

Jan 05, 2016

Artificial Seawater Based AMP1 Medium

DOI

<https://dx.doi.org/10.17504/protocols.io.c9mz45>

Bonnie Poulos¹

¹Matthew Sullivan Lab, University of Arizona, Ohio State University

VERVE Net

Sullivan Lab



Verve Team

University of Arizona

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.c9mz45>

Protocol Citation: Bonnie Poulos 2016. Artificial Seawater Based AMP1 Medium. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.c9mz45>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: July 02, 2015

Last Modified: March 17, 2018

Protocol Integer ID: 1037

Keywords: based amp1 medium, amp1 medium, artificial seawater, seawater

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 ·7H ₂ O	13.8	28 mM	Sigma M2773	Mo.Biol. 99%
	MgCl ₂ ·6H ₂ O	10.98	27 mM	AlfaAesar	99.99%
	CaCl ₂ ·2H ₂ O	2.94	10 mM	Fisher C79	ACS
	KCl	1.34	9 mM	Acros	99+%
	MQ·H ₂ O	QS to 2000 ml	na	in house	na

Macronutrients

	Macronutrient	g/100 ml	Final Conc.	Mfc.-PN	Chemical Grade
	0.1 M NaH ₂ PO ₄ ·H ₂ O	1.38	50 µM	Mallinckrodt 7892-04	ACS
	0.8 M (NH ₄) ₂ SO 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 ₂ EDTA·2H ₂ O	0.2175 g/50 ml	0.1170 µM	Sigma E4884	ACS 99-101%
	FeCl ₃ ·6H ₂ O	0.16 g/50 ml	0.1180 µM	Sigma 44944	ACS 98-102%
	ZnSO ₄ ·7H ₂ O	1.15 g/50 ml	0.0008 µM	Sigma 204986	>99.5%
	CoCl ₂ ·6H ₂ O	0.595 g/50 ml	0.0005 µM	Sigma 60820	>98%
	MnCl ₂ ·4H ₂ O	8.905 g/50 ml	0.0090 µM	M3634	ReagentPlus >99%
	Na ₂ MoO ₄ ·2H ₂ O	0.363 g/50 ml	0.0003 µM	M1651	>99.5%
	Na ₂ SeO ₃	0.865 g/50 ml	0.0010 µM	S5261	BioReagent ~98%



NiCl ₂ ·6H ₂ O	1.19 g/50 ml	0.0010 μM	223387	ReagentPlus
--------------------------------------	--------------	-----------	--------	-------------

Note: Use primary stocks of ZnSO₄, CoCl₂, MnCl₂, Na₂MoO₄, Na₂SeO₃, and NiCl₂ prepared for Pro99 trace metal working stock.

Math Calculations				
Chemical	Molar Mass (g/mol)	Amount in Amp-1	Final Conc	
NaCl	58.44 g/mol	56.22 g/2L	$\left(\frac{56.22}{2000}\right) \times \left(\frac{58.44}{1000}\right)$	= 0.481 M = 481 mM
MgSO ₄ ·7H ₂ O	246.47 g/mol	13.8 g/2L	$\left(\frac{13.8}{2000}\right) \times \left(\frac{246.47}{1000}\right)$	= 0.02799 M = 28 mM
MgCl ₂ ·6H ₂ O	203.29 g/mol	10.92 g/2L	" "	= 0.02700 M = 27 mM
CaCl ₂ ·2H ₂ O	147.01 g/mol	2.94 g/2L	" "	= 0.00999 M = 10 mM
KCl	74.55 g/mol	1.34 g/2L	" "	= 0.00898 M = 9 mM
Na ₂ H ₂ Po ₄ ·H ₂ O	184.99 g/mol	$\left(\frac{0.25 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{1.86 \text{ g}}{100 \text{ ml}}\right)$	" "	= 0.0005 M = 0.5 mM = 50 μM
(NH ₄) ₂ SO ₄	132.14 g/mol	$\left(\frac{0.25 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{10.57 \text{ g}}{100 \text{ ml}}\right)$	" "	= 0.0004 M = 0.4 mM = 40 μM
NaHCO ₃	84.01 g/mol	$\left(\frac{5 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{0.504 \text{ g}}{10 \text{ ml}}\right)$	" "	= 0.00599 M = 6 mM
HEPES	238.31 g/mol	$\left(\frac{5 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{11.9 \text{ g}}{50 \text{ ml}}\right)$	" "	= 0.009987 M = 1 mM
Na ₂ EDTA·2H ₂ O	372.24 g/mol	$\left(\frac{0.005 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{0.215 \text{ g}}{50 \text{ ml}}\right)$	" "	= 0.000001 = 0.1 μM
FeCl ₃ ·6H ₂ O	270.33 g/mol	$\left(\frac{0.005 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{0.16 \text{ g}}{50 \text{ ml}}\right)$	" "	= 1.0656×10^{-7} = 0.117 μM
			" "	= 1.1839×10^{-7} = 0.118 μM
ZnSO ₄ ·7H ₂ O	287.56 g/mol	$\left(\frac{0.005 \text{ ml}}{1 \times 10^{-5} \times 500 \text{ ml}}\right) \times \left(\frac{50 \mu\text{g}}{1.05 \text{ g}} \times (0.05 \text{ M})\right)$	" "	= 5.9932×10^{-8}
CoCl ₂ ·6H ₂ O	237.93 g/mol	$\left(\frac{0.005 \text{ ml}}{500 \text{ ml}}\right) \times \left(\frac{50 \mu\text{g}}{0.345 \text{ g}} \times (1.2 \times 10^{-2} \text{ M})\right)$	" "	= 2.5007×10^{-8} = 5×10^{-9} M
			" "	= 1.19×10^{-2}
			" "	= 0.45×10^{-4}
			" "	= 5.95×10^{-9}
			" "	= 1.3293×10^{-1}
			" "	= 1.19×10^{-4}
			" "	= 1.19×10^{-7}
			" "	= 5×10^{-9}

