



Main sol. 336

Solution A	972.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	3.00	ml

Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere 8.9 ml medium per Hungate-type tube and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas atmosphere and sterilized by filtration. Autoclave solutions D and E under 100% N₂ gas. To complete the medium add appropriate amounts of the solutions B to E to the sterile solution A. Adjust pH of complete medium to 7.0 - 7.2, if necessary.