

## Main sol. 879

NH <sub>4</sub> Cl	1.00	g
KCl	0.10	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.20	g
NaCl	0.80	g
KH <sub>2</sub> PO <sub>4</sub>	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.02	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	1.00	g
NaHCO <sub>3</sub>	1.00	g
D-Fructose	5.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, yeast extract, fructose, vitamins, and reducing agents), sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic and adjust pH to 5.0. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 15 min. Add yeast extract, fructose, vitamins (sterilized by filtration), cysteine, and sulfide from sterile stock solutions prepared under a 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile stock solution prepared under an 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust the pH of the complete medium to 5.9!