

## Modified K Medium

Keller and Guillard 1985, Keller *et al.* 1987

This is a modification of the enriched seawater medium that was designed specifically for oligotrophic (oceanic) marine phytoplankters that are poisoned by higher levels of trace metals. Steve Kibler of NOAA in North Carolina shared this recipe with us. He uses a modified K medium to grow benthic dinoflagellates. We use this recipe grow some *Gambierdiscus* strains (Note that Steve Kibler does not use tris in his recipe). The major differences between this recipe and standard K medium are as follows:

1. F/2 (aka L1) nitrate is used at twice the concentration of standard f/2 or L1. Unlike K nitrate, there is no ammonia.
2. The trace metal mix in modified K medium is made without copper.

To prepare, begin with 950 mL of filtered natural seawater, add the following components and then bring the final volume up to 1 liter with filtered natural seawater. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO <sub>3</sub>	75.00 g /L dH <sub>2</sub> O	2 mL	1.764 x 10 <sup>-3</sup> M
Na <sub>2</sub> β-glycerophosphate*	2.16 g/ L dH <sub>2</sub> O	1 mL	1.00 x 10 <sup>-5</sup> M
H <sub>2</sub> SeO <sub>3</sub>	1.29 mg/ L dH <sub>2</sub> O	1 mL	1.00 x 10 <sup>-8</sup> M
Tris-base (pH 7.2)	121.10 g/ L dH <sub>2</sub> O	1 mL	1.00 x 10 <sup>-3</sup> M
trace metal solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5 mL	---

### Trace Metal Solution

To prepare, dissolve the following components to 950 mL of dH<sub>2</sub>O (heat if necessary) and adjust the pH up with sodium hydroxide until all the components are in solution, approximately 20 pellets per liter of trace metal solution. Bring the final volume to 1 liter using dH<sub>2</sub>O.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na <sub>2</sub> EDTA • 2H <sub>2</sub> O	---	41.60g	1.11 x 10 <sup>-4</sup> M
FeCl <sub>3</sub> • 6 H <sub>2</sub> O	---	3.150 g	1.17 x 10 <sup>-5</sup> M
MnCl <sub>2</sub> • 4H <sub>2</sub> O	---	0.178 g	9.00 x 10 <sup>-7</sup> M
ZnSO <sub>4</sub> • 7H <sub>2</sub> O	23.00 g/ L dH <sub>2</sub> O	1 mL	8.00x 10 <sup>-8</sup> M
CoCl <sub>2</sub> • 6 H <sub>2</sub> O	10.00 g/ L dH <sub>2</sub> O	1 mL	5.00 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

### 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 Medium
thiamine · HCl (vit. B <sub>1</sub> )	---	200 mg	2.96 x 10 <sup>-7</sup> M
biotin (vit. H)	0.1 g/ L dH <sub>2</sub> O	10 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

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.

Keller, M.D. and Guillard, R.R.L. 1985. Factors significant to marine diatom culture. pp. 113-6. In Anderson, D.M., White, A.W. and Baden, D.G. (eds.) *Toxic Dinoflagellates*. Elsevier, New York.

Keller, M.D., Selvin, R.C., Claus, W. and Guillard, R.R.L. 1987. Media for the culture of oceanic ultraphytoplankton. *J. Phycol.* **23**: 633-638.

\*Please note that Na<sub>2</sub> β -glycerophosphate is sold as the disodium X hydrate (each batch having a different hydrate level) or as 5 hydrate. The 1987 paper used an x hydrate chemical even though the concentration of the stock was based on an anhydrous weight. Bob Guillard confirmed this for me 10Mar2011. We here at the CCMP have used an x hydrate chemical preparing a 2.16 gram per liter phosphate stock.