MNK Medium

Noël *et al*. 2004

This enriched seawater medium was developed to grow oceanic coccolithophores, but it is also a good general medium for marine phytoplankton. The authors recommend storing filtered natural seawater in a cold (4° C), dark environment for at least 3 mo. before using it. All glassware should be acid cleaned and rinsed thoroughly with high quality water (e.g., MilliQ water) before use.

To prepare, begin with 997 mL of filtered natural seawater, and add 1 mL of each stock solution. Filter sterilize (0.2 µm pore size) and store in cold, dark environment until ready to use. For best growth results, filter sterilize the medium rather than autoclaving the medium. The final pH is 8.7.

Nutrient Stock Solution

Beginning with 950 mL of high quality dH_2O, add the weight quantities of the three salts. Bring the final volume to 1 liter with high quality dH_2O. Filter sterilize (0.2 µm pore size) and store refrigerated or frozen. For convenience, the solution can be distributed into 1 mL quantities in eppendorf or cryo-vial tubes. Use 1 mL of this stock solution for each liter of medium.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$ (anhydrous)	---	20.00 g	2.35×10^{-4} M
$Na_2HPO_4 \bullet 12\ H_2O$	---	0.28 g	7.82×10^{-7} M
K_2HPO_4 (anhydrous)	---	1.00 g	5.74×10^{-6} M

PROVASOLI-GUILLARD NCMA
National Center for Marine Algae and Microbiota
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Trace Metals Solution

First, prepare primary stock solutions by dissolving each in 950 mL of high quality dH_2O, and bring the final volume to 1 liter with dH_2O. To prepare the trace metals solution, begin with 950 mL of high quality dH_2O, and add 1 mL each of the primary stock solutions. Bring the final volume to 1 liter with dH_2O. Store refrigerated or frozen. Use 1 mL of the final stock solution for each liter of medium.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Fe-Na-EDTA • $3H_20$	25.900 g L^{-1} dH_2O	1 mL	6.15 x 10^{-8} M
Mn-EDTA • $3H_2O$	33.200 g L^{-1} dH_2O	1 mL	7.49 x 10^{-8} M
Na2EDTA • $2H_2O$	3.723 g L^{-1} dH_2O	1 mL	1.00 x 10^{-8} M
$MnCl_2$ • $4H_2O$	9.000 g L^{-1} dH_2O	1 mL	4.55 x 10^{-8} M
$ZnSO_4$ • $7H_2O$	2.400 g L^{-1} dH_2O	1 mL	8.35 x 10^{-9} M
$CoSO_4$ • $7H_2O$	1.200 g L^{-1} dH_2O	1 mL	4.27 x 10^{-9} M
Na_2MoO_4 •$2H_2O$	0.720 g L^{-1} dH_2O	1 mL	2.98 x 10^{-9} M
$CuSO_4$ •$5H_2O$	0.060 g L^{-1} dH_2O	1 mL	2.40 x 10^{-10} M
Na_2SeO_3	0.030 g L^{-1} dH_2O	1 mL	1.73 x 10^{-10} M

Vitamin Solution

First, prepare the primary stock solutions for biotin and cyanocobalamin. Into 950 mL of dH_2O, add the thiamine powder and 1 mL of each primary stock solution. Bring the final volume to 1 liter with dH_2O. Filter sterilize and freeze in small volumes (e.g., 1 mL). Use 1 mL of the final stock solution for each 1 liter of medium.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Thiamine (vit. B_1)		20 mg	5.93×10^{-8} M
Biotin (vit. H)	150 mg L^{-1} dH_2O	1 mL	6.14×10^{-10} M
cyanocobalomin (vit. B_{12})	150 mg L^{-1} dH_2O	1 mL	1.11×10^{-10} M

Reference

Noël, M.-H., Kawachi, M. and Inouye, I. 2004. Induced dimorphic life cycle of a coccolithophorid, *Calyptrosphaera sphaeroidea* (Prymnesiophyceae, Haptophyta). *J. Phycol.* **40**: 112-129.