# Advanced Preparation (**HALF BATCH OF OPA**)

* **Note that many of the materials that you need for this procedure require ordering from science stores (e.g. chemicals, glassfiber filters etc.) so make sure you look at the whole protocol at least 2 weeks before sample collection**

## Working Reagent (**half batch**)

(Prepare least 24 HOURS in advance)

### Materials needed

*-****1/2 gallon*** *jug (fully opaque/wrapped to darken)*

*- scoopula*

*- stir stick*

*- 1 small closed bottle for OPA mixture*

*- 1 small glass beaker (lay mouth down on paper towel)*

*- 3 L DW*

*- 50 ml ethanol*

*-OPA (2g)*

*- sodium sulphite (1g)*

*- sodium tetraborate (40 g)*

*- dark room (as dark as possible)*

*- red head lamp or red light to provide some visibility*

*- small graduated cylinder*

*- large 1 L graduated cylinder*

*- microbalance scale*

1. Make sure everything has been acid washed
2. Wear nitrile gloves, labcoat and goggles
3. Prepare a dark location for mixing (i.e. a large tarp fort in the field ☺/dark room)

### Borate buffer (half batch)

* + Cover a large 1/2 gallon nalgene in dark plastic/ ducktape
  + Add **1L DW** (get straight from the distiller)
  + Add **40 g of sodium tetraborate**
  + Using microscale measure out 40 g of sodium tetraborate
    1. Clean scoop with kimwipe and 80% acytl alcohol (let alcohol air dry off)
    2. Tare plastic weigh boat
    3. Scoop small amounts of sodium tetraborate out until you have 40 g (if you scoop too much DO NOT add it back to the bottle)
  + Put on cap and shake vigorously to mix

### Sodium Sulphite solution (half batch)

* Use small glass beaker
* Add **1 g of sodium sulphite** to **125 ml DW**
* Stir with stir stick until dissolved
* Measure out **5 ml** and add to borate solution (use glass pipette or 10 ml pipettor if you have one)

### OPA (half batch)

* Do this in the dark as OPA is light sensitive (in a box)
* Measure out **2 g of OPA (be very very careful!)**
* Add to **50 ml of ethanol** (measure out ethanol using graduated cylinder) in nalgene bottle
* Cap and Shake to mix!
* Pour into borate solution
* Shake the whole solution vigorously for several minutes and allow to age for 24 hours before use

## Ammonium stock solution (Prepare day before sample collection)

To calibrate the fluorometer each time it is used we need to run a standard curve of different ammonium concentrations that we can then compare our sample readings too.

In the field I will make a large stock solution of 10000 uM that I then use to make daily dilutions of 200um stock solution. Other concentrations can be used but these were the easiest to make given the materials and chemical concentrations I had, and the concentrations that are appropriate to use for a standard curve given the ammonium levels that we would expect to find in our fish samples.

### Materials needed

*-2, 1L wide mouth Nalgene bottles*

*-0.661 g Ammonium sulphate*

*-Microbalance scale*

*-filter seawater (from sample collection location)*

*- small weight boat*

*- scoopula*

*- ethanol and kimwipes for cleaning equipment*

*- graduate cylinder (100 ml)*

*- 10 ml pipettor*

### 10,000 uM solution

* Using a clean scoopula measure out 0.661 g of Ammonium sulphate
* Add 1000 ml of DW to clean Nalgene using 100 ml graduate cylinder (don’t use a larger cylinder as you want the measurements to be as accurate as possible)
* Add 0.661 g of ammonium sulphtate to Nalgene, cap tightly, and shake to dissolve
* Label bottle well and refrain from opening this solution near any samples as any contamination will skew sample readings given the strong concentration on NH4 in this bottle

### 200 uM daily solution

* Using the 10 mL pipettor, add 10 ml of the 10,000 uM stock solution to a clean wide mouth nalgene
* Add 490 ml of DW to Nalgene, cap, and shake well