
pH 6.8-7.0.

n-Hexadecane is used as the sole carbon source at a concentration of 1.0 vol. %.

476. RHODOCOCCUS MEDIUM WITH PROPANE (RHODO C3)

Composition per liter:

KNO₃ 1.0 g
KH₂PO₄ 1.0 g
K₂HPO₄ 1.0 g
NaCl 1.0 g
MgSO₄ 0.2 g
CaCl₂ 0.02 g
FeCl₃ 0.001 g
pH 6.8-7.0.

Gas mixture composition: propane - air 1:5.

477. ARTIFICIAL SEAWATER (ASW)

NaCl 70 g
KCl 0.7 g
MgCl₂ × 7 H₂O 15.9 g
Yeast extract 3.0 g
Peptone 2.5 g
Distilled water 1000,0 ml
pH 7.0-7.5. Sterilization at 121°C for 15 min