

## Main sol. 292

Glycine	1.50	g
Na <sub>2</sub> SO <sub>4</sub>	3.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
NaCl	1.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
KCl	0.40	g
Yeast extract	0.20	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	2.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide), adjust pH to 6.5, then sparge with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic and dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials. After autoclaving complete the medium by adding vitamins 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. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.4 - 7.5, if necessary.