

**322. METHANOSPHERA (MCB-3) MEDIUM**

Rumen fluid, clarified (see medium 1310)	100.00	ml
Trypticase peptone (BD BBL)	2.00	g
Yeast extract	2.00	g
Na-acetate	0.50	g
Na-formate	0.50	g
Trace element solution (see medium 141)	10.00	ml
Na <sub>2</sub> SeO <sub>4</sub> solution (0.1% w/v)	1.90	ml
NiCl <sub>2</sub> x 6 H <sub>2</sub> O solution (0.1% w/v)	0.70	ml
FeSO <sub>4</sub> x 7 H <sub>2</sub> O solution (0.1% w/v in 0.1 N H <sub>2</sub> SO <sub>4</sub> )	3.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.60	g
KH <sub>2</sub> PO <sub>4</sub>	2.80	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.30	g
NH <sub>4</sub> Cl	1.00	g
NaCl	0.60	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.15	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.08	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	4.00	g
Methanol	5.00	ml
Vitamin solution (see medium 141)	20.00	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	900.00	ml

Dissolve ingredients except bicarbonate, methanol, 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. Add and dissolve bicarbonate, adjust pH of medium to 6.8 – 7.0, then dispense medium under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add methanol, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Prior to use check pH of complete medium and adjust to 6.7 - 6.9, if necessary. After inoculation add sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 1 bar overpressure.