

131. METHANOBACTERIUM THERMOAUTOTROPHICUM MEDIUM

KH ₂ PO ₄	0.300	g
(NH ₄) ₂ SO ₄	1.500	g
NaCl	0.600	g
MgSO ₄ x 7 H ₂ O	0.120	g
CaCl ₂ x 2 H ₂ O	0.080	g
FeSO ₄ x 7 H ₂ O	4.000	mg
K ₂ HPO ₄	0.150	g
Na ₂ CO ₃	4.000	g
Vitamin solution (see below)	10.000	ml
Trace element solution (see below)	10.000	ml
Resazurin	1.000	mg
Cysteine-HCl x H ₂ O	1.500	g
Na ₂ S x 9 H ₂ O	1.500	g
Distilled water	1000.000	ml

Adjust pH to 7.2.

Prepare and sterilize the medium under a strictly anaerobic hydrogen and carbon dioxide atmosphere (80:20). Concentrated cysteine and sodium sulfide solutions are sterilized separately under a nitrogen atmosphere in tightly closed vessels. Appropriate amounts of these solutions are transferred into the sterilized medium by syringe. Details of medium preparation should be taken from the original literature.

Vitamin solution:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine-HCl	10.000	mg
Thiamine-HCl x 2 H ₂ O	5.000	mg
Riboflavine	5.000	mg
Nicotinic acid	5.000	mg
Ca-pantothenate	5.000	mg
p-Aminobenzoic acid	1.000	mg
Vitamin B ₁₂	0.010	mg
Distilled water	1000.000	ml

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Trace elements solution:

Na ₂ -EDTA	0.640	g
MgSO ₄ x 7 H ₂ O	6.200	g
MnSO ₄ x 4 H ₂ O	0.550	g
NaCl	1.000	g
FeSO ₄ x 7 H ₂ O	0.100	g
CoCl ₂ x 6 H ₂ O	0.170	g
CaCl ₂ x 2 H ₂ O	0.130	g
ZnSO ₄ x 7 H ₂ O	0.180	g
CuSO ₄	0.050	g
KAl(SO ₄) ₂ x 12 H ₂ O	0.018	g
H ₃ BO ₃	0.010	g
Na ₂ MoO ₄ x 2 H ₂ O	0.011	g
NiCl ₂ x 6 H ₂ O	0.025	g

Dissolve the Na₂-EDTA in 500 ml of distilled water, and after adding the salts, make up to 1000 ml.