

141b. METHANOCULLEUS SP. MEDIUM

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	4.00	g
MgSO ₄ x 7 H ₂ O	3.45	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	6.00	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
Na-acetate	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	5.00	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 bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of final medium to 6.8 – 7.0. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at two atmospheres of pressure.

Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.

For [DSM 25996](#) adjust pH of complete medium to 6.6.