

Main sol. 1094

Solution A	943.00	ml
Solution B	13.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml

1. Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Solutions B, C, D and G are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solutions E and F are prepared under 100% N₂ gas atmosphere and autoclaved. To complete the medium add appropriate amounts of solutions B to G to the sterile solution A in the sequence indicated and let medium equilibrate overnight. Adjust pH of complete medium to 5.7, if necessary.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.
3. Note: The complete medium is not stable and can be stored only for a short time.