ARTIFICIAL SEAWATER BASED AMP1 MEDIUM

Table 2. Sea Salt and nutrient additions

Nutrient	Manufacturer/Grade	Primary Stock (M)	Dilution Factor	Final Conc.
NaCl	(2) / SigmaUltra	n/a	n/a	481 mM
$CaCl_2 \cdot 2\ H_2O$	(2) / SigmaUltra	n/a	n/a	10 mM
KCl	(2) / SigmaUltra	n/a	n/a	9 mM
$Mg\ SO_4 \cdot 7\ H_2O$	(2) / SigmaUltra	n/a	n/a	28 mM
$MgCl_2 \cdot 6\ H_2O$	(2) / SigmaUltra	n/a	n/a	27 mM
NaH_2PO_4	(2) / SigmaUltra	0.025	1:500	50 µM
$(NH_4)_2SO_4$	(2) / SigmaUltra	0.4	1:1000	400 µM
$NaHCO_3$	(2) / SigmaUltra	0.6	1:100	6 mM
HEPES	(2) / SigmaUltra	0.5	1:500	1 mM
$Na_2EDTA \cdot 2H_2O$	(2) / 99%	0.012	$1:10^5$	0.1170 µM
$FeCl_3 \cdot 6\ H_2O$	(1) / Analytic	0.012	$1:10^5$	0.1180 µM
$ZnSO_4 \cdot 7\ H_2O$	(3) / >99.5%	0.080	$1:10^8$	0.0008 µM
$CoCl_2 \cdot 6\ H_2O$	(1) / Analytic	0.050	$1:10^8$	0.0005 µM
$MnCl_2 \cdot 4\ H_2O$	(1) / Analytic	0.900	$1:10^8$	0.0090 µM
$Na_2MoO_4 \cdot 2\ H_2O$	(2) / ACS	0.030	$1:10^8$	0.0003 µM
Na_2SeO_3	(2) / ~98%	0.100	$1:10^8$	0.0010 µM
$NiCl_2 \cdot 6\ H_2O$	(1) / Analytic	0.100	$1:10^8$	0.0010 µM

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

NOTE: To avoid contamination, dry nutrients and metals should be weighed without using spatulas.

1. Prepare nutrient stocks as following using Sigma Ultra grade chemicals:

A. **25mM NaH_2PO_4 (pH 7.5)**

- Weigh out 0.300g NaH_2PO_4 on dust-free weigh paper
- Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water
- Dissolve NaH_2PO_4 by inverting flask several times
- Adjust to pH 7.5 with NaOH (Mallinckrodt #7708)
- Adjust volume to 100mL mark with Milli-Q water
- Filter with a sterile 0.2µm nylon filtration unit
- Store sterile stock at 4°C

B. **0.4M $(NH_4)_2SO_4$**

- Weigh out 5.284g $(NH_4)_2SO_4$ on dust-free weigh paper
- Transfer into 100mL volumetric flask filled with about 60mL Milli-Q water
- Dissolve $(NH_4)_2SO_4$ by inverting flask several times
- Adjust volume to 100mL mark with Milli-Q water
- Filter with a sterile 0.2µm nylon filtration unit
- Store sterile stock at 4°C

C. **0.5M HEPES (pH 7.5)**

- Weigh out 59.575g HEPES on dust-free weigh paper
- Transfer into 1L glass beaker with stir bar and 300mL Milli-Q water
- Dissolve HEPES by stirring
- Fill remaining volume to 500mL mark with Milli-Q water
- Check pH by pipetting out small amount onto pH paper for rough estimate
- Turn stirrer on and **SLOWLY** add NaOH, check pH by pipetting out small amount onto pH paper for rough estimate periodically
- When pH is around 7.5 use a calibrated pH meter probe that has been Milli-Q water rinsed and soaked several times (add NaOH as needed to obtain pH 7.5)
- Transfer to volumetric flask or graduated cylinder and adjust volume to 500mL
- Filter, using a 500mL sterile filter system (Corning code# 430773) with .22µm nylon filter and polystyrene bottle (pre-wash the filter 2-3 times with 100mL Milli-Q water to remove residual carbon) attached to a vacuum pump (use 1 inch of Hg)
- Store sterile stock at 4°C

NOTE: We have migrated to using a 3.75mM TAPS buffer as it produces less H_2O_2, but if working with high density cell concentrations then HEPES will work.

D. **Working trace metal stock**

Use the working trace metal stock from the Natural Seawater Based PRO99 medium recipe, **but ten times LESS of the stock will be added to AMP1 compared to PRO99.**

2. Prepare "Turks Island Salt Mix"[3] by adding the following:

dissolve each ingredient sequentially in 2/3 final volume of Milli-Q water

Milli-Q water	1.0L
NaCl	28.10g
Mg $SO_4 \cdot 7\ H_2O$	6.90g
$MgCl_2 \cdot 6\ H_2O$	5.49g
KCl	0.67g
$CaCl_2 \cdot 2\ H_2O$	1.47g

- Fill to appropriate volume with Milli-Q water
- Autoclave and allow to cool

[3] The Merck Index (1989) Merck&Co, Inc. (Rahway, N.J.)

3. To Turks Island Salt Mix add the following nutrients stocks:

	1L
$NaH_2PO_4 \cdot H_2O$	2mL
$(NH_4)_2SO_4$	1mL
HEPES	2mL
Working trace metal stock	10µl

4. Store this ASW basal medium at room temperature for up to one month.

5. Prepare 0.6M $NaHCO_3$ stock fresh on day of medium inoculation (**do not store**)
   - Weigh out 2.52g $NaHCO_3$ on dust-free weigh paper
   - Transfer into 50mL volumetric flask filled with 30mL Milli-Q water
   - Dissolve $NaHCO_3$ by inverting flask several times
   - Adjust volume to 50mL mark with Milli-Q water
   - Filter with a sterile 0.2µm nylon filtration unit

6. $NaHCO_3$ addition to basal medium:
   10 mL of 0.6M $NaHCO_3$ per 1L basal medium