



## Protocol for Determination of Chlorophyll by Fluorometry

### Laboratory Equipment and Materials:

96% ethanol  
15ml centrifuge tubes  
5ml pipet  
25ul pipet  
10% HCl  
Quartz test tubes  
\*Turner TD-700 fluorometer

### EXTRACTION

1. Transfer filter with chlorophyll sample into a clean 15mL centrifuge tube
2. Add 10mL of 96% ethanol to each centrifuge tube
3. Refrigerate for 24hr in dark container

### FLUOROMETER PREPARATION

1. Let fluorometer warm up for at least 30min and make sure sample cell is in the chlorophyll position (“A” should read right-side-up)
2. Invert the cold extracted samples 3X to remove any stratification of concentrations
3. Let extracted samples sit in a dark container for ~1hr to allow them to come to room temperature to let any particulates resettle

### FLUORESCENCE MEASUREMENT

1. Rinse quartz test tube 3X with 96% ethanol (*Thorough rinsing of the test tube between samples is critical to prevent premature acidification of next sample.*)
2. Pipet 3mL of sample from centrifuge tube to rinsed test tube
3. Place test tube in sample cell, close lid, allow reading to settle, and record “FU”
4. If FU is greater than 500, dilute the sample in the test tube with 3mL of 96% ethanol, inverting with clean parafilm and read again
5. Rinse pipet with 96 % ethanol

\*Our TD-700 is configured for the non-acidification method, which measures active chlorophyll a, i.e. pheophytin correction is not necessary.