

**395a. BROCKIA LITHOTROPHICA MEDIUM**

|   |         |    |
|---|---------|----|
| NH <sub>4</sub> Cl                            | 0.33    | g  |
| KH <sub>2</sub> PO <sub>4</sub>               | 0.33    | g  |
| KCl   | 0.33    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O        | 0.44    | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O        | 0.70    | g  |
| NaCl  | 0.50    | g  |
| Trace element solution SL-10 (see medium 320) | 1.00    | ml |
| Na-resazurin solution (0.1% w/v)              | 0.50    | ml |
| Sulfur, powdered                              | 10.00   | g  |
| NaHCO <sub>3</sub>                            | 0.80    | g  |
| Vitamin solution (see medium 141)             | 10.00   | ml |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O        | 0.50    | g  |
| Distilled water                               | 1000.00 | ml |

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4, and dispense medium under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins (sterilized by filtration) and sulfide are added to the medium from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 6.5, if necessary.