ATCC Medium: 2744 F/2-Si

### F2 Medium:

NaNO <sub>3</sub>	1.0mL
NaH <sub>2</sub> PO <sub>4</sub> x H <sub>2</sub> O	I.0mL
*Na <sub>2</sub> SiO <sub>3</sub> x 9H <sub>2</sub> O (if required-see note)	).1.0mL
*Carolina Biological Seawater Cat.163	390 (filtered and processed according to
instruction below)	950.0mL

To prepare, begin with 950mL of filtered seawater and add the stock solutions as listed above. Bring final solution to 1 liter. Autoclave at 121°C.

### \*\*Filtration of Seawater:

To each liter of natural seawater add 5.0 g of activated charcoal, shake and leave overnight. Filter through Whatman 934-HA glass fiber filter. Treatment with the carbon will remove potentially toxic organic contaminants

\*\*Note: If the alga to be grown does not require Silica, then it is recommended that the Silica be omitted because it enhances precipitation.

# Aseptically add:

Trace Mineral solution (see below)......1.0mL Vitamin Solution (see below)......0.5mL Aseptically dispense into desired vessels.

## Stock solutions:

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO <sub>3</sub>	75 g/L dH₂O	1 mL	8.82 x 10 <sup>-4</sup> M
NaH <sub>2</sub> PO <sub>4</sub> H <sub>2</sub> O	5 g/L dH₂O	1 mL	3.62 x 10 <sup>-5</sup> M
Na <sub>2</sub> SiO <sub>3</sub> 9H <sub>2</sub> O	30 g/L dH₂O	1 mL	1.06 x 10 <sup>-4</sup> M
Trace metal solution	(see recipe below)	1 mL	

Vitamin solution (see recipe below)	0.5 mL	
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## F/2 Trace Metal Solution

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
FeCl <sub>3</sub> 6H <sub>2</sub> O		3.15 g	1.17 x 10 <sup>-5</sup> M
Na <sub>2</sub> EDTA 2H <sub>2</sub> O		4.36 g	1.17 x 10 <sup>-5</sup> M
CuSO <sub>4</sub> 5H <sub>2</sub> O	9.8 g/L dH <sub>2</sub> O	1 mL	3.93 x 10 <sup>-8</sup> M
Na <sub>2</sub> MoO <sub>4</sub> 2H <sub>2</sub> O	6.3 g/L dH <sub>2</sub> O	1 mL	2.60 x 10 <sup>-8</sup> M
ZnSO4 7H <sub>2</sub> O	22.0 g/L dH₂O	1 mL	7.65 x 10 <sup>-8</sup> M
CoCl <sub>2</sub> 6H <sub>2</sub> O	10.0 g/L dH₂O	1 mL	4.20 x 10 <sup>-8</sup> M
MnCl <sub>2</sub> 4H <sub>2</sub> O	180.0 g/L dH <sub>2</sub> O	1 mL	9.10 x 10 <sup>-7</sup> M

To prepare, begin with 950 mL of  $dH_2O$ , add the components and bring final volume to 1 liter with  $dH_2O$ . Filter Sterilize. Store stock solutions in refrigerator or freezer.

<sup>\*\*</sup>Note that the original medium (Guillard and Ryther 1962) used ferric sequestrene; we have substituted  $Na_2EDTA \cdot 2H_2O$  and  $FeCl_3 \cdot 6 H_2O$ .

# **F/2 Vitamin Solution:**

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine HCl (vit. B <sub>1</sub> )		200 mg	2.96 x 10 <sup>-7</sup> M
biotin (vit. H)	1.0 g/L dH <sub>2</sub> O	1 mL	2.05 x 10 <sup>-9</sup> M
cyanocobalamin (vit. B <sub>12</sub> )	1.0 g/L dH <sub>2</sub> O	1 mL	3.69 x 10 <sup>-10</sup> M

To prepare final vitamin solution, begin with 950 mL of  $dH_2O$ , dissolve the thiamine (200mg), add 1 mL (each) of the primary stocks and bring final volume to 1 liter with  $dH_2O$ . Filter Sterilize. Store stock solutions in refrigerator or freezer