

Main sol. 837

Solution A	880.00	ml
Solution B	50.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	50.00	ml
Solution F	1.00	ml

1. Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 100% N₂ gas atmosphere. Solutions C and F are prepared under 80% N₂ and 20% CO₂ gas atmosphere. Solutions D and E are prepared under 100% N₂ gas atmosphere. Solution C is autoclaved and solutions D, E and F are sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 6.9, if necessary.

2. Note: For transfers use 5 - 10% (v/v) inoculum.