Media for Symbiont Culturing

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Uses Bigelow f/2 kit

<https://ncma.bigelow.org/mkf250l#.XPaGiNNKjSw>

# f/2 culture media

1. 950 mL artificial seawater (ASW)

+ 1 mL NaNO3 stock

+ 1 mL NaH2PO4 stock

+ 1 mL trace metal solution

1. Fill to 1L with ASW
2. Autoclave liquid cycle.
3. + 0.5 mL vitamin solution to cooled liquid
4. Store at room temperature.

# f/2 + 5 mg/mL GeO2 culture media

*Germanium oxide (GeO2) is useful for limiting diatoms because it inhibits silica biomineralization.*

**Option 1**: Add 5 mg GeO2 to each 1 L of f/2 culture media if you have a balance that is sensitive enough to weigh 5 mg.

**Option 2**: Make a 500 mg/L GeO2 stock solution and add as a component to fresh f/2 media.

1. Dissolve 25 mg of GeO2 in 50 mL ASW.

Stock concentration = 25 mg / 50 mL = 50 mg / 100 mL = 500 mg / 1000 mL = 500 mg/L (100X more concentrated than you want it to be in the final solution).

1. Make f/2 + GeO2

950 mL artificial seawater (ASW)

+ 1 mL NaNO3 stock

+ 1 mL NaH2PO4 stock

+ 1 mL trace metal solution

+ 10 mL 500 mg/L GeO2 stock

1. Fill to 1L with ASW
2. Autoclave liquid cycle.
3. + 0.5 mL vitamin solution to cooled liquid
4. Store at room temperature.

# Agar media for plates

1. Transfer 300 mL of f/2 or f/2+GeO2 to a flask.
2. Add 3 g agar (e.g. Sigma 1296).

### To melt the agar…

Option 1 (if not sterile):

Add a stir bar to the flask and autoclave using a liquid cycle.

Allow to cool while stirring until the liquid is safe to handle, but before gel begins to form.

Option 2 (if already sterile):

Microwave the flask in 1 minute intervals until the agar melts.

Swirl the flask under running water until the liquid is cool enough to handle safely.

### Pouring plates....

It’s a good idea to label the contents of your plates before you pour them. You can stack a sleeve of plates and quickly mark lines down the side to label them.

I suggest a labeling convention:

One black line = f/2 only

Two black lines = f/2 + GeO2

1. Set the bottom (smaller) half of petri dishes on a clean surface that will not be disturbed for the next half hour or so.
2. Quickly, but gently, pour equal portions of molten agar into each dish. You are aiming for ~30 mL. Use a serological pipet for consistency.

Tip: Pour slowly until the dish is ~75% full and allow the remainder of the dish to fill as the liquid spreads.

1. Allow the plates to solidify with their lids off (this is why it’s important that the area is clean and undisturbed… you don’t want someone to come by and contaminate your plates!)
2. Add the lids to the petri dish and stack them to return them to their sleeve.
3. Tape the sleeve closed and add a tape label to the sleeve that includes the contents with your initials and date.
4. Store the sleeve of dishes \***upside down**\* in the 4°C fridge.