ESAW Medium

(**E**nriched **S**eawater, **A**rtificial **W**ater)

Systems Education Experiences
Baliga Lab at Institute for Systems Biology

This is a medium designed to mimic natural seawater that coastal and open ocean phytoplankton live in (e.g. diatoms *Thalasiossira pseudonana* aka "THAPS").
This recipe is for a final volume of **1L** medium (you may multiple the recipe for any quantity you need)

1.) Determine the final volume of media you want to create and calculate the proper grams and volumes to add.

2.) Collect all needed materials:

2 mixing jars (or flasks) large enough to split the final volume of media in (eg. If making 1L of media, use 1L flask and a 500mL flask)
graduated cylinder filled with DI H20 to proper final volume
weigh boats and spatulas
gloves (optional)
Erlenmeyer flask (2L) that is sterile
Filter tops to filter media through (or if you do not have filters, see other techniques in the media prep folder)
labeling tape
pen

3.) Dissolve Salt I (anhydrous salts) first in ~600mL of DI water
Weigh out each salt and place in flask (don't forget to tare the scale between each weigh)
**Before adding the stock salts, dissolve dry salts in the water.
Once dissolved, then add the stock salt solutions.

Component	Stock Solution	Quantity in 1L final medium	Molar Concentration in Final	
Salt I ***Anhydrous salts***				
NaCl		**21.194 g**	3.63×10^{-1}	
Na_2SO_4		**3.550 g**	2.50×10^{-2}	
KCl		**0.599 g**	8.03×10^{-3}	
$NaHCO_3$		**0.174 g**	2.07×10^{-3}	
KBr**	stock	****1mL**	7.25×10^{-4}	86.3g/L
H_3BO_3	stock	****1mL**	3.72×10^{-4} M	23g/L
NaF	stock	****1mL**	6.67×10^{-5}	2.8g/L

4.) Dissolve Salt II (hydrated salts) in ~400mL of water

Salt II ***Hydrated salts***	Stock Solution	Quantity	Molar Concentration in Final	
$MgCl_2$ $6H_2O$		**9.592 g**	4.71×10^{-2} M	
$CaCl_2$ $2H_2O$		**1.344 g**	9.14×10^{-3} M	
$SrCl_2$ $6H_2O$	stock	****1mL**	8.18×10^{-5} M	21.89g/L

5.) Combine dissolved salt I and II, and then add Trace Metals, Vitamins and Major Nutrients (NO3, PO4, SIO3).
**Pay attention to whether you are creating a media that is limited in one nutrient!!

Trace Metals	**1mL**
Vitamins	**1mL**

For full "f/2" Media				For Nitrate limiting Media				For Silica limiting Media				For Phosphate limiting Media			
Major Nutrients				**Major Nutrients**				**Major Nutrients**				**Major Nutrients**			
$NaNO_3$	75 g L^{-1} dH_2O	**1 mL**	88uM	$NaNO_3$	75 g L^{-1} dH_2O	**0.5mL**	44uM	$NaNO_3$	75 g L^{-1} dH_2O	**1 mL**	88uM	$NaNO_3$	75 g L^{-1} dH_2O	**1 mL**	88uM
$NaH_2PO_4.H_20$	5 g L^{-1} dH_2O	**1 mL**	36.2uM	$NaH_2PO_4.$ H_20	5 g L^{-1} dH_2O	**1 mL**	36.2uM	$NaH_2PO_4.$ H_20	5 g L^{-1} dH_2O	**1 mL**	36.2uM	$NaH_2PO_4.$ H_20	5 g L^{-1} dH_2O	**0.071mL**	2.5uM
$Na_2SiO_3.9H_2O$	30 g L^{-1} dH_2O	**1mL**	106uM	$Na_2SiO_3.9$ H_2O	30 g L^{-1} dH_2O	**1mL**	106uM	$Na_2SiO_3.9$ H_2O	30 g L^{-1} dH_2O	**0.333mL**	37.5uM	$Na_2SiO_3.9$ H_2O	30 g L^{-1} dH_2O	**1mL**	106uM

5.) Filter sterilization is recommended. (See Media Prep folder for more information if needed.)
6.) Label the media with [ESAW] [condition (f/2, N-, Si- or P-)] [date]
7.) Let media sit overnight before use. The final pH is 8.2.

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