



## Main sol. 347

<b>Solution A</b>	921.00	ml
<b>Solution B</b>	30.00	ml
<b>Solution C</b>	1.00	ml
<b>Solution D</b>	25.00	ml
<b>Solution E</b>	10.00	ml
<b>Solution F</b>	10.00	ml
<b>Solution G</b>	10.00	ml

Dissolve ingredients of solution A, adjust pH to 7.0, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is filter-sterilized and then equilibrated with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for at least 15 min. Solution C is prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Solutions D, E, F and G are autoclaved under 100% N<sub>2</sub> gas. To complete the medium, appropriate amounts of the solutions B to G are added to solution A in the sequence indicated. Adjust pH of complete medium to 7.2, if necessary.