Artificial Seawater Based AMP1 Medium

Bonnie Poulos

Abstract

Citation: Bonnie Poulos Artificial Seawater Based AMP1 Medium. **protocols.io**
dx.doi.org/10.17504/protocols.io.c9mz45
Published: 04 Jan 2016

Guidelines

Turk's Island Salt Mix

Chemical	g/2 L	Final Conc.	Mfc.-PN	Chemical Grade
NaCl	56.22	481 mM	Fisher S271	ACS
$MgSO_4 \cdot 7H_2O$	13.8	28 mM	Sigma M2773	Mo.Biol. 99%
$MgCl_2 \cdot 6H_2O$	10.98	27 mM	AlfaAesar	99.99%
$CaCl_2 \cdot 2H_2O$	2.94	10 mM	Fisher C79	ACS
KCl	1.34	9 mM	Acros	99+%
$MQ \cdot H_2O$	QS to 2000 ml	na	in house	na

Macronutrients

Macronutrient	g/100 ml	Final Conc.	Mfc.-PN	Chemical Grade
0.1 M $NaH_2PO_4 \cdot H_2O$	1.38	50 µM	Mallinckrodt 7892-04	ACS
0.8 M $(NH_4)_2SO_4$	10.57	400 µM	VWR-BDH 0216	ACS 99%

Buffers

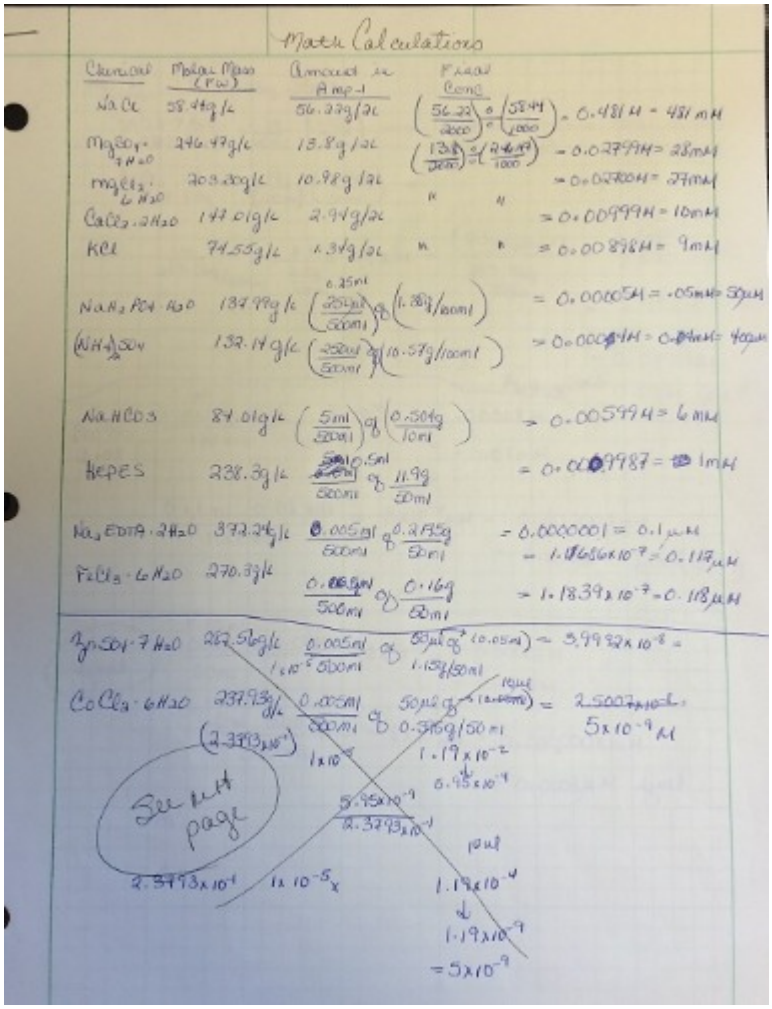
Buffer	Stock	Final Conc.	Mfc.-PN	Chemical Grade
0.6 M $NaHCO_3$	0.504 g/10 ml	6 mM	Sigma S6014	ACS 99.7-100.3%
1 M HEPES	11.9 g/50 ml	1 mM	Fisher BP310	Mol. Biol.

