

# *P*reparation of seawater media for Ectocarpus culture

## Natural Sea Water

1. If possible collect seawater by boat at some distance from the coast.
2. Filter at 0.7 micron to remove any suspended particles.
3. Aliquot into plastic bottles and autoclave (avoid using glass bottles because a precipitate can form).
4. Store at 13°C.



Filtration apparatus used in Roscoff

## Artificial Sea Water

1. Dissolve the constituents in 800 ml of milliQ water. in a 1 liter beaker
2. Adjust to pH 7.8 with NaOH or HCl if necessary (this can take quite a long time)
3. Adjust the volume to 1 litre with milliQ water and transfer to a 1 liter bottle.
4. Autoclave and store at 4°C

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*Additional information:*

**Starr, R.C. and Zeikus, J.A. (1993).** *UTEX-*  
*The culture collection of algae at the*  
*University of Texas at Austin. J. Phycol.* **29**  
*(suppl.), 1-106.*

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## SOLUTIONS

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### ARTIFICIAL SEA WATER

<u>Reagent</u>	<u>Quantity (for 1 litre)</u>	<u>Final concentration</u>
NaCl	26.29 g	450 mM
KCl	0.74 g	10 mM
CaCl <sub>2</sub>	0.99 g	9 mM
MgCl <sub>2</sub> (6H <sub>2</sub> O)	6.09 g	30 mM
MgSO <sub>4</sub> (7H <sub>2</sub> O)	3.94 g	16 mM

Adjust to pH 7.8, autoclave and store at 4°C.

### PROVASOLI SOLUTION

<u>Solution 1 (10x)</u>	<u>Quantity (for 1 litre)</u>	<u>Final concentration</u>
H <sub>3</sub> BO <sub>3</sub> (MW=61.83)	1.9 g	30.7 mM
FeCl <sub>3</sub> (MW=162.21)	0.05 g	0.3 mM
MnSO <sub>4</sub> (H <sub>2</sub> O) (MW=169.02)	0.273 g	1.6 mM
ZnSO <sub>4</sub> (7 H <sub>2</sub> O) (MW=287.54)	0.0367 g	0.127 mM
CoSO <sub>4</sub> (7 H <sub>2</sub> O) (MW=281.1)	0.008 g	28 µM
0.5 M EDTA pH8 (MW=292.24)	11.4 ml	5.7 mM

<u>Solution 2 (10x)</u>	<u>Quantity (for 500 ml)</u>
Vitamin B12 (cyanocobalamine)	3.35 mg
Thiamine hydrochloride (vitamin B1) (MW=337.27)	165 mg
Biotin C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S (MW=244.31)	1.65 mg
TRIS = Trisma base C <sub>4</sub> H <sub>11</sub> NO <sub>3</sub> (MW=121.14)	166.5 g

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<u>Solution 3 (10x)</u>	<u>Quantity (for 1 litre)</u>	<u>Final concentration</u>
(NH <sub>4</sub> ) <sub>2</sub> Fe(SO <sub>4</sub> ) <sub>2</sub> (6H <sub>2</sub> O) (MW=392.14)	1.17 g	3 mM
0.5 M EDTA pH8	6.8 ml	3.4 mM

<u>Solution 4 (10x)</u>	<u>Quantity (for 1 litre)</u>	<u>Final concentration</u>
NaNO <sub>3</sub> (MW=84.99)	23 g	270 mM

<u>Solution 5 (10x)</u>	<u>Quantity (for 1 litre)</u>	<u>Final concentration</u>
C <sub>3</sub> H <sub>7</sub> Na <sub>2</sub> O <sub>6</sub> P(5H <sub>2</sub> O) "glycerophosphate" (MW=216.04)	3.33 g	15.4 mM

Prepare each stock solution separately, autoclave and store at 4°C in a glass bottle. Use a dark bottle for solution 2.

For 1 litre of Provasoli solution add 100 ml each of solution 1, 3, 4 and 5 plus 10 ml of solution 2 to milliQ water (starting pH should be between 9.6 and 9.8).

Adjust to pH 7.8 with concentrated HCl (37%) and adjust the volume to 1 litre with milliQ water.

Aliquot into small glass bottles (20, 50, 100 or 200 ml), autoclave and store at 4°C.

#### PROVASOLI ENRICHED SEAWATER (PES) (Starr and Zeikus 1993)

<u>Reagent</u>	<u>Quantity (for 1 litre)</u>
Natural seawater	1 l
Provasoli solution	20 ml

If possible seawater should be collected by boat at some distance from the coast. Filter the seawater at 5 µm. Aliquot into Nalgene™ bottles (in glass bottles a precipitate can form), autoclave and store at 13°C. We use half-strength PES, i.e. 10 ml of Provasoli solution is added to 1l of autoclaved seawater.

*The filtered seawater and the Provasoli solution are autoclaved separately in order to avoid precipitation.*