

Main sol. 195d

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	13.00	ml

1. Solution A is sparged with 80% H₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclaved. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is autoclaved under 100% N₂ gas. Solutions D and E are prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. After inoculation add 1 bar overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.