

318: METHANOSARCINA THERMOPHILA (BCYT) MEDIUM

KH ₂ PO ₄	0.30	g
NaCl	0.60	g
MgCl ₂ x 6 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.08	g
Trace element solution	10.00	ml
NH ₄ Cl	1.00	g
Yeast extract (OXOID)	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
KHCO ₃	2.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, vitamins, methanol, cysteine and sulfide, 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 or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), methanol (50% v/v solution), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% 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 6.8, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
FeCl ₂ x 4 H ₂ O	1.00	g
MnCl ₂ x 4 H ₂ O	0.10	g
CoCl ₂ x 6 H ₂ O	0.03	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnCl ₂	0.10	g
CuCl ₂	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
NiCl ₂ x 6 H ₂ O	0.10	g
NaCl	1.00	g
Na ₂ SeO ₃ x 5 H ₂ O	0.03	g
Na ₂ WO ₄ x 2 H ₂ O	0.04	g
Distilled water	1000.00	ml

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

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

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