

## Main sol. 135c

NH <sub>4</sub> Cl	1.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
K <sub>2</sub> HPO <sub>4</sub>	0.45	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
<b>Modified Wolin's mineral solution</b>	20.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	1.00	g
D-Fructose (optional)	10.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine and sulfide. Sparge medium 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 anoxic Hungate-type tubes or serum vials and autoclave. Add fructose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. The pH of the complete medium should be at 6.5.
2. Note: For autotrophic growth fructose is omitted and a gas atmosphere of 80% H<sub>2</sub> and 20% CO<sub>2</sub> is used.