

Main sol. 684

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|---|---------|----|
| Na ₂ HPO ₄ x 2 H ₂ O | 0.53 | g |
| KH ₂ PO ₄ | 0.41 | g |
| NH ₄ Cl | 0.30 | g |
| CaCl ₂ x 2 H ₂ O | 0.11 | g |
| MgCl ₂ x 6 H ₂ O | 0.10 | g |
| NaCl | 0.30 | g |
| Trace element solution SL-10 | 1.00 | ml |
| Selenite-tungstate solution | 1.00 | ml |
| Yeast extract | 0.20 | g |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| Na ₂ CO ₃ | 1.50 | g |
| Na ₂ -fumarate | 3.20 | g |
| Wolin's vitamin solution (10x) | 1.00 | ml |
| Na ₂ S x 9 H ₂ O | 0.50 | g |
| Distilled water | 1000.00 | ml |

1. Dissolve ingredients (except carbonate, vitamins, fumarate and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding vitamins, fumarate and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Stock solutions of vitamins and fumarate are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2. After inoculation pressurize culture vials to 0.7 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture.

2. Note: A white precipitate forms in this medium after autoclaving, which has however no negative effect on growth.