

**VON STOSCH'S ENRICHED SEAWATER MEDIUM**

von Stosch's Enrichment (as cited by Ott, 1966)

The seawater should be filtered (Whatman's #1) to remove large organic particles and sand. Then sterilize by autoclaving (time: 100 ml requires 10 minutes; 2 liters requires 40 minutes; 3 liters requires 50 minutes; and 5 liters requires 70 minutes).

To each liter of seawater, then add the following:

<b>Salts</b>	<b>1 liter of seawater</b>
(1) $\text{NaNO}_3$	42.50 mg
(2) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	10.75 mg
(3) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	278.00 $\mu\text{g}$
(4) $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	19.80 $\mu\text{g}$
(5) $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	3.72 mg
<b>Vitamins</b>	
(6a) Thiamine-HCl	0.20 mg
(6b) Biotin	1.00 $\mu\text{g}$
(6c) $\text{B}_{12}$	1.00 $\mu\text{g}$

It is convenient to prepare a stock solution of each salt in distilled water; of such concentration that 1 ml of the stock solution gives the required concentration of each ingredient. The three vitamins may be incorporated in the same stock solution, which should be refrigerated. The salts and vitamins after preparation into stock solutions should be filter sterilized.

- I. To make stock solutions use deionized distilled water and clean volumetric flasks.
- II. Filter each stock solution through separate 0.22  $\mu\text{m}$  Millipore filters. Each solution will have to be sterilized separately.
- III. Aseptically pour filtered volume of liquid into autoclaved stock bottles.

<b>1-liter stock solution</b>	<b>2-liter stock solution</b>
(1) 42.500 grams	85.000 grams
(2) 10.750	21.500
(3) 0.278	0.556
(4) 0.0198	0.039
(5) 3.720	7.440
(6a) 0.200	0.400
(6b) 0.001	0.002
(6c) 0.001	0.002