

**Main sol. 1011c**

NaCl	30.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.18	g
KCl	0.33	g
NH <sub>4</sub> Cl	0.25	g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O	0.01	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Na-pyruvate	0.50	g
Na-lactate	0.50	g
Yeast extract	0.10	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
<b>Na-dithionite solution (5% w/v)</b>	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, pyruvate, lactate, yeast extract, thiosulfate, vitamins and dithionite), then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add pyruvate, lactate, yeast extract, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate and dithionite from sterile anoxic stock solutions prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Solutions of vitamins, thiosulfate and dithionite are sterilized by filtration. Adjust pH of the complete medium to 6.7.

2. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.