

Main sol. 503f

Solution A	952.00	ml
Solution B	25.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

Dissolve compounds of solution A except sulfur and sparge it with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Distribute under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Solution B is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas and sterilized by filtration. Solutions D, E, and F are autoclaved under 100% N₂ gas. 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 7.2 - 7.4, if necessary.