

**321. METHANOCORPUSCULUM AGGREGANS MEDIUM**

NH <sub>4</sub> Cl	1.00	g
K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	0.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
Mineral solution (see below)	50.00	ml
Na-formate	5.00	g
Na-acetate	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Trace element solution (see medium 141)	10.00	ml
Sludge fluid (see medium 119) <i>or</i>		
Rumen fluid, clarified (see medium 1310)	5.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
L-Cysteine-HCl x H <sub>2</sub> O	0.20	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.20	g
Distilled water	935.00	ml

Dissolve ingredients except carbonate, cysteine and sulfide, then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Prior to use check pH of complete medium and adjust to 6.8 – 7.0, if necessary. After inoculation add sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.

*Mineral solution:*

KH <sub>2</sub> PO <sub>4</sub>	6.00	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	6.00	g
NaCl	12.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	2.60	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.16	g
Distilled water	1000.00	ml