

**f/2 medium\* (Guillard and Ryther 1963)**

*Updated April 2007*

**FOUR STOCK SOLUTIONS (1-4)**

**OBS! For all solutions, use sterilized distilled deionized water!**

<b><u>1. NaNO<sub>3</sub> stock solution</u></b>	NaNO <sub>3</sub>	<b>For 1L</b> 75.0 g	<b>For 0.5L</b> 37.5 g
<b><u>2. NaH<sub>2</sub>PO<sub>4</sub> stock solution</u></b>	NaH <sub>2</sub> PO <sub>4</sub>	5.0 g	2.5 g

**3. Trace Metals stock solution**

To distilled water add the following:

	<b>For 1L</b>	<b>For 0.5L</b>
Na <sub>2</sub> EDTA	4.36 g	2.18 g
FeCl <sub>3</sub> •6H <sub>2</sub> O (Ferric Chloride)	3.15 g	1.575 g
Primary Metals Stocks (below)	1ml of each of the five	0.5ml of each of five

Primary Trace Metals stock solutions (make up five separate stocks)

To the chosen volume of sterile distilled deionized water add the following:

	<b><u>100ml</u></b>	<b><u>50ml</u></b>	<b><u>10ml</u></b>
CuSO <sub>4</sub> •5H <sub>2</sub> O	1.0 g	0.50 g	<b>0.10 g</b>
ZnSO <sub>4</sub> •7H <sub>2</sub> O	2.2 g	1.10 g	<b>0.22 g</b>
CoCl <sub>2</sub> •6H <sub>2</sub> O	1.0 g	0.50 g	<b>0.10 g</b>
MnCl <sub>2</sub> •4H <sub>2</sub> O	1.8 g	0.90 g	<b>0.18 g</b>
NaMoO <sub>4</sub> •2H <sub>2</sub> O	0.63 g	0.315g	<b>0.063 g</b>

**4. Vitamin Stock solution**

**Light sensitive – keep covered in foil!**

	<b>For 1.0 L</b>	<b>For 0.5L</b>
Biotin	10.0 mL of 0.1 mg•mL <sup>-1</sup> solution (1mg in 10ml)	5.0 mL
Vitamin B <sub>12</sub>	1.0 mL of 1.0 mg•mL <sup>-1</sup> solution (1mg in 1ml)	0.5 mL
Thiamine HCl	0.2g	0.1 g

**Lastly: Making Final Medium**

To 950 mL of 0.22 µM filtered seawater (FSW) add:

		<b><u>To make 100 tubes:</u></b>
NaNO <sub>3</sub> Stock solution	1.0 mL	100 ml
NaH <sub>2</sub> PO <sub>4</sub> Stock Solution	1.0 mL	100 ml
Trace Metals Stock Solution	1.0 mL	100 ml
Vitamin Stock Solution	0.5 mL	50 ml

Filter sterilize at 0.22 µM before use and store at 4° C. \* Si has been removed from this recipe to reduce the growth of contaminating diatoms.

**Tip:** Make up a larger batch, just multiply each stock by how many tubes you want to set up. For example, for 100 tubes of 3.5ml total of f/2 final medium **stock**, add 100ml of each of the first three stocks and 50 ml of the Vitamin stock. That gives a total of 350 ml, which gives 100 15ml falcon tubes of 3.5 ml each of f/2 final medium **stock**, each of which is ready to make up one each of 1.0L **working** f/2 media (one tube of 3.5 ml plus 950ml of filtered seawater).