

## 1180. METHYLOMICROBIUM MEDIUM

|  |         |    |
|--|---------|----|
| NaCl                                   | 30.00   | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O | 0.20    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O | 0.02    | g  |
| KNO <sub>3</sub>                       | 1.00    | g  |
| Trace elements (see below)             | 1.00    | ml |
| Distilled water                        | 1000.00 | ml |

Dispense the medium into the culture vessels. For small volumes serum bottles or Hungate tubes may be used. The gas phase to liquid ratio should be about 10:1. Use a gas phase containing air, 50% methane and autoclave at 121°C for 15 minutes. When the growth medium is cool the NaHCO<sub>3</sub>, the Na<sub>2</sub>CO<sub>3</sub> and phosphate buffer are added. Liquid cultures should be grown with shaking.

To the sterile medium add from sterile stock solutions:

|                                      |      |      |
|--------------------------------------|------|------|
| NaHCO <sub>3</sub> (1M)              | 50.0 | ml/l |
| Na <sub>2</sub> CO <sub>3</sub> (1M) | 5.0  | ml/l |

Phosphate buffer: 20.0 ml/l

|  |        |    |
|--|--------|----|
| KH <sub>2</sub> PO <sub>4</sub>                        | 14.0   | g  |
| Na <sub>2</sub> HPO <sub>4</sub> x 12 H <sub>2</sub> O | 30.0   | g  |
| Distilled water  | 1000.0 | ml |

Final pH of the medium: 8.5-9.0

### *Trace elements:*

|  |         |    |
|--|---------|----|
| EDTA                                   | 5.00    | g  |
| CuCl <sub>2</sub> x 5 H <sub>2</sub> O | 0.10    | g  |
| FeSO <sub>4</sub> x 7 H <sub>2</sub> O | 2.00    | g  |
| ZnSO <sub>4</sub> x 7 H <sub>2</sub> O | 0.10    | g  |
| NiCl <sub>2</sub> x 6 H <sub>2</sub> O | 0.02    | g  |
| CoCl <sub>2</sub> x 6 H <sub>2</sub> O | 0.20    | g  |
| Na <sub>2</sub> MoO <sub>4</sub>       | 0.03    | g  |
| MnCl <sub>2</sub> x 4 H <sub>2</sub> O | 0.03    | g  |
| H <sub>3</sub> BO <sub>3</sub>         | 0.03    | g  |
| Distilled water                        | 1000.00 | ml |