# Artificial Seawater Medium

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**Abstract**

Modified from Harrison et al., 1980. A broad spectrum artifical seawater medium for coastal and open ocean phytoplankton. The final salt concentration is ~34 ppt.

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## Guidelines

Prepare solutions separately, autoclave (or filter sterilize as needed), and then mix to prevent precipitation of salts. When autoclaving, use a bottle at least twice the size of the needed volume (e.g. use a 1 L bottle to autoclave 500 mL). Solutions should sit for 24-48 hr after autoclaving to allow for gaseous exchanges. N, P, Si, and trace element components are from the Bigelow NCMAM L1 medium; Vitamin components are from the Bigelow NCMAM f/2 medium. Trace metal and vitamin solutions can be made ahead of time and aliquots stored frozen.

## Before start

Acid washed all glassware with 10% HCl and rinse well (3-5 times with deionized or ultrapure water depending on downstream application). Prepare L1 medium components, L1 trace element solution and f/2 vitamin mix ahead of time.

## Protocol

### Prepare

***Step 1.***
Prepare solution 1.

PROTOCOL

. ASM Solution 1

CONTACT: Christa Smith

***Step 1.1.***
Dissolve 10.595 g NaCl into the bottle.

***Step 1.2.***
Dissolve 1.775 g $Na_2SO_4$ into the bottle.

***Step 1.3.***



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Dissolve 0.2795 g KCl into the bottle.

***Step 1.4.***
Dissolve 43.1 mg Kbr into the bottle.

***Step 1.5.***
Dissolve 0.1145 g $H_3BO_3$ into the bottle.

***Step 1.6.***
Dissolve 1.375 mg NaF into the bottle.

***Step 1.7.***
Dissolve 86.75 mg $NaHCO_3$ into the bottle.

***Step 1.8.***
Measure 500 mL $dH_2O$ into a 1 L glass bottle.

***Step 1.9.***
Mix final solution well and set aside until ready to autoclave.

## Prepare

***Step 2.***
Prepare solution 2.

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. ASM Solution 2

CONTACT: Christa Smith

***Step 2.1.***
Dissolve 4.796 g $MgCl_2 \cdot 6H_2O$ into bottle.

***Step 2.2.***
Dissolve 0.672 g $CaCl_2 \cdot 2H_2O$ into bottle.

***Step 2.3.***
Dissolve 10.95 mg $SrCl_2 \cdot 6H_2O$ into bottle.

***Step 2.4.***
$NaNO_3$: 1 ml primary stock solution (75.00 g/L)

***Step 2.5.***



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$NaH_2PO_4 \cdot H_2O$: 1 ml primary stock solution (5.00 g/L)

***Step 2.6.***

$Na_2SiO_3 \cdot 9H_2O$ (optional): 1 ml primary stock solution (30.00 g/L)

NOTES

**Christa Smith** 14 Sep 2017

Only add if phytoplankton requires silicates.

***Step 2.7.***

Measure 500 mL $dH_2O$ into a 1 L glass bottle.

***Step 2.8.***

Mix final solution well and set aside until ready to autoclave.

Autoclave

***Step 3.***

Autoclave solutions 1 and 2 and let sit 24-48 hours for gas exchange.

Mix

***Step 4.***

Combine solutions 1 and 2.

NOTES

**Christa Smith** 13 Sep 2017

If a solution has some precipitate, consider filtering solution(s) before or after combining through a 0.2 micron PES membrane.

Mix

***Step 5.***

Add 1 mL of trace element solution.

PROTOCOL

. L1 Trace Element Solution

CONTACT: Christa Smith

***Step 5.1.***

Add 1 mL primary stock of $MnCl_2 \cdot 4H_2O$ (178.10 g/L in $dH_2O$) into the bottle.

***Step 5.2.***

Add 1 mL primary stock of $ZnSO_4 \cdot 7H_2O$ (23.00 g/L in $dH_2O$) into the bottle.



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***Step 5.3.***
Add 1 mL primary stock of $CoCl_2 \cdot 6H_2O$ (11.90 g/L in $dH_2O$) into the bottle.

***Step 5.4.***
Add 1 mL primary stock of $CuSO_4 \cdot 5H_2O$ (2.50 g/L in $dH_2O$) into the bottle.

***Step 5.5.***
Add 1 mL primary stock of $Na_2MoO_4 \cdot 2H_2O$ (19.90 g/L in $dH_2O$) into the bottle.

***Step 5.6.***
Add 1 mL primary stock of $H_2SeO_3$ (1.29 g/L in $dH_2O$) into the bottle.

***Step 5.7.***
Add 1 mL primary stock of $NiSO_4 \cdot 6H_2O$ (2.63 g/L in $dH_2O$) into the bottle.

***Step 5.8.***
Add 1 mL primary stock of $Na_3VO_4$ (1.84 g/L in $dH_2O$) into the bottle.

***Step 5.9.***
Add 1 mL primary stock of $K_2CrO$ (1.94 g/L in $dH_2O$) into the bottle.

***Step 5.10.***
Bring final volume to 1 L with $dH_2O$.

***Step 5.11.***
Meausre 950 mL $dH_2O$ into a 1 L glass bottle.

***Step 5.12.***
Filter sterilize final trace element solution through a 0.2 micron PES membrane.

***Step 5.13.***
Dissolve 3.15 g $FeCl_3 \cdot 6H_2O$ into the bottle.

***Step 5.14.***
Dissolve 4.36 g $Na_2EDTA \cdot 2H_2O$ into the bottle.

***Step 5.15.***
Store frozen in 1 mL aliquots.

Mix

***Step 6.***



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Add 0.5 mL of f/2 vitamin solution.

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. f/2 Vitamin Solution

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***Step 6.1.***
Measure 950 mL $dH_2O$ into a 1 L glass bottle.

***Step 6.2.***
Add 10 mL primary stock solution of biotin (vit. H) (0.1 g/L in $dH_2O$) into the bottle.

***Step 6.3.***
Add 1 mL primary stock solution of cyanocobalamin (vit. B12) (1.0 g/L in $dH_2O$) into the bottle.

***Step 6.4.***
Bring the final volume to 1 L with $dH_2O$.

***Step 6.5.***
Filter sterilize final vitamin solution through a 0.2 micron PES membrane.

***Step 6.6.***
Dissolve 200 mg thiamine·HCl (vit. B1) into the bottle.

***Step 6.7.***
Store frozen in 0.5 mL aliquots.

## Store

***Step 7.***
Mix well and store for up to 2 weeks at 4 Celsius.

NOTES

**Christa Smith** 14 Sep 2017
Final salt concentration is ~34 ppt.



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