

## Ostreococcus Medium

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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 <sub>3</sub>	37.50 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	4.42 x 10 <sup>-4</sup> M
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O	2.50 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	1.81 x 10 <sup>-5</sup> M
Na <sub>2</sub> citrate · 2H <sub>2</sub> O	29.41 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	1.00 x 10 <sup>-4</sup> M
H <sub>2</sub> SeO <sub>3</sub>	1.29 mg L <sup>-1</sup> dH <sub>2</sub> O	4.5 mL	4.50 x 10 <sup>-8</sup> 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 mL dH<sub>2</sub>O add the following components and bring final volume to 1 liter with dH<sub>2</sub>O. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Ostreococcus Medium
Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	---	4.36 g	5.85 x 10 <sup>-6</sup> M
FeCl <sub>3</sub> · 6H <sub>2</sub> O	---	3.15 g	5.85 x 10 <sup>-6</sup> M
MnCl <sub>2</sub> · 4 H <sub>2</sub> O	178.10 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	4.55 x 10 <sup>-7</sup> M
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	23.00 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	4.00 x 10 <sup>-8</sup> M
CoCl <sub>2</sub> · 6H <sub>2</sub> O	11.90 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	2.50 x 10 <sup>-8</sup> M
CuSO <sub>4</sub> · 5H <sub>2</sub> O	2.50 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	5.0x 10 <sup>-9</sup> M
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	19.9 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	4.11 x 10 <sup>-8</sup> M
H <sub>2</sub> SeO <sub>3</sub>	1.29 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	5.00 x 10 <sup>-9</sup> M
NiSO <sub>4</sub> · 6H <sub>2</sub> O	2.63 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	5.00 x 10 <sup>-9</sup> M
Na <sub>3</sub> VO <sub>4</sub>	1.84 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	5.00 x 10 <sup>-9</sup> M
K <sub>2</sub> CrO <sub>4</sub>	1.94 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	5.00 x 10 <sup>-9</sup> 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<sub>2</sub>O, 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<sub>2</sub>O. At the NCMA we autoclave to sterilize. Store in refrigerator or freezer.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Ostreococcus Medium
thiamine · HCl (vit. B <sub>1</sub> )	---	200 mg	5.92 x 10 <sup>-7</sup> M



biotin (vit. H)	0.1g L <sup>-1</sup> dH <sub>2</sub> O	10 mL	4.10 x 10 <sup>-9</sup> M
cyanocobalamin (vit. B <sub>12</sub> )	1.0 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	7.38 x 10 <sup>-10</sup> M

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