

## Main sol. 1301

KH <sub>2</sub> PO <sub>4</sub>	0.41	g
Na <sub>2</sub> HPO <sub>4</sub>	0.43	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.11	g
NaCl	0.30	g
NH <sub>4</sub> Cl	0.30	g
<b>Trace element solution</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
D-Glucose	1.80	g
<b>Wolin's vitamin solution</b>	2.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, glucose, vitamins and reducing agents) 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, vitamins, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of the complete medium to 7.0-7.2, if necessary.