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Artificial Seawater Based AMP1 Medium

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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

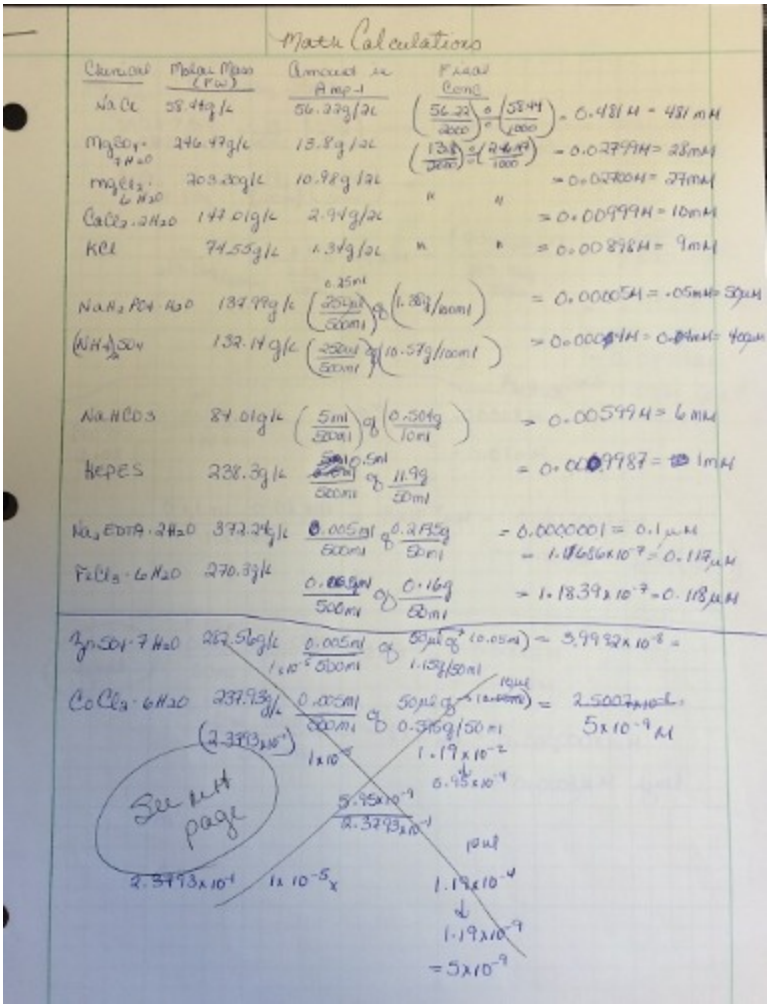
	Buffer	Stock	Final Conc.	Mfc.-PN	Chemical Grade
	0.6 M $NaHCO_3$	0.504 g/10 ml	6 mM	Sigma S6014	ACS 99.7-100.3%
	1 M HEPES	11.9 g/50 ml	1 mM	Fisher BP310	Mol. Biol.

