

**The Liu Laboratory protocol — Symbiodiniaceae
Arts & Sci Washington University in St. Louis**

f/2 media

Table 1: f/2 Stock Solution Chemical Composition

Components	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO ₃	75 g/L dH ₂ O	1 mL	8.82 x10 ⁻⁴ M
NaH ₂ PO ₄ H ₂ O	5 g/L dH ₂ O	1 mL	3.62 x 10 ⁻⁵ M
Na ₂ CO ₃	30 g/L dH ₂ O	1 mL	1.06 x 10 ⁻⁴ M
Trace Metal Solution	See table 2	1 mL	---
Vitamin Solution	See Table 3	0.5 mL	---

Filtered seawater to 1L and autoclave

Table 2: Trace Metal Composition

Components	Stock Solution	Quantity	Molar Concentration in Final Medium
FeCl ₃ 6(H ₂ O)	---	3.15 g	1.17 x10 ⁻⁵ M
Na ₂ (EDTA)2(H ₂ O)	---	4.36 g	1.17 x10 ⁻⁵ M
CuSO ₄ 5(H ₂ O)	9.8 g/L dH ₂ O	1 mL	3.93x10 ⁻⁸ M
Na ₂ MoO ₄ 2(H ₂ O)	6.3 g/L dH ₂ O	1 mL	2.60 x10 ⁻⁸ M
MnCl ₂ 4(H ₂ O)	180.0 g/L dH ₂ O	1 mL	9.10 x10 ⁻⁷ M
CoCl ₂ 6(H ₂ O)	10.0 g/L dH ₂ O	1 mL	4.20 x10 ⁻⁸ M
ZnSO ₄ 7(H ₂ O)	22.0 g/L dH ₂ O	1 mL	7.65 x10 ⁻⁸ M

To prepare, begin with 950 mL of dH₂O, add the components and bring final volume to 1 liter with dH₂O. Autoclave. Note that the original medium (Guillard and Ryther 1962) used ferric sequestrene; we have substituted Na₂EDTA · 2H₂O and FeCl₃ · 6 H₂O

Table 3: Vitamin Composition

Components	Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine HCl (Vit-B1)	---	200 mg	2.96 x10 ⁻⁷ M
Biotin (Vitamin H)	0.1 g/L dH ₂ O	10 mL	2.05 x10 ⁻⁹ M
Cyanocobalamin (VitB12)	1 g/L dH ₂ O	1 mL	3.69 x10 ⁻¹⁰ M

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH₂O, 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 dH₂O. We autoclave to sterilize. Store in refrigerator or freezer.

References

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26- 60. In Smith W.L. and Chanley M.H (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA.
Guillard, R.R.L. and Ryther, J.H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can. J. Microbiol. 8: 229-239.