

1597: METHANOSALSUM NATRONOPHILUM MEDIUM

Solution A	750.00	ml
Solution B	250.00	ml
Solution C	1.00	ml
Solution D	1.00	ml
Solution E	5.00	ml
Solution F	3.00	ml
Solution G	5.00	ml
Solution H	10.00	ml
Solution I	1.00	ml
Solution J	10.00	ml

1. Sterilize solutions A and B in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of solution A after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 - 4 days. It is best to remove precipitates by decantation before using solution A for medium preparation.

2. Combine solution A with solution B and sparge medium with 100% N₂ gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions C to J are sterilized separately under 100% N₂ gas. Vitamins and coenzyme M should be sterilized by filtration. To complete the medium appropriate amounts of solutions C to J are added to the combined sterile solutions A and B in the sequence as indicated. Final pH of the medium should be 9.5.

3. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

Solution A

Na ₂ CO ₃	71.25	g
NaHCO ₃	11.25	g
NaCl	4.00	g
K ₂ HPO ₄	0.75	g
Distilled water	750.00	ml

Solution B

NaCl	30.00	g
K ₂ HPO ₄	0.25	g
KCl	0.25	g
Distilled water	250.00	ml

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Solution C

Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
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Solution D

Selenite-tungstate solution	1.00	ml
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Solution E

NH ₄ Cl	0.20	g
MgSO ₄ x 7 H ₂ O	0.25	g
Distilled water	5.00	ml

Solution F

Methanol	3.00	ml
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Solution G

Yeast extract	0.02	g
Distilled water	5.00	ml

Solution H

2-Mercaptoethanesulfonic acid (coenzyme M)	0.15	g
Distilled water	10.00	ml

Solution I

Wolin's vitamin solution (10x)	1.00	ml
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Solution J

Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	10.00	ml

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
FeSO ₄ x 7 H ₂ O	2.20	g
ZnSO ₄ x 7 H ₂ O	0.10	g
MnCl ₂ x 4 H ₂ O	0.03	g
H ₃ BO ₃	0.03	g
CoCl ₂ x 6 H ₂ O	0.20	g
CuCl ₂ x 2 H ₂ O	0.03	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g

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Distilled water	1000.00	ml
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pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	1000.00	ml

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

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