

## Main sol. 740

|                                                     |         |    |
|-----------------------------------------------------|---------|----|
| NH <sub>4</sub> Cl                                  | 0.50    | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O              | 0.16    | g  |
| K <sub>2</sub> HPO <sub>4</sub>                     | 1.60    | g  |
| Na <sub>2</sub> HPO <sub>4</sub> x H <sub>2</sub> O | 1.00    | g  |
| <b>Modified Wolin's mineral solution</b>            | 10.00   | ml |
| Yeast extract (OXOID)                               | 2.00    | g  |
| Trypticase peptone (BD BBL)                         | 2.00    | g  |
| Sodium resazurin (0.1% w/v)                         | 0.50    | ml |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O              | 0.06    | g  |
| D-Glucose                                           | 3.00    | g  |
| <b>Wolin's vitamin solution (10x)</b>               | 1.00    | ml |
| L-Cysteine HCl x H <sub>2</sub> O                   | 0.30    | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O              | 0.30    | g  |
| Distilled water                                     | 1000.00 | ml |

Dissolve ingredients except calcium chloride, glucose, vitamins, cysteine and sulfide. Sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins should be sterilized by filtration. The pH of the complete medium should be 6.8 - 7.0.