Main sol. 195

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

1. Solution A is sparged with 80% N_2 and 20% CO_2 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 and autoclaved. Solutions C and E are autoclaved separately under 100% N_2 gas. Solution B is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of 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. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.