NATURAL SEAWATER-BASED PRO99 MEDIUM

Chisholm Lab

Abstract

Citation: Chisholm Lab NATURAL SEAWATER-BASED PRO99 MEDIUM. **protocols.io** dx.doi.org/10.17504/protocols.io.c7zzp5
Published: 04 Jan 2016

Guidelines

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)	Sigma Cat. No.
$NaH_2PO_4 \cdot H_2O$	(1) / ACS	0.025	1:500	50	(MK7892-04)
NH_4Cl	(1) / ACS	0.50	1:625	800	(MK3384-12)
$Na_2EDTA \cdot 2\ H_2O$	(2) / 99%	0.012	$1:10^4$	1.17	E4884-100g
$FeCl_3 \cdot 6\ H_2O$	(1) / Analytic	0.012	$1:10^4$	1.18	44944-50g
$ZnSO_4 \cdot 7\ H_2O$	(3) / >99.5%	0.080	$1:10^7$	0.008	204986-10g
$CoCl_2 \cdot 6\ H_2O$	(1) / Analytic	0.050	$1:10^7$	0.005	60820-50g
$MnCl_2 \cdot 4\ H_2O$	(1) / Analytic	0.900	$1:10^7$	0.090	M3634-100g
$Na_2MoO_4 \cdot 2\ H_2O$	(2) / ACS	0.030	$1:10^7$	0.003	M1651-100g
Na_2SeO_3	(2) / ~98%	0.100	$1:10^7$	0.010	S5261-25g
$NiCl_2 \cdot 6\ H_2O$	(1) / Analytic	0.100	$1:10^7$	0.010	223387-25g

Manufacturer Index: (1) Mallinckrodt, (2) Sigma, (3) Fluka **Note 2/6/12:** Mallinckrodt and Fluka no longer available; ordered all reagents from Sigma at same grade as specified.

Note 4/28/08:
Matt suggested higher concentrations of NH_4Cl and NaH_2PO_4 so only use 0.5ml per 1L seawater:
1.6M NH_4Cl 2.14g/25ml or 8.56g/100ml
0.1M NaH_2PO_4-H_2O 0.345g/25ml or 1.38g/100ml
Trace metal working stock 100µl

Protocol

Seawater Preparation

Step 1.
Prepare a glass filter funnel and flask by cleaning with acid and Milli-Q water

NOTES

VERVE Team 29 Jun 2015

Clean the system after every 10 filtrations.

Seawater Preparation

Step 2.

Filter raw seawater through 47mm Whatman GF/F stacked on top of a 47mm 0.2µm polycarbonate filter

NOTES

VERVE Team 29 Jun 2015

Make sure there are no bubbles/creases.

Seawater Preparation

Step 3.

Autoclave seawater in a Teflon bottle (60 minutes for 2L)

DURATION

01:00:00

Seawater Preparation

Step 4.

Allow to cool overnight

DURATION

18:00:00

Stock and Trace Metal Preparation

Step 5.

Prepare 0.5M NH_4Cl solution

PROTOCOL

. 0.5M NH4Cl

CONTACT: Bonnie Poulos

Step 5.1.

Weigh out 2.67g NH_4Cl using dust-free weigh paper

Step 5.2.

Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water

Step 5.3.

Dissolve NH_4Cl by inverting flask several times

Step 5.4.

Adjust volume to 100mL mark with Milli-Q water

Step 5.5.

Using a polycarbonate syringe, filter through 0.2µm syringe filter (Acrodisc, Pall #2006-01) into sterile container in a laminar flow hood

Step 5.6.

Store sterile stock at 4°C

Stock and Trace Metal Preparation

Step 6.

Prepare 0.025M $NaH_2PO_4 \cdot H_2O$ solution

PROTOCOL

. 0.025M NaH2PO4·H2O

CONTACT: Bonnie Poulos

Step 6.1.
Weigh out 0.345g $NaH_2PO_4 \cdot H_2O$ using dust-free weigh paper

Step 6.2.
Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water

Step 6.3.
Dissolve NaH2PO4 by inverting flask several times

Step 6.4.
Adjust volume to 100mL mark with Milli-Q water

Step 6.5.
Using a polycarbonate syringe, filter through 0.2µm syringe filter into sterile container in a laminar flow hood.

