

Main sol. 503e

Solution A	972.00	ml
Solution B	10.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml

Sparge solution A with 80% H₂ and 20% CO₂ gas for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and D are prepared under 100% N₂ gas and sterilized by filtration. Solution E is autoclaved under 100% N₂ gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 6.0 - 6.5, if necessary.