

Chlorophyll a extraction following EPA Method 445.0 rev. 1.2

Materials Needed:

- ☐ sample filters
- ☐ 2 filter forceps
- ☐ mortar and pestle
- ☐ 1 mL pipet
- ☐ 1 mL pipet tips
- ☐ 100 uL pipet
- ☐ 100 uL pipet tips
- ☐ 90% acetone solution
- ☐ squirt bottle with 90% acetone
- ☐ kim wipes
- ☐ 15 ml screw-cap centrifuge tube with foil covers
- ☐ 0.1 N HCl

Procedure:

1. Remove filters from the -80 freezer, keep in dark. Label the centrifuge tubes with sample information on sides and cap. Work with one filter at a time, keep other filters in freezer in 4109.
2. Rip filter into small pieces and put in mortar.
3. Use 1 mL pipet to add 4 mL of aqueous acetone solution to mortar.
4. Grind the filter for about 1 minute, until it has been converted into a slurry. Note: Do not overheat the sample.
5. Use forceps to scrape slurry into a 15-mL screw-cap centrifuge tube.
6. Before placing another filter in the mortar, use the 1 mL pipet to add 6 mL of acetone solution to the mortar. Use the forceps to scrape around the mortar and get bits of filter off the edges and into the acetone solution. Use the pipet to transfer the acetone solution and filter bits to centrifuge tube. Use forceps to scrape and last bits of filter into the centrifuge tube.
7. Clean the mortar, pestle, and forceps with DI water and acetone solution from squirt bottle. Do a final rinse with squirt bottle. Dry with kim wipes.
8. ***Cap the tube and shake it vigorously.*** Place in the refrigerator in 4109 before proceeding to the next filter extraction. Record the time you place the tube in the refrigerator.
9. Repeat steps 2-9 for each filter.
10. Leave tubes to steep in the dark at 4 C for 2 – 24 hours. ***Shake the tubes at least once during the steeping period.***
11. After steeping is complete, ***shake the tubes vigorously*** and centrifuge samples (in Chaparral) for 5 min at 1000 g and at 4 C. Bring extra 15 mL tubes to balance centrifuge. Leave samples in dark on bench top for 30 min so they can reach ambient temperature. *****Note: sometimes centrifuge lid won't open/gets stuck when you press the button. There is a power switch on the back left side, try closing lid completely and turning off and on again.***

Chlorophyll a measurement

12. Turn on fluorometer, push "Chl-A" button, press "OK" to confirm, press "Calibrate", press "Use stored calibration", select "ST9-4-19", press "Select".
13. Pipet 2 mL of the supernatant of the extracted sample into a sample cuvette. Wear gloves and wipe sides of cuvette with kim wipe. ***Be very careful not to pipet any bits of filter into the cuvette. The longer the tubes sit after being centrifuged, the more likely some of the filter will get into the supernatant.***
14. Insert a sample and press the "Measure Fluorescence" button.
15. Enter the volume filtered (from yellow Chl a notebook) and extraction volume (2 mL) when prompted.
16. Press "OK" to measure the fluorescence before acidification.
17. Acidify the sample by adding 60 uL of 0.1 N HCl, wait 90 seconds while gently inverting the vial about 10 times and insert into the instrument (with gloves on).
18. Press "OK" to measure the fluorescence after acidification.
19. The corrected chlorophyll a and pheophytin a concentrations in the whole water sample, according to EPA Method 445.0 equations will be displayed on the home screen. Record these values in lab notebook.
20. Repeat steps 13-19 a second time before moving on to next sample.