

Main sol. 816

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|-------------------|--------|----|
| Solution A | 920.00 | ml |
| Solution B | 1.00 | ml |
| Solution C | 1.00 | ml |
| Solution D | 50.00 | ml |
| Solution E | 10.00 | ml |
| Solution F | 5.00 | ml |
| Solution G | 5.00 | ml |
| Solution H | 10.00 | ml |

Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B, C, F, G, and H are autoclaved separately under 100% N₂ gas. Solution D is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere and autoclaved. Solution E is prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be checked and adjusted to 7.6 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v), if necessary.