

274. METHANOLACINIA MEDIUM

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	2.75	g
MgSO ₄ x 7 H ₂ O	3.40	g
NH ₄ Cl	1.50	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	6.31	g
Trace element solution (see medium 141)	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O solution (0.1% w/v)	2.00	ml
NaHCO ₃	5.00	g
Na-acetate x 3 H ₂ O	4.15	g
Na-resazurin solution (0.1% w/v)	0.50	ml
MOPS (3-(N-morpholino) propane-sulfonic acid)	20.93	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except MOPS, vitamins, cysteine and sulfide in a total volume of 890 ml. The MOPS buffer is dissolved separately in 90 ml of distilled water, the pH is adjusted to 7.0 with 2 M KOH, then add the buffer to the salt solution. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then dispense medium in anoxic Hungate-type tubes under 80% H₂ and 20% CO₂ gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 7.0, if necessary. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure.