

825. METHANOBACTERIUM II MEDIUM

NH ₄ Cl	1.00	g
K ₂ HPO ₄	0.30	g
KH ₂ PO ₄	0.30	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Trace element solution (see medium 141)	10.00	ml
Na-acetate	0.50	g
Yeast extract	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	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, 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. 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 6.8 - 7.0, if necessary. After inoculation pressurize cultivation vessels to 2 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.