Step 6.6.
Store sterile stock at 4°C

Stock and Trace Metal Preparation

Step 7.
Prepare primary trace metal stocks

PROTOCOL

. Primary trace metal stocks

CONTACT: Bonnie Poulos

Step 7.1.
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 $NiCl_2 \cdot 6H_2O$

NOTES

Bonnie Poulos 30 Nov 2015
Before culturing cyanobacteria, read the publication by LR Moore et al. (2007) Limnol. Ocenogr. 5:353-362.

Bonnie Poulos 30 Nov 2015
All reagents used for culturing cyanobacteria should be the highest quality to avoid contamination by trace metals. Do not use metal spatulas for dispensing the chemicals (use plastic, teflon, or dust-free weighing paper to dispense). Store reagents in acid-washed teflon or polycarbonate containers.

Bonnie Poulos 30 Nov 2015

Use the highest quality chemicals that are dedicated for cyanobacteria work.
Do not use metal spatulas to transfer chemicals; use plastic or teflon spatulas or dust-free weigh paper to weigh out chemicals.
All re-useable glassware or plastic should be acid-washed and then autoclaved.
Bonnie Poulos 30 Nov 2015
Do not use metal spatulas; use plastic or teflon spatulas or dust-free weigh paper to weigh out chemicals and purchase the highest quality chemicals

Step 7.2.
Transfer each trace metal into separate 100mL volumetric flasks containing about 60 mL Milli-Q water

NOTES
Bonnie Poulos 30 Nov 2015
Do not use metal spatulas; use plastic or teflon spatulas or dust-free weigh paper to weigh out chemicals and purchase the highest quality chemicals
Bonnie Poulos 30 Nov 2015
Alternatively, if prepaing 50 mL volumes, use half the amount of chemical listed and adjust volume to 50 mL mark with Milli-Q water

Step 7.3.
Dissolve contents by placing stopper in top and inverting flask several times

Step 7.4.
Adjust volume to 100 mL mark with Milli-Q water

Step 7.5.
Store each stock in acid-washed Teflon or polycarbonate (i.e., Nalgene) bottles at 4°C

Stock and Trace Metal Preparation

Step 8.
Prepare trace metal working stock

PROTOCOL

. Cyanobacteria Trace Metal Mixture (CTMM)

CONTACT: Bonnie Poulos

Step 8.1.
Weigh out 0.435 g $Na_2EDTA{\cdot}2H_2O$ using dust-free weigh paper

REAGENTS
Ethylenediaminetetraacetic acid disodium salt dihydrate E4884 by Sigma Aldrich

Step 8.2.
Transfer into 100 mL volumetric flask filled with 60 mL Milli-Q water

Step 8.3.
Dissolve Na_2EDTA by inserting the stopper and inverting flask several times

NOTES
Bonnie Poulos 11 Aug 2015
May have to heat 5 min at 80°C to dissolve.

Step 8.4.
Weigh out 0.32 g $FeCl_3{\cdot}6H_2O$ using dust-free weigh paper

Step 8.5.

Dissolve $FeCl_3$ into same volumetric flask and mix by inverting several times

Step 8.6.
Individually add and dissolve 100 µl of each of the six primary trace metal stocks described in the Primary Trace Metal Stocks protocol. The six primary trace metals to add are $ZnSO_4$, $CoCl_2$, $MnCl_2$, Na_2MoO_4, Na_2SeO_3 and $NiCl_2$.

LINK:
https://www.protocols.io/view/Primary-trace-metal-stocks-c8hzt5

Step 8.7.
Adjust volume to 100mL mark with Milli-Q water

Step 8.8.
Using a polycarbonate syringe, filter through 0.2µm syringe filter into a sterile, acid-washed teflon or polycarbonate container in a laminar flow hood

Step 8.9.
Store sterile CTMM (cyanobacteria trace metal mixture) at 4°C

Step 9.
To make up the media, add following volumes of sterile nutrients and trace metal stock to one liter of the autoclaved seawater

Nutrient	Volume
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

NOTES
VERVE Team 11 Aug 2015
It is important to dissolve each nutrient sequentially.
VERVE Team 11 Aug 2015
Note 4/28/08:
Matt suggested higher concentrations of NH4Cl and NaH2PO4 so only use 0.5ml per 1L seawater:
1.6M NH4Cl 2.14g/25ml or 8.56g/100ml
0.1M NaH2PO4-H2O 0.345g/25ml or 1.38g/100ml
Trace metal working stock 100µl

Step 10.
Store at room temperature for up to one month