



Ostreococcus Medium

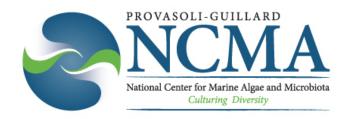
Brian Palenik (unpublished)

This enriched seawater medium is derived from f/2 medium and L1 medium (Guillard and Ryther 1962; Guillard and Hargraves 1993). It is specifically designed to grow the picoplanktonic green alga, *Ostreococcus*. The medium adds sodium citrate and additional Selenium and vitamins, but compared to L1 medium, it uses half-strength nitrate, phosphate, and trace metals.

To prepare, begin with 950 mL of filtered natural seawater. Add the quantity of each component as indicated below, and then bring the final volume to 1 liter using filtered natural seawater. The trace element solution and vitamin solutions are given below. Autoclave. Final pH should be 8.0 to 8.2.

Component	Stock Solution	Quantity	Molar Concentration in
			Final Medium
NaNO ₃	37.50 g L ⁻¹ dH ₂ O	1 mL	4.42 x 10 ⁻⁴ M
NaH ₂ PO ₄ ⋅ H ₂ O	2.50 g L ⁻¹ dH ₂ O	1 mL	1.81 x 10 ⁻⁵ M
Na₂ citrate· 2H₂O	29.41 g L ⁻¹ dH ₂ O	1 mL	$1.00 \times 10^{-4} \mathrm{M}$
H_2SeO_3	1.29 mg L ⁻¹ dH ₂ O	4.5 mL	$4.50 \times 10^{-8} \mathrm{M}$
trace element solution	(see recipe below)	0.5 mL	
vitamin solution	(see recipe below)	1.0 mL	

L1 Trace Element Solution- final concentration in Ostreococcus medium based on 0.5mL additions per liter of medium



To $950 \text{ mL} dH_2O$ add the following components and bring final volume to 1 liter with dH_2O . Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Ostreococcus Medium
Na ₂ EDTA · 2H ₂ O		4.36 g	5.85 x 10 ⁻⁶ M
FeCl₃ · 6H ₂ O		3.15 g	$5.85 \times 10^{-6} M$
MnCl ₂ ·4 H ₂ O	178.10 g L ⁻¹ dH ₂ O	1 mL	4.55 x 10 ⁻⁷ M
$ZnSO_4 \cdot 7H_2O$	23.00 g L ⁻¹ dH ₂ O	1 mL	$4.00 \times 10^{-8} M$
CoCl ₂ · 6H ₂ O	11.90 g L ⁻¹ dH ₂ O	1 mL	2.50 x 10 ⁻⁸ M
CuSO ₄ · 5H ₂ O	2.50 g L ⁻¹ dH ₂ O	1 mL	5.0x 10 ⁻⁹ M
$Na_2MoO_4 \cdot 2H_2O$	19.9 g L ⁻¹ dH ₂ O	1 mL	4.11 x 10 ⁻⁸ M
H_2SeO_3	1.29 g L ⁻¹ dH ₂ O	1 mL	5.00 x 10 ⁻⁹ M
NiSO ₄ · 6H ₂ O	2.63 g L ⁻¹ dH ₂ O	1 mL	5.00 x 10 ⁻⁹ M
Na ₃ VO ₄	1.84 g L ⁻¹ dH ₂ O	1 mL	5.00 x 10 ⁻⁹ M
K ₂ CrO ₄	1.94 g L ⁻¹ dH ₂ O	1 mL	5.00 x 10 ⁻⁹ M

f/2 Vitamin Solution

(Guillard and Ryther 1962, Guillard 1975)

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH_2O , dissolve the thiamine, add the amounts of the primary stocks as indicated in the quantity column below, and bring final volume to 1 liter with dH_2O . At the NCMA we autoclave to sterilize. Store in refrigerator or freezer.

Component	Primary Stock	Quantity	Molar Concentration in
	Solution		Final Ostreococcus Medium
thiamine \cdot HCl (vit. B_1)		200 mg	5.92 x 10 ⁻⁷ M



biotin (vit. H)	0.1g L ⁻¹ dH ₂ O	10 mL	4.10 x 10 ⁻⁹ M
cyanocobalamin (vit. B ₁₂)	1.0 g L ⁻¹ dH ₂ O	1 mL	7.38 x 10 ⁻¹⁰ M

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