

**946. NAUTILIA MEDIUM**

|   |        |    |
|---|--------|----|
| Synthetic seawater (2 x conc.) (see medium 795) | 500.00 | ml |
| NH <sub>4</sub> Cl                              | 0.33   | g  |
| KH <sub>2</sub> PO <sub>4</sub>                 | 0.33   | g  |
| Trace element solution (see medium 141)         | 10.00  | ml |
| Na-resazurin solution (0.1% w/v)                | 0.50   | ml |
| Sulfur, powdered                                | 10.00  | g  |
| NaHCO <sub>3</sub>                              | 2.50   | g  |
| Na-formate                                      | 3.00   | g  |
| Vitamin solution (see medium 141)               | 10.00  | ml |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O          | 0.60   | g  |
| Distilled water                                 | 500.00 | ml |

Dissolve ingredients (except sulfur, bicarbonate, formate, vitamins and sulfide), then 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 that contain already the appropriate amount of sulfur. For sterilization heat cultivation vessels for 1 - 2 hours to 90 – 100°C in a water bath on each of three successive days. Prior to inoculation add bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and formate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.