

**122. HUNGATEICLOSTRIDIUM MEDIUM**

|   |         |    |
|---|---------|----|
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>   | 1.30    | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O  | 2.60    | g  |
| KH <sub>2</sub> PO <sub>4</sub>   | 1.43    | g  |
| K <sub>2</sub> HPO <sub>4</sub>   | 5.50    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O  | 0.13    | g  |
| Na <sub>2</sub> -β-glycerophosphate x 5 H <sub>2</sub> O (MERCK 35675)                              | 6.00    | g  |
| 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> ) | 1.10    | ml |
| L-Glutathione reduced   | 0.25    | g  |
| Yeast extract   | 4.50    | g  |
| Na-resazurin solution (0.1% w/v)  | 0.50    | ml |
| Cellobiose  | 5.00    | g  |
| Distilled water   | 1000.00 | ml |

Dissolve ingredients except cellobiose, sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Then adjust pH to 7.0 - 7.2, distribute under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave.

Cellobiose is added to the sterile medium from an anoxic 10% (w/v) stock solution prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose (Avicel or MN 301, MACHEREY-NAGEL).

*Note: A white precipitate forms after mixing the ingredients of this medium, but this has no negative effect on growth.*