NATURAL SEAWATER-BASED PRO99 MEDIUM

NOTE: We observe maximal growth with open ocean seawater (i.e. Sargasso seawater), though we can routinely grow *Prochlorococcus* in some coastal seawater (from Cape Cod, MA) as well.

Table 1. Nutrient additions to filtered, autoclaved seawater

Nutrient	Manufacturer/Grade	Primary Stock (M)	Dilution Factor	Final Conc. (μM)
$NaH_2PO_4 \cdot H_2O$	(1) / ACS	0.025	1:500	50
NH_4Cl	(1) / ACS	0.50	1:625	800
$Na_2EDTA \cdot 2\ H_2O$	(2) / 99%	0.012	$1:10^4$	1.17
$FeCl_3 \cdot 6\ H_2O$	(1) / Analytic	0.012	$1:10^4$	1.18
$ZnSO_4 \cdot 7\ H_2O$	(3) / >99.5%	0.080	$1:10^7$	0.008
$CoCl_2 \cdot 6\ H_2O$	(1) / Analytic	0.050	$1:10^7$	0.005
$MnCl_2 \cdot 4\ H_2O$	(1) / Analytic	0.900	$1:10^7$	0.090
$Na_2MoO_4 \cdot 2\ H_2O$	(2) / ACS	0.030	$1:10^7$	0.003
Na_2SeO_3	(2) / ~98%	0.100	$1:10^7$	0.010
$NiCl_2 \cdot 6\ H_2O$	(1) / Analytic	0.100	$1:10^7$	0.010

Manufacturer Index: (1) Mallinckrodt, (2) Sigma, (3) Fluka

PREPARATION:

1. Prepare a glass filter funnel and flask by cleaning with acid and Milli-Q water, as described on p. 7 (clean the system after every 10 filtrations). Filter raw seawater through 47mm Whatman GF/F stacked on top of a 47mm 0.2μm polycarbonate filter (make sure there are no bubbles/creases).

   Autoclave seawater in a Teflon bottle (60 minutes for 2L) and allow to cool overnight

2. Prepare primary stocks of NH_4Cl, $NaH_2PO_4 \cdot H_2O$, and trace metals as follows:

   A. **0.5M NH_4Cl**
   - Weigh out 2.67g NH_4Cl using dust-free weigh paper
   - Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water
   - Dissolve NH_4Cl by inverting flask several times

- Adjust volume to 100mL mark with Milli-Q water
- Using a polycarbonate syringe, filter through 0.2µm syringe filter (Acrodisc, Pall #2006-01) into sterile container in a laminar flow hood.
- Store sterile stock at 4°C

B. **0.025M $NaH_2PO_4 \cdot H_2O$**

- Weigh out 0.345g $NaH_2PO_4 \cdot H_2O$ using dust-free weigh paper
- Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water
- Dissolve NaH_2PO_4 by inverting flask several times
- Adjust volume to 100mL mark with Milli-Q water
- Using a polycarbonate syringe, filter through 0.2µm syringe filter into sterile container in a laminar flow hood.
- Store sterile stock at 4°C

C. **Trace Metal Stocks**

i. Primary trace metal stocks

- Using dust-free weigh paper, weigh out:

2.30g	$ZnSO_4 \cdot 7H_2O$
1.19g	$CoCl_2 \cdot 6H_2O$
17.81g	$MnCl_2 \cdot 4H_2O$
0.726g	$Na_2MoO_4 \cdot 2H_2O$
1.73g	Na_2SeO_3
2.38g	$NiCl_2 \cdot 6H_2O$

- Transfer each trace metal into separate 100mL volumetric flasks containing about 60 mL Milli-Q water
- Dissolve contents by inverting flask several times
- Adjust volume to 100mL mark with Milli-Q water
- Store each stock in a cleaned Teflon bottle at 4°C

ii. Trace metal working stock

- Weigh out 0.435g $Na_2EDTA \cdot 2\ H_2O$ using dust-free weigh paper
- Transfer into 100mL volumetric flask filled with 60mL Milli-Q water
- Dissolve Na_2EDTA by inverting flask several times
- Weigh out 0.32g $FeCl_3 \cdot 6\ H_2O$ using dust-free weigh paper
- Dissolve $FeCl_3$ into same volumetric flask by inverting several times
- Individually add and dissolve 100µl of the Primary trace metal stocks
- Adjust volume to 100mL mark with Milli-Q water
- Using a polycarbonate syringe, filter through 0.2µm syringe filter into sterile container in a laminar flow hood
- Store sterile stock at 4°C

3. To make up the media, add following volumes of sterile nutrients and trace metal stock to one liter of the autoclaved seawater. It is important to *dissolve each nutrient sequentially*.

Filtered Seawater		1000.0mL
0.5M	NH_4Cl	1.6mL
0.025M	$NaH_2PO_4 \cdot H_2O$	2.0mL
Trace metal working stock		100µL

4. Store at room temperature for up to one month.