Main sol. 383b

Solution A	972.00	ml
Solution B	20.00	ml
Solution C	1.00	ml
Solution D	13.00	ml

1. Solution A is sparged with 80% H_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then dispensed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is prepared under 100% N_2 gas and filter-sterilized. 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. After inoculation pressurize vials to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

3. 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.