

ESAW Medium

(Harrison et al. 1980, modified in Berges et al. 2001)

This is an enriched artificial seawater medium designed for coastal and open ocean phytoplankton. The recipe is from Berges et al. (2001) and has been modified from the earlier version (Harrison et al. 1980). Modifications include adding borate only in the salt solution (not in trace metals), an inorganic phosphate in place of glycerophosphate, and the silicate stock solution is made at half strength without acidification to facilitate dissolution. Three trace elements have been added: $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, Na_2SeO_3 and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. The iron is now added, solely as chloride (to remove ammonium), from a separate stock with equimolar EDTA.

The anhydrous and hydrated salts must be dissolved separately; masses assume specific gravity = 1.021 at 20°C. Dissolve specified quantity of the anhydrous salts in 600 mL of d H_2O and dissolve the hydrated salts in 300 mL d H_2O . Combine salt solutions I and II, and then add 1 mL of the nitrate and phosphate solutions, 2 mL of the silicate solution, 1 mL of the Iron-EDTA solution, 1 mL of the Trace Metals Solution II and 1 mL of the vitamin stock solution. Bring the final volume to 1 liter with d H_2O . Filter sterilization is recommended, e.g., a 147 mm Millipore GS filter (pore size 0.22 μm) with a Gelman A/E prefilter. Autoclaving the final medium often causes precipitants to form. If autoclaving is necessary, autoclave the two salt solutions separately, and when they are completely cooled, aseptically combine them. The 1 or 2 mL nutrient additions should be added using a 0.2 μm pore size sterile filter and a syringe. The medium should be bubbled with filtered air for 12 hours before use. The final pH is 8.2.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Salt solution I			
<i>Anhydrous salts</i>			
NaCl		21.194 g	3.63×10^{-1} M
Na ₂ SO ₄		3.550 g	2.50×10^{-2} M
KCl		0.599 g	8.03×10^{-3} M
NaHCO ₃		0.174 g	2.07×10^{-3} M
KBr		0.0863 g	7.25×10^{-4} M
H ₃ BO ₃		0.0230 g	3.72×10^{-4} M
NaF		0.0028 g	6.67×10^{-5} M
Salt solution II			
<i>Hydrated salts</i>			
MgCl ₂ 6H ₂ O		9.592 g	4.71×10^{-2} M
CaCl ₂ 2H ₂ O		1.344 g	9.14×10^{-3} M
SrCl ₂ 6H ₂ O		0.0218 g	8.18×10^{-5} M
Major Nutrients			
NaNO ₃	46.67 g L ⁻¹ dH ₂ O	1 mL	5.49×10^{-4} M
NaH ₂ PO ₄ ·H ₂ O	3.094 g L ⁻¹ dH ₂ O	1 mL	2.24×10^{-5} M
Na ₂ SiO ₃ ·9H ₂ O	15 g L ⁻¹ dH ₂ O	2 mL	1.06×10^{-4} M
Iron-EDTA			
Stock Solution			
Na ₂ EDTA·2H ₂ O	---	2.44 g	6.56×10^{-6} M
FeCl ₃ ·6H ₂ O	1.77 g L ⁻¹ dH ₂ O	1 mL	6.55×10^{-6} M
Trace Metals II	(see recipe below)	1 mL	
Stock Solution			
Vitamin Stock Solution	(see recipe below)	1 mL	---

Trace Metals Solution II

Prepare primary stock solutions. To prepare final Trace Metals Solution II, begin with 900 mL of dH₂O and add the indicated quantities of trace elements. Bring the final volume to 1 liter with dH₂O. For each liter of ESAW medium, add 1 mL of the Trace Metals Solution II.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA.2H ₂ O	---	3.09 g	8.30 x 10 ⁻⁶ M
ZnSO ₄ .7H ₂ O	---	0.073 g	2.54 x 10 ⁻⁷ M
CoSO ₄ .7H ₂ O	---	0.016 g	5.69 x 10 ⁻⁸ M
MnSO ₄ .4H ₂ O	---	0.54 g	2.42 x 10 ⁻⁶ M
Na ₂ MoO ₄ .2H ₂ O ^g	1.48 g L ⁻¹ dH ₂ O	1 mL	6.12 x 10 ⁻⁹ M
Na ₂ SeO ₃ ^g	0.173 g L ⁻¹ dH ₂ O	1 mL	1.00 x 10 ⁻⁹ M
NiCl ₂ . 6H ₂ O ^g	1.49 g L ⁻¹ dH ₂ O	1 mL	6.27 x 10 ⁻⁹ M

Vitamin Stock Solution

To prepare, begin with 900 mL dH₂O, add 100 mg of thiamine and 1 mL each of the biotin and cyanocobalamin solutions, and then bring to 1 liter with dH₂O. Filter sterilize and store frozen in small volumes.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B ₁)	---	0.1 g	2.96 x 10 ⁻⁷ M
biotin (vit. H)	1.0 g L ⁻¹ dH ₂ O	1 mL	4.09 x 10 ⁻⁹ M
cyanocobalamin (vit. B ₁₂)	2.0 g L ⁻¹ dH ₂ O	1 mL	1.48 x 10 ⁻⁹ M



Berges, J.A., Franklin, D.J. and Harrison, P.J. 2001. Evolution of an artificial seawater medium: improvements in enriched seawater, artificial water over the past two decades. *J. Phycol.* 37: 1138-1145.

Harrison, P.J., Waters, R.E., and Taylor, F.J.R. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.* 16: 28-35.