

## 110a. CMC MEDIUM (N<sub>2</sub>/CO<sub>2</sub>)

|                                   |         |    |
|-----------------------------------|---------|----|
| Casitone                          | 30.0    | g  |
| Yeast extract                     | 5.0     | g  |
| K <sub>2</sub> HPO <sub>4</sub>   | 5.0     | g  |
| Na-resazurin solution (0.1% w/v)  | 0.5     | ml |
| L-Cysteine-HCl x H <sub>2</sub> O | 0.5     | g  |
| D-Glucose                         | 4.0     | g  |
| Cellobiose                        | 1.0     | g  |
| Maltose                           | 1.0     | g  |
| Starch (soluble)                  | 1.0     | g  |
| Na <sub>2</sub> CO <sub>3</sub>   | 1.5     | g  |
| Meat filtrate (see below)         | 1000.00 | ml |

Dissolve ingredients, except cysteine, carbohydrates and carbonate, boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere, add 0.5 g/l L-cysteine-HCl x H<sub>2</sub>O and dispense under same gas atmosphere into Hungate-type tubes (for strains demanding meat particles put those first into the tube, use 1 part meat particles to 4 or 5 parts fluid). Autoclave at 121°C for 20 min. After autoclaving add glucose, cellobiose, maltose and starch 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. Adjust pH of medium to **pH 7**, if necessary.

### *Meat filtrate:*

|                        |        |    |
|------------------------|--------|----|
| Ground beef (fat free) | 500.0  | g  |
| NaOH 1 N               | 25.0   | ml |
| Distilled water        | 1000.0 | ml |

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1000.0 ml.