

Main sol. 346

Solution A	966.00	ml
Solution B	20.00	ml
Solution C	11.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	1.00	ml
Solution G	1.00	ml
Solution H	1.70	ml

Spurge solution A with 100% N₂ gas for 30 - 45 min to make it anoxic. Thereafter, dispense 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. Anoxic stock solutions C, D, E, F, and H are prepared under 100% N₂ gas atmosphere and solution G under 80% N₂ and 20% CO₂ gas atmosphere. Filter sterilize solutions D, F and G. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 7.2 - 7.5, if necessary.