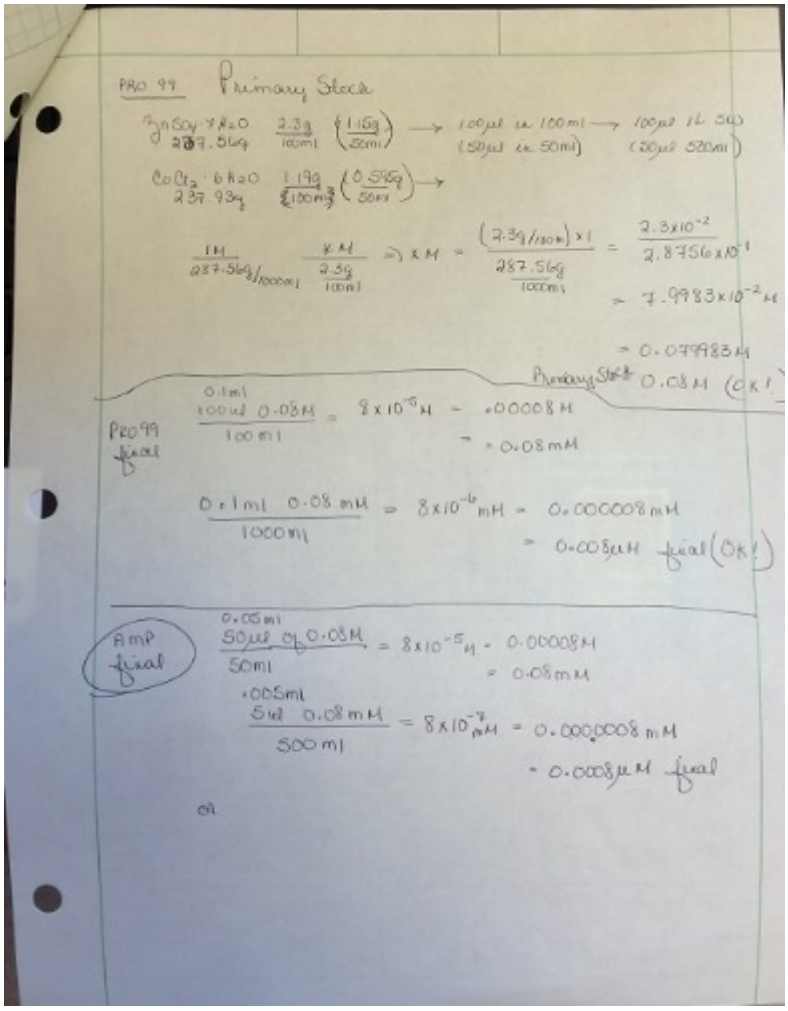
Trace Metal Mix Working Stock

Trace Metal	Primary Stock	Final Conc.	Mfc.-PN	Chemical Grade
$Na_2EDTA \cdot 2H_2O$	0.2175 g/50 ml	0.1170 µM	Sigma E4884	ACS 99-101%
$FeCl_3 \cdot 6H_2O$	0.16 g/50 ml	0.1180 µM	Sigma 44944	ACS 98-102%
$ZnSO_4 \cdot 7H_2O$	1.15 g/50 ml	0.0008 µM	Sigma 204986	>99.5%
$CoCl_2 \cdot 6H_2O$	0.595 g/50 ml	0.0005 µM	Sigma 60820	>98%
$MnCl_2 \cdot 4H_2O$	8.905 g/50 ml	0.0090 µM	M3634	ReagentPlus >99%
$Na_2MoO_4 \cdot 2H_2O$	0.363 g/50 ml	0.0003 µM	M1651	>99.5%
Na_2SeO_3	0.865 g/50 ml	0.0010 µM	S5261	BioReagent ~98%

$NiCl_2 \cdot 6H_2O$	1.19 g/50 ml	0.0010 µM	223387	ReagentPlus

Note: Use primary stocks of $ZnSO_4$, $CoCl_2$, $MnCl_2$, Na_2MoO_4, Na_2SeO_3, and $NiCl_2$ prepared for Pro99 trace metal working stock.





