

## Main sol. 1263

|  |         |    |
|--|---------|----|
| NaCl                                     | 30.00   | g  |
| KCl                                      | 0.20    | g  |
| K <sub>2</sub> HPO <sub>4</sub>          | 0.20    | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O   | 0.10    | g  |
| NH <sub>4</sub> Cl                       | 1.00    | g  |
| <b>Modified Wolin's mineral solution</b> | 10.00   | ml |
| Sodium resazurin (0.1% w/v)              | 0.50    | ml |
| Na <sub>2</sub> CO <sub>3</sub>          | 2.76    | g  |
| NaHCO <sub>3</sub>                       | 24.00   | g  |
| Yeast extract                            | 0.50    | g  |
| Cellobiose                               | 5.00    | g  |
| <b>Wolin's vitamin solution</b>          | 2.00    | ml |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O   | 0.40    | g  |
| Distilled water                          | 1000.00 | ml |

Dissolve ingredients (except carbonate, bicarbonate, yeast extract, cellobiose, vitamins and sulfide), then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add solid carbonate and bicarbonate to the medium, dissolve and adjust to pH 9.4. Dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum bottles and autoclave. To the autoclaved medium add yeast extract, cellobiose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Stock solutions of vitamins and cellobiose should be sterilized by filtration.