

Main sol. 1597

Solution A	750.00	ml
Solution B	250.00	ml
Solution C	1.00	ml
Solution D	1.00	ml
Solution E	5.00	ml
Solution F	3.00	ml
Solution G	5.00	ml
Solution H	10.00	ml
Solution I	1.00	ml
Solution J	10.00	ml

1. Sterilize solutions A and B in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of solution A after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 - 4 days. It is best to remove precipitates by decantation before using solution A for medium preparation.
2. Combine solution A with solution B and sparge medium with 100% N₂ gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions C to J are sterilized separately under 100% N₂ gas. Vitamins and coenzyme M should be sterilized by filtration. To complete the medium appropriate amounts of solutions C to J are added to the combined sterile solutions A and B in the sequence as indicated. Final pH of the medium should be 9.5.
3. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.