

784a. METHANOCALCULUS NATRONOPHILUS MEDIUM

KH ₂ PO ₄	0.2	g
MgCl ₂ x 6 H ₂ O	0.1	g
NH ₄ Cl	1.0	g
KCl	0.2	g
NaCl	60.0	g
Trace element solution SL-11 (see medium 722)	1.0	ml
Na-acetate	0.2	g
Na-resazurin solution (0.1% w/v)	0.5	ml
Na ₂ CO ₃	68.0	g
NaHCO ₃	38.0	g
Vitamin solution (see medium 141)	10.0	ml
Na ₂ S x 9 H ₂ O	1.0	g
Distilled water	1000.0	ml

Dissolve ingredients except carbonate, bicarbonate, vitamins and sulfide, then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve carbonates and sulfide while gassing the head space only. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add vitamins from an anoxic stock solution sterilized by filtration. Adjust pH of the complete medium to 9.0 – 9.5.