



Main sol. 503

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

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 80% N₂ and 20% CO₂ gas atmosphere. Solution 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 7.2 - 7.4, if necessary.