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Calibrating 10-AU fluorometer to quantify extracted chl-a

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1 Works for me dx.doi.org/10.17504/protocols.io.2ubgesn

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ABSTRACT

This method is for use for calibrating the 10-AU flurometer using chl-a extracts. The method also provides guidelines for extracting chl-a and calculating absorbance using a spectrophotometer, making dilutions of extracts that can be used to create differetn chl-a concentrations

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

S.W. Jeffrey, G.F. Humphrey, New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton, Biochemie und Physiologie der Pflanzen, Volume 167, Issue 2, 1975, Pages 191-194, ISSN 0015-3796, https://doi.org/10.1016/S0015-3796(17)30778-3.

Shelby's Notes for Fluorometer.pdf

Sam's Notes for Fluorometer.pdf

MATERIALS TEXT

97% acetone
spinach or green plant as source for chlorophyll extracts

SAFETY WARNINGS

97% acetone is flammable.

See MSDS safety and hazard warnings.

BEFORE STARTING

- *Fixed Application* can be found under "general tests" on the spectrophotometer.
- Make sure the cuvette is in the correct position for measurements. Misplacement may cause an error such as a negative absorbance measurement.
- Spinach only has chlorophyll a. If using an organism that has more pigments, will have to subtract those from total absorbance (i.e. chlorophyll a - chlorophyll c).

Chlorophyll Extraction

1 Extract chlorophyll from **1 g** of gound spinach with **5 ml** of 90% acetone at **-20 °C** overnight.

Spectrophotometry

2 Use the Biomate 5 spectrophotometer to measure the adsorption of extracted chlorophyll by adding **1 ml** to a quartz cuvette.

- Make sure the cuvette is in the correct position in the machine.
- Zero the machine by blanking with **1 ml** of 90% acetone.
- Measure "multi λ" with λ1 = 664 nm and λ2 = 647 nm. (Jeffrey 1975)

3 Based on the absorbance value from **go to step #2** : dilute the chlorophyll extract so that the absorbance is in optimum range for the fluorometer. Optimum rangle is Abs λ1 and λ2= 0.3-0.85.

Example:
Undiluted
Abs λ1= 1.88
Abs λ2= 1.324

20-fold dilution:
Abs λ1= 0.736
Abs λ2= 0.479

Fluorometry

4 Calculate the concentration of diluted chlorophyll extract using the absorbance values from the spectrophotometer and the equation:

concentration of chlorophyll a = 11.93(λ1) - 1.93(λ2)

(Jeffrey 1975)

If using an organism that has more pigments, will have to subtract those from total absorbance (i.e. chlorophyll a - chlorophyll c). The spinach we used did not require subtraction of chlorophyll c.

Example:

11.93(0.736) - 1.93(0.479) = 7.85601 ug/ml

Calibrate Fluorometer

5 Convert answer from **go to step #4** to ug/L, because this is the readout for the fluorometer.

6 Turn on the 10-AU Fluorometer and allow the instrument to warm up for at least 30 minutes.

7 Create solutions of varying concentrations to test the fluorometer readings. Use C1V1=C2V2 to make solutions at different concentrations based on expected field concentrations, for example: 20, 10, 5, 1 and 0.5 ug/L. Also, create an acetone blank (with no chlorophyll).

For example:
7,856 ug/L (C1)
For a 5 ug/L concentration (C2) in 7 mls acetone (V2)

(7,856 ug/L)(V1)=(5 ug/L)(7 ml)
V1= 4.45519 ug

8 Use the concentration most expected in the field to set as the solid standard.

Chlorophyll (ug/l)	Rhodamine WT (ppb)	Range	% FS (+/-5)
240 - 180	100 - 75	High	80%
180 - 160	75 - 65	High	70%
160 - 130	65 - 55	High	60%
130 - 110	55 - 45	High	50%
110 - 80	45 - 35	High	40%
80 - 60	35 - 25	High	30%
60 - 40	25 - 15	High	20%
24 - 18	10 - 7.5	Medium	80%
18 - 16	7.5 - 6.5	Medium	70%
16 - 13	6.5 - 5.5	Medium	60%
13 - 11	5.5 - 4.5	Medium	50%
11 - 8.0	4.5 - 3.5	Medium	40%
8.0 - 6.0	3.5 - 2.5	Medium	30%
6.0 - 4.0	2.5 - 1.5	Medium	20%
2.4 - 1.8	1.0 - 0.75	Low	80%
1.8 - 1.6	0.75 - 0.65	Low	70%
1.6 - 1.3	0.65 - 0.55	Low	60%
1.3 - 1.1	0.55 - 0.45	Low	50%
1.1 - 0.8	0.45 - 0.35	Low	40%
0.8 - 0.6	0.35 - 0.25	Low	30%
0.6 - 0.4	0.25 - 0.15	Low	20%

Table 1 is an example of how to set the scale for a given linear range of the 10-AU.

For this example we are using a 20µg/L standard.

Table 1 is an example of how to set the sensitivity scale for the 10-AU. Use the table to determine the appropriate range and %FS for the calibration standard.

9 Clear past calibrations on screen #2.6.

10 Access screen #2.43 and set range control to "manual" and range to the appropriate level for the standard chosen. For example, we set range control to "Man" and "Med."

11 Access screen #3.2. Loosen the sensitivity locking screw using an allen wrench. (The sensitivity lock is the small screw to the left of the number pad.)

12 Insert and cover your selected standard concentration. Use a coin or tool to slowly turn the sensitivity knob (located to the right of the number pad, under the power button) to adjust the %FS to the value appropriate for the standard concentration according to Table 1 in go to step #8. A deviation of 5% less than or greater than the table is acceptable. When %FS reaches the designated value, tighten the sensitivity lock.

At this point, sensitivity is set.

Example:
For 5 ug/L.
FS = 21%

13 Access screen #2.43 again, and set range control to "auto."

14 Access screen #2.1. Insert and cover the acetone blank created in go to step #7. Press "1" to Run Blank. Wait for the reading to stabilize for about 8 seconds, and then press "0." The instrument will take 15 seconds to complete blanking.

At this point, blank is set.

15 Calibrate with your standard:

15.1 Access screen #2.2 and input the actual concentration of your standard. Return to screen #2.0

15.2 Insert and cover the standard.

15.3 Run standard by pressing "3." Wait about 8 seconds for reading to stabilize.

15.4 Press the "*" to set the calibration point. After about 15 seconds, a message with appear that says "Finished".

16 Test the 10-AU fluorometer using the concetrations created in go to step #7 to assure appropriate readout.

Example:

Expected	Observed
5 ug/L	5.01 ug/L
1 ug/L	1.11 ug/L
Blank	0.0 ug/L