

**Main sol. 311**

NH <sub>4</sub> Cl	0.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
NaCl	2.25	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O (0.1% w/v in 0.1 N H <sub>2</sub> SO <sub>4</sub> )	2.00	ml
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	2.00	g
Casitone	2.00	g
Betaine x H <sub>2</sub> O	6.70	g
Sodium resazurin (0.1% w/v)	0.50	ml
K <sub>2</sub> HPO <sub>4</sub>	0.35	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
Na <sub>2</sub> CO <sub>3</sub>	1.00	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 phosphates, carbonate, vitamins, 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 phosphates, vitamins (sterilized by filtration), cysteine and sulfide to the medium after autoclaving from sterile 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 complete medium to pH 7.0, if necessary.