PRO 99 Primary Stock

$\text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O} \quad \frac{2.3 \text{ g}}{100 \text{ ml}} \left(\frac{1.15 \text{ g}}{50 \text{ ml}} \right) \rightarrow 100 \mu\text{l in } 100 \text{ ml} \rightarrow 100 \mu\text{l in } 50 \text{ ml}$
 $(50 \mu\text{l in } 50 \text{ ml}) \quad (50 \mu\text{l in } 50 \text{ ml})$

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O} \quad \frac{1.19 \text{ g}}{257.93 \text{ g}} \left(\frac{0.585 \text{ g}}{50 \text{ ml}} \right) \rightarrow$

$\frac{1 \text{ M}}{257.93 \text{ g} / 1000 \text{ ml}} \cdot \frac{\text{g M}}{2.3 \text{ g}} \Rightarrow \text{M} = \frac{(2.3 \text{ g} / 100 \text{ ml}) \times 1}{257.93 \text{ g} / 1000 \text{ ml}} = \frac{2.3 \times 10^{-2}}{2.5793 \times 10^1}$
 $= 7.9983 \times 10^{-2} \text{ M}$
 $= 0.079983 \text{ M}$

Primary Stock 0.08 M (OK!)

PRO 99 final

$\frac{0.1 \text{ ml}}{100 \mu\text{l}} \cdot \frac{0.08 \text{ M}}{100 \text{ ml}} = 8 \times 10^{-5} \text{ M} = 0.00008 \text{ M}$
 $= 0.08 \text{ mM}$

$\frac{0.1 \text{ ml}}{100 \text{ ml}} \cdot \frac{0.08 \text{ mM}}{1000 \text{ ml}} = 8 \times 10^{-6} \text{ mM} = 0.000008 \text{ mM}$
 $= 0.008 \mu\text{M}$ final (OK!)

Amp final

$\frac{0.05 \text{ ml}}{50 \mu\text{l}} \cdot \frac{0.08 \text{ M}}{50 \text{ ml}} = 8 \times 10^{-5} \text{ M} = 0.00008 \text{ M}$
 $= 0.08 \text{ mM}$

$\frac{0.05 \text{ ml}}{5 \mu\text{l}} \cdot \frac{0.08 \text{ mM}}{500 \text{ ml}} = 8 \times 10^{-6} \text{ mM} = 0.000008 \text{ mM}$
 $= 0.008 \mu\text{M}$ 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

-  HEPES **Fisher Scientific Catalog #BP310**
-  Ethylenediaminetetraacetic acid disodium salt dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E4884**
-  Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944**
-  HEPES **Fisher Scientific Catalog #BP310**
-  Ethylenediaminetetraacetic acid disodium salt dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E4884**
-  Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944**

Protocol materials

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



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 $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ 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 $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ 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 μ l 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 μ m syringe filter into sterile, acid washed container in laminar flow hood
- 19 Store sterile stock at 4°C
- 20 Dispense 5 μ l Stock Trace Metal Mix to 500 ml bottle of Turk's Island Salt Mix when preparing final medium