

**Main sol. 935a**

NH <sub>4</sub> Cl	0.33	g
KCl	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
NaCl	25.00	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
KNO <sub>3</sub>	0.50	g
Na-acetate	2.00	g
Yeast extract	0.15	g
NaHCO <sub>3</sub>	0.30	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate and vitamins), then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Adjust pH to 7.0 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins from an anoxic stock solution prepared under 100% N<sub>2</sub> gas and sterilized by filtration and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. The pH of the complete medium should be 7.0.