**Minimal Basal Medium**

Moran Lab, updated 25 July 2022

Prepare and combine (details below):

1. Artificial Seawater (ASW) Solution (final medium should be 20 ppt)
2. FeEDTA Stock (100 µM in final medium)
3. 250 mL Basal Medium per liter
4. 1 ml Vitamin Supplement per liter
5. Add dH2O to reach 1 liter total medium

* Filter-sterilize prepared medium through 0.2-micron filter into sterile bottle

This medium does not contain an organic carbon source. We add organics to 12 mM C-equivalents for our experiments (e.g. 2 mM glucose (C6H12O6) = 12 mM C-equivalent)

**1) ASW (20 ppt)**

Prepare Solution 1 and Solution 2 each in 500 mL dH2O separately and combine sterilely after autoclaving to avoid precipitation. Below is the 1X (20 ppt) solution. Prepare at 2X (40 ppt) and use 500 mL per liter in the above recipe (or up to 10X and dilute accordingly). Can keep S1 and S2 separate until adding to final medium if precipitation is a problem.

Solution 1 (500 ml):

|  |  |
| --- | --- |
| **Compound** | **g/L** |
| NaCl | 11.58 |
| Na2SO4 | 1.94 |
| KCl | 0.328 |
| Kbr | 0.0472 |
| H3BO3 | 0.01252 |
| NaF | 0.001504 |
| NaHCO3 | 0.0948 |

Solution 2 (500 ml):

|  |  |
| --- | --- |
| **Compound** | **g/L** |
| MgCl2·6H2O | 5.24 |
| CaCl2·2H2O | 0.734 |
| SrCl2·6H2O | 0.012 |

**2) FeEDTA:** make a 20 mM stock solution, filter sterilize (do not autoclave), and add to 100 µM final concentration.

**Basal Medium and Vitamin Supplement components**

1. **Basal Medium**

* 150 ml 1 M Tris HCL pH 7.5
* 0.34 g NH4Cl
* 182.98 mg K2HPO4
* 375 ml dH2O

*Note: Use 250 mL per L of this solution*

1. **Vitamin Supplement (for minimal basal medium)**

* 100 ml dH2O
* 2 mg biotin
* 2 mg folic acid
* 10 mg pyridoxine-HCl
* 5 mg riboflavin
* 5 mg thiamine
* 5 mg nicotinic acid
* 5 mg pantothenic acid
* 0.1 mg cyanocobalamin
* 5 mg *p*-aminobenzoic acid

*Note: Do not autoclave vitamin mix.*

*Note: Save extra vitamin stocks in 1 mL aliquots at –20˚C.*