

892. METHANOCALCULUS PUMILUS MEDIUM

KH ₂ PO ₄	0.40	g
NH ₄ Cl	0.90	g
MgCl ₂ x 6 H ₂ O	0.36	g
NaCl	10.00	g
Na-acetate	0.50	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Trace element solution (see medium 318)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	5.00	g
Vitamin solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
L-Cysteine-HCl x H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve carbonate, adjust pH to 7.2 then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials 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 7.3 - 7.5, if necessary. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 1.5 - 2 bar overpressure.