

## Main sol. 559

<b>Solution A</b>	932.00	ml
<b>Solution B</b>	30.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	2.00	ml
<b>Solution E</b>	10.00	ml
<b>Solution F</b>	10.00	ml
<b>Solution G</b>	10.00	ml

1. Boil solution A for 3 min., then cool to room temperature under 100% N<sub>2</sub> gas atmosphere. Dispense the medium under same gas atmosphere in culture vessels and autoclave. Autoclave separately solutions E and F under 100% N<sub>2</sub> and solution B under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C, D, and G are prepared under N<sub>2</sub> gas atmosphere and sterilized by filtration.

2. Solutions B to G are added to the sterile, cooled solution A in the sequence as indicated. The final pH of the medium should be 7.2-7.4. After inoculation add 25 mg sodium dithionite per liter medium from a 2.5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized.