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% H$_2$ and 20% CO$_2$ 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.