

**Main sol. 1292**

NH <sub>4</sub> Cl	1.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.30	g
KCl	0.10	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
NaCl	0.50	g
Na-acetate	0.20	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	2.50	g
D-Glucose	2.50	g
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

Dissolve ingredients (except carbonate, glucose, cysteine and sulfide) and sparge medium with 80% N<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 and autoclave. Add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of the complete medium to 7.2, if necessary.