

**1329. METHANOSAETA PELAGICA MEDIUM**

|  |         |    |
|--|---------|----|
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O     | 3.05    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O     | 0.15    | g  |
| NH <sub>4</sub> Cl                         | 0.54    | g  |
| NaCl                                       | 20.00   | g  |
| Na-acetate                                 | 6.56    | g  |
| Trace elements solution (see medium 318)   | 2.00    | ml |
| Yeast extract (OXOID)                      | 1.50    | g  |
| Trypticase peptone (BD BBL)                | 0.40    | g  |
| Na-resazurin solution (0.1% w/v)           | 0.50    | ml |
| KH <sub>2</sub> PO <sub>4</sub>            | 1.19    | g  |
| K <sub>2</sub> HPO <sub>4</sub>            | 0.21    | g  |
| NaHCO <sub>3</sub>                         | 2.50    | g  |
| 2-Mercaptoethanesulfonic acid (coenzyme M) | 0.14    | g  |
| Vitamins solution (see medium 141)         | 10.00   | ml |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O     | 0.36    | g  |
| Distilled water                            | 1000.00 | ml |

Dissolve ingredients (except phosphates, bicarbonate, coenzyme M, vitamins and sulfide) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add phosphates, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of the complete medium to 7.5, if necessary.