

## Main sol. 135a

|  |       |   |
|--|-------|---|
| NaCl                                   | 20.00 | g |
| NH <sub>4</sub> Cl                     | 1.00  | g |
| KH <sub>2</sub> PO <sub>4</sub>        | 0.33  | g |
| K <sub>2</sub> HPO <sub>4</sub>        | 0.45  | g |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O | 0.10  | g |

|  |       |    |
|--|-------|----|
| <b>Modified Wolin's mineral solution</b> | 20.00 | ml |
|--|-------|----|

|                      |      |    |
|----------------------|------|----|
| Yeast extract        | 2.00 | g  |
| Resazurin (0.1% w/v) | 0.50 | ml |
| NaHCO <sub>3</sub>   | 5.00 | g  |
| Ethylene glycol      | 1.25 | g  |

|                                       |      |    |
|---------------------------------------|------|----|
| <b>Wolin's vitamin solution (10x)</b> | 1.00 | ml |
|---------------------------------------|------|----|

|  |         |    |
|--|---------|----|
| L-Cysteine HCl x H <sub>2</sub> O      | 0.50    | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O | 0.50    | g  |
| Distilled water                        | 1000.00 | ml |

Dissolve ingredients except bicarbonate, ethylene glycol, vitamins, cysteine and sulfide, bring to the boil and cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add bicarbonate (solid) and equilibrate the medium with the gas until a pH of around 7.4 is reached. Then distribute under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add ethylene glycol, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 7.0 - 7.2, if necessary.