Main sol. 383

Solution A	952.00	ml
Solution B	20.00	ml
Solution C	20.00	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 in anoxic cultivation vials and autoclaved under the same gas atmosphere. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas and filter-sterilized. Solution E is autoclaved under 100% N_2 gas. 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 7.0 – 7.2.

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% inoculum. Incubate all strains in the dark.