**Marine Basal Medium**

Per 1 L:

1. 250 mL Basal Medium
2. 50 ml FeEDTA Stock (1.36 mM)
3. 699 ml Sea Salt Solution
4. 1 ml Vitamin Supplement (see next page for recipe)
5. Make and autoclave solutions 1,2 and 3 separately (never autoclave vitamin supplement or carbon source)(see recipes below). Also autoclave extra, empty bottles and/or graduated cylinders that will be needed for mixing afterwards
6. Let autoclaved components cool to at least ~50 Celsius before combining
7. Mix components in appropriate proportions, as outlined above, using sterile technique. Remember to add carbon source, if needed, before mixing (see note below).

*Note:*

* *You will have leftover FeEDTA and Basal Medium stock solutions that you can save for later*
* *Carbon source: to add carbon to the medium, autoclave the Sea Salt Solution and let cool to at least 50 Celsius as stated above. Take out however many ml are necessary to dissolve the carbon source you want to add. Dissolve carbon in this aliquot of Sea Salt Solution. Filter (0.2 µm filter) the carbon solution back into the stock Sea Salt Solution.*
  + *When using DMSP as the sole carbon source, we use 1mM (or 0.134 g/L) of DMSP*

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**[See next page for component recipes]**

**Marine Basal Medium (cont.)**

1. **Sea Salt Solution**

* 699 ml dH2O
* 20 g Sigma Sea Salts
* 15g Bacto Agar (only if making plates)
* (Carbon source added here post-autoclaving – see note above)

1. **FeEDTA Stock**

* 50 mg FeEDTA
* 100 ml H2O

1. **Basal Medium**

* 150 ml 1 M Tris HCL pH 7.5 (growth is very pH sensitive so you need to make sure this is accurate)(increase or decrease the pH solution as needed by adding small, measured amounts of concentrated NaOH and/or HCl solutions)
* 0.34 g NH4Cl
* 182.98 mg K2HPO4
* 375 ml dH2O

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

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Filter-sterilie (0.2 µm filter) and store at -20 Celsius. It is a good idea to make small aliquots to store for future use so you can just pull out a tube as needed (I make mine up in 1 ml aliquots so I can just pull out one tube to use for each 1 L medium) so as to avoid multiple freeze-thaws and to avoid contaminating the stock.

*Note:*

* *You can increase or decrease the amounts proportionally to make your stocks larger or smaller as needed, to either make more medium at a time or to avoid excess stocks*
* *I would suggest for each component to add reagents individually and slow enough to allow for complete dissolution*