



Main sol. 135a

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| NaCl | 20.00 | g |
| NH ₄ Cl | 1.00 | g |
| KH ₂ PO ₄ | 0.33 | g |
| K ₂ HPO ₄ | 0.45 | g |
| MgSO ₄ x 7 H ₂ O | 0.10 | g |
| Modified Wolin's mineral solution | 20.00 | ml |
| Yeast extract | 2.00 | g |
| Resazurin (0.1% w/v) | 0.50 | ml |
| NaHCO ₃ | 5.00 | g |
| Ethylene glycol | 1.25 | g |
| Wolin's vitamin solution (10x) | 1.00 | ml |
| L-Cysteine HCl x H ₂ O | 0.50 | g |
| Na ₂ S x 9 H ₂ 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% N₂ and 20% CO₂ 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% N₂ gas. Adjust pH of complete medium to 7.0 - 7.2, if necessary.