Pro99 Medium

(Moore et al, 2007)

This medium was developed specifically for *Prochlorococcus*, but it can be used for other oceanic species tolerating high ammonia concentrations (e.g, *Bolidomonas*) and no vitamin requirement. All containers should be acid cleaned and rinsed with high quality H_2O (e.g., Milli-Q). Seawater should be collected from the oligotrophic open ocean (e.g., Sargasso Sea water), taking the usual precautions to avoid contamination. Ultrapure grade reagents should be used. This recipe was developed in Dr. Penny Chisholm's Lab (MIT), and it used smaller volumes of stock solutions. Good sterile technique is required when growing axenic strains, and a laminar flow hood is recommended.

To prepare, add one liter of oligotrophic open ocean seawater into a Teflon-lined container, autoclave and cool before adding nutrients. Aseptically, add 1 mL each of the NaH_2PO_4, NH_4Cl and trace element solutions.

The ammonium chloride and sodium phosphate solutions should be prepared by adding the amounts indicated below, and after they are dissolved, the solution should be sterile filtered into a sterile container. The two stocks should be stored in a 4° C refrigerator.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaH_2PO_4 \cdot H_2O$	6.90 g L^{-1} dH_2O	1.0 mL	5.0 x 10^{-5} M
NH_4Cl	42.80 g L^{-1} dH_2O	1.0 mL	8.0 x 10^{-4} M
Trace Elements	(see recipe below)	1.0 mL	---

PRO99 Trace Element Solution

Primary stocks of most metals and selenium are prepared first, as indicated below. To prepare primary stocks, add the indicated amount of the component to 1 liter of high quality water. Next, the trace element solution is prepared by dissolving the EDTA in 1 liter of high quality water, pH to 8.0-8.4, dissolve the iron, and finally by adding 1 mL of

each primary stock. The final trace element solution should be sterile filtered into a clean, sterile container and stored at 4° C in a refrigerator.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na_2 EDTA • $2H_2O$	---	0.436 g	1.17×10^{-6} M
$FeCl_3$ • $6H_2O$	---	0.316 g	1.17×10^{-6} M
$ZnSO_4$ • $7H_2O$	2.30 g L^{-1} dH_2O	1 mL	8.00×10^{-9} M
$CoCl_2$ • $6H_2O$	1.19 g L^{-1} dH_2O	1 mL	5.00×10^{-9} M
$MnCl_2$ • $4H_2O$	17.80 g L^{-1} dH_2O	1 mL	9.00×10^{-8} M
Na_2MoO_4 • $2H_2O$	0.73 g L^{-1} dH_2O	1 mL	3.00×10^{-9} M
Na_2SeO_3	1.29 g L^{-1} dH_2O	1 mL	7.46×10^{-8} M
$NiSO_4$ • $6H_2O$	2.63 g L^{-1} dH_2O	1 mL	1.00×10^{-8} M