

317. METHANOSARCINA THERMOPHILA MEDIUM

NH ₄ Cl	1.00	g
K ₂ HPO ₄	0.40	g
MgCl ₂ x 6 H ₂ O	0.20	g
Mineral solution (see below)	50.00	ml
Trace element solution (see medium 141)	10.00	ml
Yeast extract	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Methanol	5.00	ml
L-Cysteine-HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	950.00	ml

Dissolve ingredients except carbonate, 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 vilas and autoclave. Add methanol, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate 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 - 7.0, if necessary.

Minerals solution:

KH ₂ PO ₄	6.00	g
(NH ₄) ₂ SO ₄	6.00	g
NaCl	12.00	g
MgSO ₄ x 7 H ₂ O	2.60	g
CaCl ₂ x 2 H ₂ O	0.16	g
Distilled water	1000.00	ml