

## Main sol. 895

|  |         |    |
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| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>  | 1.00    | g  |
| NH <sub>4</sub> Cl   | 0.40    | g  |
| Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O                         | 0.10    | g  |
| K <sub>2</sub> HPO <sub>4</sub>  | 0.50    | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O   | 0.10    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O   | 0.05    | g  |
| NaCl   | 10.00   | g  |
| FeSO <sub>4</sub> x 7 H <sub>2</sub> O (0.1% w/v in 0.1 N H <sub>2</sub> SO <sub>4</sub> ) | 2.00    | ml |
| <b>Modified Wolin's mineral solution</b>   | 10.00   | ml |
| Yeast extract (BD Bacto)   | 0.25    | g  |
| Tryptone (BD Bacto)  | 0.25    | g  |
| Sodium resazurin (0.1% w/v)  | 0.50    | ml |
| L-Cysteine HCl x H <sub>2</sub> O  | 0.50    | g  |
| Na <sub>2</sub> CO <sub>3</sub>  | 3.00    | g  |
| Starch (soluble)   | 2.00    | g  |
| <b>Wolin's vitamin solution (10x)</b>  | 1.00    | ml |
| Distilled water  | 1000.00 | ml |

Dissolve all ingredients except cysteine, carbonate, starch and vitamins. Sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic, add cysteine and adjust pH to 7.5, then dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add starch and vitamins from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 9.3 - 9.5, if necessary.