L1 Medium

Guillard and Hargraves (1993) - please see note at the bottom of this page

This enriched seawater medium is based upon f/2 medium (Guillard and Ryther 1962) but has additional trace metals. It is a general-purpose marine medium for growing coastal algae.

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.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75.00 g L^{-1} dH_2O	1 mL	8.82×10^{-4} M
$NaH_2PO_4 \cdot H_2O$	5.00 g L^{-1} dH_2O	1 mL	3.62×10^{-5} M
$Na_2SiO_3 \cdot 9\ H_2O$	30.00 g L^{-1} dH_2O	1 mL	1.06×10^{-4} M
trace element solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5mL	---

L1 Trace Element Solution

To 950 mL dH_2O add the following components and bring final volume to 1 liter with dH_2O. Autoclave.

$Na_2EDTA \cdot 2H_2O$ should be added first and brought to pH 8.0-8.4 to assure it is fully dissolved. $FeCl_3 \cdot 6H_2O$ should be added next assure it is also fully dissolved prior to the addition of the remaining components.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$Na_2EDTA \cdot 2H_2O$	---	4.36 g	1.17×10^{-5} M
$FeCl_3 \cdot 6H_2O$	---	3.15 g	1.17×10^{-5} M
$MnCl_2 \cdot 4\ H_2O$	178.10 g L^{-1} dH_2O	1 mL	9.00×10^{-7} M
$ZnSO_4 \cdot 7H_2O$	23.00 g L^{-1} dH_2O	1 mL	8.00×10^{-8} M
$CoCl_2 \cdot 6H_2O$	11.90 g L^{-1} dH_2O	1 mL	5.00×10^{-8} M
$CuSO_4 \cdot 5H_2O$	2.50 g L^{-1} dH_2O	1 mL	1.00×10^{-8} M
$Na_2MoO_4 \cdot 2H_2O$	19.9 g L^{-1} dH_2O	1 mL	8.22×10^{-8} M
H_2SeO_3	1.29 g L^{-1} dH_2O	1 mL	1.00×10^{-8} M
$NiSO_4 \cdot 6H_2O$	2.63 g L^{-1} dH_2O	1 mL	1.00×10^{-8} M
Na_3VO_4	1.84 g L^{-1} dH_2O	1 mL	1.00×10^{-8} M
K_2CrO_4	1.94 g L^{-1} dH_2O	1 mL	1.00×10^{-8} 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_2O, 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_2O. 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_1)	---	200 mg	2.96×10^{-7} M
biotin (vit. H)	0.1g L^{-1} dH_2O	10 mL	2.05×10^{-9} M
cyanocobalamin (vit. B_{12})	1.0 g $L^{-1}$$dH_2O$	1 mL	3.69×10^{-10} 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 Hargraves, P.E. 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia* **32:** 234-236.

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.

Please note:

In April 2013, it was brought to our attention that the recipe for L1 medium on the NCMA website differs from the original publication of L1 medium(Guillard, R. R. L., and P. E. Hargraves. "Stichochrysis immobilis is a diatom, not a chrysophyte." *Phycologia* 32.3 (1993): 234-236). The intended final concentration for K_2CrO_4 was 10^{-9} molar. There is an inconsistency in the 1993 paper as the directions for making the chromium stock solution that are in that paper result in a 10^{-8}molar final concentration in the medium. We have been using those directions for making our L1 trace metal mix for almost 15 years, resulting in a final concentration of 10^{-8}molar. We plan to continue to do so as a matter of consistency.

This error was corrected by Guillard in 2003.The corrected recipe can be found in the Manual on Harmful Marine Microalgae,Edited by G.M. Hallegraeff, D.M. Anderson and A.D. Cembella,Technical director: H.O. Enevoldsen,Oceanographic Methodology series, 2nd revised edition, 2003, 978-92-3-103871-6, UNESCO Publishing. Note that this L1 recipe uses a different vanadium compound (ammonium metavanadate, NH_4VO_3) to make the vanadium stock solution than the 1993 paper, which used sodium orthovanadate, Na_3VO_4. According to Sigma Aldrich, the concentration of vanadium ionic species depends upon the pH and redox potential of the environment. Sodium orthovanadate is more soluble in water than ammonium metavanadate. The concentration of vanadium in both recipes is more or less the same. Since the concentration of vanadium in seawater is 1.0-4.0 X 10^{-8}molar with an average of 3.2 X 10^{-8}molar (Abbasse, Ghiasse, Baghdad Ouddane, and Jean Fischer. "Determination of trace levels of dissolved vanadium in seawater by use of synthetic complexing agents and inductively coupled plasma–atomic emission spectroscopy (ICP–AES)." *Analytical and bioanalytical chemistry* 374.5 (2002): 873-878), the L1 trace metal addition of vanadium should not elevate the concentration of vanadium in L1 medium much above that of natural seawater.