

120b. METHANOMICROCOCCLUS MEDIUM

K ₂ HPO ₄	0.35	g
KH ₂ PO ₄	0.23	g
NH ₄ Cl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.25	g
NaCl	2.25	g
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract (OXOID)	2.00	g
Casitone (BD BBL)	2.00	g
Na-acetate	2.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
2-Mercaptoethanesulfonate (coenzyme M)	0.14	g
Vitamin solution (see medium 141)	10.00	ml
Methanol	10.00	ml
L-Cysteine-HCl x H ₂ O	0.36	g
Na ₂ S x 9 H ₂ O	0.36	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, coenzyme M, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Then add and dissolve bicarbonate, adjust pH to 7.2 and dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N₂ gas atmosphere as concentrated solutions in tightly closed tubes. Vitamins and coenzyme M are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 7.0 – 7.2, if necessary. After inoculation, pressurize culture vessels with sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure.

For [DSM 22503](#) supplement medium with 6.00 g/l NaCl. Do not add overpressure of 80% H₂ and 20% CO₂ gas mixture.