

1084. METHERMICOCOCUS (METHANOGEN) MEDIUM

KCl	0.34	g
NH ₄ Cl	0.25	g
K ₂ HPO ₄	0.20	g
NaCl	24.00	g
MgCl ₂ x 6 H ₂ O	10.20	g
Yeast extract	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Sludge fluid (see medium 119)	5.00	ml
Methanol	8.00	ml
2-Mercaptoethanesulfonic acid (coenzyme M)	2.50	g
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 carbonate, sludge fluid, methanol, coenzyme M, 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 same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sludge fluid, methanol, coenzyme M (sterilized by filtration), 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. Adjust pH of complete medium to 6.0 - 6.5, if necessary.