

**1596. METHANOCALCULUS ALKALIPHILUS MEDIUM**

NaCl	3.00	g
K <sub>2</sub> HPO <sub>4</sub>	1.00	g
NaHCO <sub>3</sub>	20.00	g
Na <sub>2</sub> CO <sub>3</sub>	15.00	g
NH <sub>4</sub> Cl	0.20	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
Trace element solution (see medium 1369)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-formate	3.40	g
Na-acetate	0.16	g
Yeast extract (OXOID)	0.02	g
Vitamin solution (see medium 141)	10.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve sodium chloride and hydrogenphosphate, then sparge solution with 100% N<sub>2</sub> gas for at least 30 – 45 min to make it anoxic. Add and dissolve hydrogencarbonate and carbonate while gassing the head space only, then dispense under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add ammonium chloride, magnesium sulfate, trace elements, formate, acetate, yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins should be sterilized by filtration. The pH of the complete medium should be 9.5.

*Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.*