Materials

Ethylenediaminetetraacetic acid disodium salt dihydrate E4884 by Sigma Aldrich

Sodium Chloride S271 by Fisher Scientific

Magnesium sulfate heptahydrate M2773 by Sigma Aldrich

Calcium Chloride Dihydrate C79 by Fisher Scientific

Sodium bicarbonate S6014 by Sigma Aldrich

HEPES BP310 by Fisher Scientific

Iron(III) chloride hexahydrate 44944 by Sigma Aldrich

Zinc sulfate heptahydrate 204986 by Sigma Aldrich

Cobalt(II) chloride hexahydrate 60820 by Sigma Aldrich

Manganese(II) chloride tetrahydrate M3634 by Sigma Aldrich

Sodium molybdate dihydrate M1651 by Sigma Aldrich

Sodium selenite S5261 by Sigma Aldrich

Nickel(II) chloride hexahydrate 223387 by Sigma Aldrich

Protocol

Turk's Island Salt Mix

Step 1.

Dissolve each salt completely before adding the next one

NOTES

VERVE Team 12 Aug 2015

Refer to table in guidelines for full list of salts.

Turk's Island Salt Mix

Step 2.

Dispense into 500 ml acid-washed polycarbonate bottles

Turk's Island Salt Mix

Step 3.

Autoclave 30 min.

DURATION

00:30:00

Macronutrients

Step 4.

pH NaH_2PO_4 to 7.5 using 1M NaOH

NOTES

VERVE Team 01 Jul 2015

About 8ml for 100ml volume.

VERVE Team 01 Jul 2015

Prepare each one separately.

Macronutrients

Step 5.

Filter sterilize each solution using 0.2 µm syringe filter into new, sterile 50ml centrifuge tubes or acid washed and sterile polycarbonate bottles

Macronutrients

Step 6.

Store at 4°C

NOTES

VERVE Team 01 Jul 2015

Dispense 250 µl of each per 500 ml bottle of Turk's Island Salt Mix when preparing final medium.

Buffers

Step 7.

pH HEPES to 7.5 using 1M NaOH

REAGENTS

HEPES BP310 by Fisher Scientific

NOTES

VERVE Team 01 Jul 2015

About 9 ml for 50 ml volume.

VERVE Team 01 Jul 2015

Prepare each one separately.

Buffers

Step 8.

Filter sterilize each solution using 0.2 µm syringe filter into new, sterile 15 or 50 ml centrifuge tubes or acid washed and sterile polycarbonate bottles

Buffers

Step 9.

Store at 4°C

NOTES

VERVE Team 01 Jul 2015

The $NaHCO_3$ should be made monthly.

Buffers

Step 10.

Dispense 5 ml of $NaHCO_3$ and 0.5 ml HEPES per 500 ml bottle of Turk's Island Salt Mix when preparing final medium

Trace Metal Mix Working Stock

Step 11.

Weigh out 0.2175 g $Na_2EDTA{\cdot}2H_2O$ using dust free paper

AMOUNT

0 g Additional info:

REAGENTS

Ethylenediaminetetraacetic acid disodium salt dihydrate E4884 by Sigma Aldrich

Trace Metal Mix Working Stock

Step 12.

Transfer to acid washed 50 ml volumetric flask filled with 40 ml MQ-water

Trace Metal Mix Working Stock

Step 13.

Dissolve EDTA by inverting flask several times

NOTES

VERVE Team 01 Jul 2015

May have to heat 5 min. at 80°C to dissolve.

Trace Metal Mix Working Stock

Step 14.

Weigh out 0.16 g $FeCl_3{\cdot}6H_2O$ using dust free paper

AMOUNT

0 µl Additional info:

REAGENTS

Iron(III) chloride hexahydrate 44944 by Sigma Aldrich

Trace Metal Mix Working Stock

Step 15.

Dissolve iron chloride into same volumetric flask by inverting several times

Trace Metal Mix Working Stock

Step 16.

Individually add and dissolve 50 µl each of the $ZnSO_4$, $CoCl_2$, $MnCl_2$, Na_2MoO_4, $NaSeO_3$, and $NiCl_2$

Primary Trace Metal Stocks

Trace Metal Mix Working Stock

Step 17.

Adjust volume to 50 ml mark with MQ-water

Trace Metal Mix Working Stock

Step 18.

Filter through a 0.2 µm syringe filter into sterile, acid washed container in laminar flow hood

Trace Metal Mix Working Stock

Step 19.

Store sterile stock at 4°C

Trace Metal Mix Working Stock

Step 20.

Dispense 5 µl Stock Trace Metal Mix to 500 ml bottle of Turk's Island Salt Mix when preparing final medium