

## DISCRETE CHLOROPHYLL FILTRATION PROCEDURE

### GET IT TOGETHER:

- square 125ml PP bottle or square 250ml clear PC bottle for measuring sample volume
- 0.45um 47mm HAWP filters
- magnetic filtration towers
- forceps
- clean glass scintillation vials with inner-poly lids not inner-foil lids - acetone reacts with foil
- Sharpie

### SAMPLE COLLECTION:

- Use the square 125ml **PP** bottle to measure sample.
- Rinse bottle and cap 3 times with water from the 4L carboy.
- Fill bottle to brim and cap – this volume is 188ml. Filter samples immediately.
- If Chl concentration is known to be low, use 250ml **PC** bottle - brim volume is 330ml.

### SAMPLE FILTERING:

#### Blank Preparation:

- Place a blank filter (HAWP 0.45um) in a glass scintillation vial. Label the lid with the date and “BLANK”. Do a blank for every box of filters.

#### Sample Preparation:

- Be sure 47mm magnetic towers have been rinsed with Nanopure and are clean.
- Place filter on base with tweezers; do not use fingers, seat magnetic funnel, do not crease filter.
- Shake/swirl sample container, then pour seawater sample into filter funnel.
- Turn vacuum pump on; vacuum should be around 10 mm Hg. Pump only until filter dries, do not maintain vacuum on dry filters.
- Carefully fold the filter in thirds with tweezers and place in glass scintillation vial, cap tightly
- Label cap of scintillation vial as per labeling protocol. Keep samples in the dark until all samples have been filtered. If samples are not to be extracted immediately, place tray in freezer and keep filters frozen until they can be processed (up to 3 months).
- Add 10ml of 90% acetone to all vials (don't forget to calibrate dispenser!), CAP TIGHTLY, shake to dissolve filter. Cover with foil and place in freezer, KEEP UPRIGHT, samples will leak if tipped over. The acetone extraction volume can be altered to alleviate the need for dilutions with a pipetter during sample reading.
- Extract in the freezer for 24-48 hours. Note time on log.
- Shake the samples vigorously once during this period. Do not shake 2 hours prior to reading – particulate matter will be resuspended in sample volume.
- Instructions for using the 10-AU digital fluorometer are in the “Chl Reading” protocol.

### PREPARE REAGENTS

#### 90% acetone - use volumetric flasks for preparation to ensure volume accuracy

- Measure 100ml of Nanopure into a 1000ml volumetric flask.
- Fill flask to 1000ml volumetric mark with acetone - use a disposable pipette if necessary.
- Cover with stopper or parafilm and invert several times.
- Make sure that volume of liquid is at correct mark on neck of flask.
- Pour contents of flask into bottle marked 90% ACETONE.

#### 10% HCl

- Measure 10ml HCl into a 100ml volumetric flask.
- Fill to 100ml volumetric mark with Nanopure.

### THINGS TO REMEMBER:

- 1) Do not pre-fill glass scintillation vials with 90% acetone, acetone evaporates!
- 2) Calibrate acetone dispenser every 15-20 vials.
- 3) Record sample information on log sheet and assign consecutive numbers to samples/blanks prior to processing. Using consecutive numbers makes labelling the vials easier.
- 4) Be sure everyone is following same procedure.

### LOG EVERYTHING:

Record ALL information to log sheet. Make a note on the log sheet if any problems were encountered during filtration; i.e. a torn filter, leaking funnel - ANYTHING. Fill out entire log sheet – it may take a little time now, but it's worth it in the long run! Check your labelling.

### CLEAN EVERYTHING:

Rinse all sample bottles 3X with Nanopure and shake out excess water. Rinse all filter towers and frits 3x with Nanopure.