

**Main sol. 846**

NH <sub>4</sub> Cl	0.30	g
K <sub>2</sub> HPO <sub>4</sub>	0.20	g
KH <sub>2</sub> PO <sub>4</sub>	0.30	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
KCl	0.50	g
NaCl	1.00	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
L-Serine	1.05	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

Dissolve ingredients (except carbonate, serine, vitamins, cysteine, and sulfide), then 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 the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add serine, vitamins, 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 atmosphere. The vitamin solution should be sterilized by filtration. Adjust the pH of the complete medium to 7.2, if necessary.