

## Main sol. 385

|                   |        |    |
|-------------------|--------|----|
| <b>Solution A</b> | 952.00 | ml |
| <b>Solution B</b> | 20.00  | ml |
| <b>Solution C</b> | 10.00  | ml |
| <b>Solution D</b> | 10.00  | ml |
| <b>Solution E</b> | 1.00   | ml |
| <b>Solution F</b> | 10.00  | ml |

1. Solution A is sparged with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C and F are autoclaved under 100% N<sub>2</sub> gas. Solutions D and E are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. The pyrocatechol stock solution has to be prepared always freshly prior to use. To complete the medium appropriate amounts of the solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 6.9 - 7.1.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 10 - 20% inoculum.