120b. METHANOMICROCOCCUS MEDIUM

K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
NaCl	2.25	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v in 0.1 N H_2SO_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_2O	0.36	g
Na_2S x 9 H_2O	0.36	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, coenzyme M, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% H_2 and 20% CO_2 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% H_2 and 20% CO_2 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% N_2 gas atmosphere as concentrated solutions in tightly closed tubes. Vitamins and coenzyme M are prepared under 100% N_2 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% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.

For DSM 22503 supplement medium with 6.00 g/l NaCl. Do not add overpressure of 80% H_2 and 20% CO_2 gas mixture.