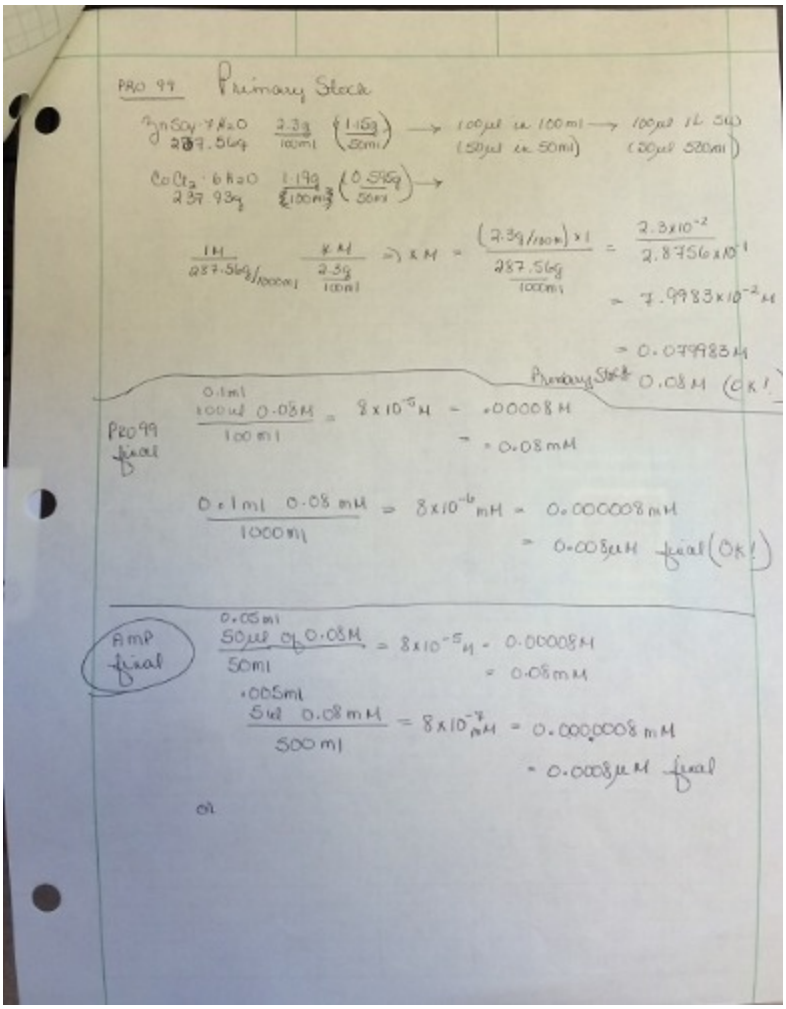
Trace Metal Mix Working Stock

	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.




PRO 99 Primary Stock
MnSO4·7H2O 2.3g/100ml (1.15g/50ml) → 100µl in 100ml → 100µl in 1L SW (50µl in 50ml) (50µl in 500ml)
CoCl2·6H2O 1.19g/100ml (0.595g/50ml) →
1M/287.56g/1000ml x M/2.3g/100ml => x M = (2.3g/100ml) x 1 / 287.56g/1000ml = 2.3x10^-2 / 2.8756x10^1
= 7.9983x10^-2 M
= 0.07998 M
Primary Stock 0.08 M (OK!)
PRO 99 final
0.1ml 100µl 0.08M / 100ml = 8x10^-5 M = .00008 M
= 0.08 mM
0.1ml 0.08 mM / 1000ml = 8x10^-6 mM = 0.000008 mM
= 0.008µM final (OK!)
AMP final
0.05ml 50µl of 0.08M / 50ml = 8x10^-5 M = 0.00008 M
= 0.08 mM
.005ml 5µl 0.08 mM / 500ml = 8x10^-7 mM = 0.0000008 mM
= 0.0008µM final
or

Materials

MATERIALS

- Ethylenediaminetetraacetic acid disodium salt dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E4884
- Sodium Chloride **Fisher Scientific Catalog #**S271
- Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2773
- Calcium Chloride Dihydrate **Fisher Scientific Catalog #**C79
- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S6014
- HEPES **Fisher Scientific Catalog #**BP310
- Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**44944
- Zinc sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**204986
- Cobalt(II) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**60820
- Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3634
- Sodium molybdate dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M1651
- Sodium selenite **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S5261
- Nickel(II) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**223387

STEP MATERIALS

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Turk's Island Salt Mix

1 Dissolve each salt completely before adding the next one

Note

Refer to table in guidelines for full list of salts.

2 Dispense into 500 ml acid-washed polycarbonate bottles

3 Autoclave 30 min.

00:30:00

Macronutrients

4 pH NaH_2PO_4 to 7.5 using 1M NaOH

Note

About 8ml for 100ml volume.

Note

Prepare each one separately.

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

6 Store at 4°C

Note

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

Buffers

7 pH HEPES to 7.5 using 1M NaOH

Note

About 9 ml for 50 ml volume.

Note

Prepare each one separately.

HEPES **Fisher Scientific Catalog #**BP310

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

9 Store at 4°C

Note

The $NaHCO_3$ should be made monthly.

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

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

0 µL

Ethylenediaminetetraacetic acid disodium salt dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E4884

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

13 Dissolve EDTA by inverting flask several times

Note

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

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

0 µL

Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**44944

15 Dissolve iron chloride into same volumetric flask by inverting several times

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

17 Adjust volume to 50 ml mark with MQ-water

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

19 Store sterile stock at 4°C

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