

## Main sol. 833

<b>Solution A</b>	892.00	ml
<b>Solution B</b>	10.00	ml
<b>Solution C</b>	2.00	ml
<b>Solution D</b>	35.00	ml
<b>Solution E</b>	20.00	ml
<b>Solution F</b>	40.00	ml
<b>Solution G</b>	3.00	ml
<b>Solution H</b>	1.00	ml

1. Sparge solution A with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B and H are autoclaved separately under 100% N<sub>2</sub> gas. Solutions C, E, F and G are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Solution D is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.3 - 7.6, if necessary.

2. Note: Before inoculation, the medium must be completely reduced, i.e. resazurin must be colorless. The 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) can help to reduce the medium.