

## Main sol. 913

<b>Artificial sea water</b>	750.00	ml
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
Yeast extract	2.00	g
MOPS (SIGMA)	5.00	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
<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	240.00	ml

Dissolve ingredients except vitamins and sulfide, adjust pH to 7.0, then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (to 30% of volume) and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.0, if necessary.