



Main sol. 837

Solution A	870.00	ml
Solution B	50.00	ml
Solution C	20.00	ml
Solution D	10.00	ml
Solution E	50.00	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. Solution C is prepared under 80% N₂ and 20% CO₂ gas atmosphere. Solutions D and E are prepared under 100% N₂ gas atmosphere and sterilized by filtration. 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 6.9, if necessary. Prior to inoculation medium is reduced by addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized).
2. Note: For transfers use 5 - 10% (v/v) inoculum.