

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

<u>Nutrient</u>	<u>Manufacturer/Grade</u>	<u>Primary Stock (M)</u>	<u>Dilution Factor</u>	<u>Final Conc. (μM)</u>
NaH ₂ PO ₄ ·H ₂ O	(1) / ACS	0.025	1:500	50
NH ₄ Cl	(1) / ACS	0.50	1:625	800
Na ₂ EDTA·2 H ₂ O	(2) / 99%	0.012	1:10 ⁴	1.17
FeCl ₃ ·6 H ₂ O	(1) / Analytic	0.012	1:10 ⁴	1.18
ZnSO ₄ ·7 H ₂ O	(3) / >99.5%	0.080	1:10 ⁷	0.008
CoCl ₂ ·6 H ₂ O	(1) / Analytic	0.050	1:10 ⁷	0.005
MnCl ₂ ·4 H ₂ O	(1) / Analytic	0.900	1:10 ⁷	0.090
Na ₂ MoO ₄ ·2 H ₂ O	(2) / ACS	0.030	1:10 ⁷	0.003
Na ₂ SeO ₃	(2) / ~98%	0.100	1:10 ⁷	0.010
NiCl ₂ ·6 H ₂ O	(1) / Analytic	0.100	1:10 ⁷	0.010

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

PREPARATION:

1. Prepare primary stocks of NH₄Cl, NaH₂PO₄·H₂O, and trace metals as follows:

A. 0.5M NH₄Cl

- Weigh out 2.67g NH₄Cl using dust-free weigh paper
- Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water
- Dissolve NH₄Cl by inverting flask several times
- Adjust volume to 100mL mark with Milli-Q water
- Filter using a.2μm nylon filtration unit
- Store sterile stock at 4°C

B. 0.025M NaH₂PO₄·H₂O

- Weigh out 0.345g NaH₂PO₄·H₂O using dust-free weigh paper
- Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water
- Dissolve NaH₂PO₄ by inverting flask several times
- Adjust volume to 100mL mark with Milli-Q water
- Filter using a.2μm nylon filtration unit
- 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 ·7H ₂ O
1.19g	CoCl ₂ ·6H ₂ O
17.81g	MnCl ₂ ·4H ₂ O
0.726g	Na ₂ MoO ₄ ·2H ₂ O
1.73g	Na ₂ SeO ₃
2.38g	NiCl ₂ ·6H ₂ O

- 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 Na₂EDTA·2 H₂O using dust-free weigh paper
- Transfer into 100mL volumetric flask filled with 60mL Milli-Q water
- Dissolve Na₂EDTA by inverting flask several times
- Weigh out 0.32g FeCl₃·6 H₂O using dust-free weigh paper
- Dissolve FeCl₃ 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
- Filter using a 2µm nylon filtration unit
- Store sterile stock at 4°C

2. To make up the media, add following volumes of sterile nutrients and trace metal stock to one liter of the autoclaved or 0.2µm filtered seawater. **It is important to *dissolve each nutrient sequentially.***

Filtered Seawater		1000.0mL
0.5M	NH ₄ Cl	1.6mL
0.025M	NaH ₂ PO ₄ ·H ₂ O	2.0mL
	Trace metal working stock	100µL

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