



## Main sol. 1114

<b>Solution A</b>	972.00	ml
<b>Solution B</b>	10.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	1.00	ml

1. Sparge solution A with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into serum vials (to 20% of volume) and autoclave. Solution B is autoclaved separately under 100% N<sub>2</sub> gas and solution C under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions D and E are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. The pH of the complete medium should be 6.5.

2. After inoculation cultivation vials are pressurized with an amount of sterile air that is equivalent to 30 vol% of the cultivation vial.