

**332. METHANOCULLEUS BOURGENSE MEDIUM**

NH <sub>4</sub> Cl	1.0	g
K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	0.4	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.1	g
Na-formate	5.0	g
Na-acetate	1.0	g
Trypticase peptone (BD BBL)	1.0	g
Yeast extract (OXOID)	1.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.5	g
Na <sub>2</sub> CO <sub>3</sub>	1.5	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.2	g
Distilled water	1000.0	ml

Dissolve ingredients except cysteine, carbonate and sulfide. 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 cysteine, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

*Note: After growth has started and the culture is becoming turbid add sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 0.5 - 1 bar overpressure.*