

Required Materials

- Trilogy Laboratory Fluorometer (P/N: 7200-000)
- Chlorophyll a Acidification Module (P/N: 7200-040)
- 12x75mm round bottom glass test tubes (P/N: 10-029A)
- 12mm round vial adaptor (included with Trilogy accessories) (P/N: 016-0810)
- Liquid Chlorophyll a Standard – available from Turner Designs (P/N: 10-850)

Optional Materials

- Solid Secondary Standard (refer to User Manual for instruction) (P/N: 8000-952)

Procedure

1. Lift the Trilogy Laboratory Fluorometer's lid.
2. Snap the Chlorophyll a Acidification Module into the Trilogy Laboratory Fluorometer.
3. Insert the 12mm round vial adapter into the module.
4. Close the lid and turn on the fluorometer.
5. Press the "Chl-A" button on the touch screen to choose the acidification module.
6. Press "OK" after confirming the correct module is snapped in to go to the home screen.
7. From the home screen choose the "Calibrate" button.
8. Select "Run New Calibration" and choose "µg/L".
9. Insert a 90% acetone blank solution and press "OK". Wait for measuring to complete.
10. Remove the blank and insert a standard of known concentration. *(Note: If you will enter more than one standard you must enter them in order of increasing concentration and we recommend the first standard entered to be no less than 1µg/L).*
11. Enter the concentration using the keypad on the touch screen and press "OK".
12. Press "OK" to measure before acidification. Wait for measuring to complete.
13. Acidify the standard by adding 0.03 mL of 0.1 N HCl for every mL of standard, wait 90 seconds while gently inverting the vial about 10 times and insert into the instrument.
14. Press "OK" to measure after acidification. Wait for measuring to complete.
15. Acid Ratio will be displayed on the screen, press "OK" to proceed. *(Note: An acceptable acid ratio is >1.7; An optimal acid ratio is 1.9-2.0).*
If your acid ratio is <1.7, then you can try one of the following three steps to obtain an acceptable acid ratio:
 - a. Remake the standard concentration used as your first standard in the calibration
 - b. Use a higher standard concentration as your first standard in the calibration (up to 5 µg/L)
 - c. Obtain a new parent standard to remake all of your standard concentrations in the calibration
16. You may enter up to 5 standards increasing in concentration, or proceed with current calibration. *(Note: Acid ratio is only calculated for the first standard entered.)*
17. Save the calibration.
18. Extract all filtered samples as described in [EPA Method 445.0](#), rev. 1.2 (September 1997), section 11.1.
19. Insert a sample and press the "Measure Fluorescence" button.

20. Enter the volume filtered and extraction volume when prompted.
21. Press “OK” to measure the fluorescence before acidification.
22. Acidify the sample by adding 0.03 mL of 0.1 N HCl for every mL of sample, wait 90 seconds while gently inverting the vial about 10 times and insert into the instrument.
23. Press “OK” to measure the fluorescence after acidification.
24. The corrected chlorophyll *a* and pheophytin *a* concentrations in the whole water sample, according to [EPA Method 445.0](#) equations in sections 12.2 and 12.3, will be displayed on the home screen.