

*ESAW Medium (Enriched Seawater, Artificial Water) (8.2 pH)*

**Materials**

- 2 500 mL mixing jars (or flasks)
- 1 L Graduated cylinder
  - Filled with 1 L distilled water (dH<sub>2</sub>O, DI water)
- 1 L Bottle
- 2 L sterile Erlenmeyer flask (for control purposes)
- Filter top
- Labeling tape and Pen
- 2 Stirring Utensils
- Micropipettes and tips: p1000, p200
- .01 g – 300 g capable Scale
- Weigh boats and spatulas

**Salt I (Anhydrous Salts)**

- 21.194 g NaCl (3.63 x 10<sup>-1</sup> M)
- 3.550 g Na<sub>2</sub>SO<sub>4</sub> (2.50 x 10<sup>-2</sup> M)
- 0.599 g KCl (8.03 x 10<sup>-3</sup> M)
- 0.174 g NaHCO<sub>3</sub> (2.07 x 10<sup>-3</sup> M)

**Salt II (Hydrated Salts)**

- 9.592 g MgCl<sub>2</sub>·6H<sub>2</sub>O (4.71 x 10<sup>-2</sup> M)
- 1.344 g CaCl<sub>2</sub>·2H<sub>2</sub>O (9.14 x 10<sup>-3</sup> M)
- 1 mL 1 M Trace Metals
- 1 mL 1 M Vitamins

**Major Nutrients for full “f/2” Media (Regular)**

- 1 mL 75 g L<sup>-1</sup> dH<sub>2</sub>O NaNO<sub>3</sub> (88 μM)
- 1 mL 5 g L<sup>-1</sup> dH<sub>2</sub>O NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (36.2 μM)
- 1 mL 30 g L<sup>-1</sup> dH<sub>2</sub>O Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O (106 μM)

**Major Nutrients for Silica limiting Media**

- 1 mL 75 g L<sup>-1</sup> dH<sub>2</sub>O NaNO<sub>3</sub> (88 μM)
- 1 mL 5 g L<sup>-1</sup> dH<sub>2</sub>O NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (36.2 μM)
- ⅓ mL 30 g L<sup>-1</sup> dH<sub>2</sub>O Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O (37.5 μM)

**Salt I (Stock Salts)**

- 1 mL 1 M KBr (7.25 x 10<sup>-4</sup> M)
- 1 mL 1 M H<sub>3</sub>BO<sub>3</sub> (3.72 x 10<sup>-4</sup> M)
- 1 mL 1 M NaF (6.67 x 10<sup>-5</sup> M)

**Salt II (Stock Salts)**

- 1 mL 1 M SrCl<sub>2</sub>·6H<sub>2</sub>O (8.18 x 10<sup>-5</sup> M)

**Major Nutrients for Nitrate limiting Media**

- 0.5 mL 75 g L<sup>-1</sup> dH<sub>2</sub>O NaNO<sub>3</sub> (44 μM)
- 1 mL 5 g L<sup>-1</sup> dH<sub>2</sub>O NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (36.2 μM)
- 1 mL 30 g L<sup>-1</sup> dH<sub>2</sub>O Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O (106 μM)

**Major Nutrients for Phosphate limiting Media**

- 1 mL 75 g L<sup>-1</sup> dH<sub>2</sub>O NaNO<sub>3</sub> (88 μM)
- 0.071 mL 5 g L<sup>-1</sup> dH<sub>2</sub>O NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (2.5 μM)
- 1 mL 30 g L<sup>-1</sup> dH<sub>2</sub>O Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O (106 μM)

**Procedure**

1. Dissolve Salt I (Anhydrous Salts) ingredients in <600 mL of distilled water
  - a. Don't forget to reset the scale to zero between each weigh
2. Dissolve Salt I (Stock Salts) into the same flask
3. Dissolve Salt II (hydrated salts) into a new flask with <400mL of water
4. Dissolve Salt II (Stock Salts) into the same flask
5. Combine dissolved the two mixtures in a 2 L flask
6. Add Trace Metals and Vitamins
7. Add Major Nutrients based on the type of Media made
  - a. Make sure everything is dissolved before continuing
8. Add filter top to 1 L bottles and pour mixture through
9. Add distilled water until the bottle is filled to 1 L
10. Let media sit overnight before use

Note: Remember to label the bottle and only open it in sterile areas