

## Main sol. 1280

<b>Solution A</b>	954.00	ml
<b>Solution B</b>	10.00	ml
<b>Solution C</b>	13.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	10.00	ml
<b>Solution F</b>	10.00	ml

1. Dissolve ingredients of solution A, adjust pH to 5.0 with 0.1 N HCl and sparge with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense solution A under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Solutions B - F are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Prior to inoculation complete the medium by adding appropriate amounts of solutions B to F to the sterile solution A in the sequence as indicated.

2. Adjust pH of the complete medium to 5.1 with a sterile anoxic solution of 1 N HCl. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

3. Note: Use 10-20% (v/v) as inoculum.