



Main sol. 194b

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|-------------------|--------|----|
| Solution A | 962.00 | ml |
| Solution B | 30.00 | ml |
| Solution C | 1.00 | ml |
| Solution D | 10.00 | ml |

Dissolve ingredients of solution A and adjust pH to 6.8, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30 - 45 min to make it anoxic. Distribute solution under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solution D is autoclaved under 100% N₂ gas. Solutions B to D are added to the sterile, cooled solution A in appropriate amounts in the sequence as indicated. Final pH of the medium should be 6.8 - 7.0.