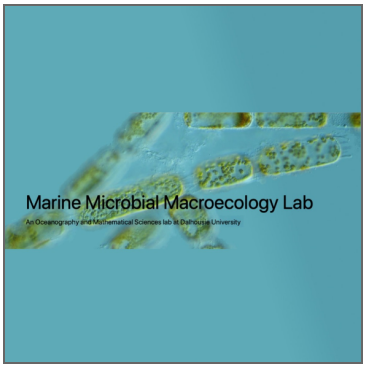

VERSION 2

MAR 27, 2024

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Created: Jul 21, 2023

Last Modified: Mar 27, 2024

Measure chlorophyll-a and pheophytin-a by Turner Designs V.2

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ABSTRACT

Here we describe a protocol for measuring chlorophyll-a and pheophytin-a from microalgae by using Turner Designs (10-AU)

CITATIONS

STEP 3.1

Shoaf WT, Lium BW. Improved extraction of chl and b from algae using dimethyl sulfoxide https://doi.org/10.4319/lo.1976.21.6.0926

GUIDELINES

1. The entire procedure should be carried out as much as possible in subdued light (Green) to prevent photodecomposition.
2. All glassware should be clean and acid free to prevent chlorophyll-a degradation.
3. Waste disposal:
   Follow all laboratory waste disposal guidelines regarding the disposal of acetone, DMSO solutions.

PROTOCOL integer ID: 85320

Funders Acknowledgement:
Simons Foundation
Grant ID: 549937
Simons Foundation
Grant ID: 723789

PROTOCOL MATERIALS

Chlorophyll-a standard **Catalog #** E-541-0-850 Step 26.1

Magnesium carbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M7179-500G
Step 1.1

Acetone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**270725-4X2L Step 2.2

DMSO (CAS grade) Step 3.1

12 N Hydrochloric acid Step 4.1

Chlorophyll-a from spinach **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C5753-1MG
Step 5.1

Prepare reagent

1 Saturated magnesium carbonate solution

1.1 Add 10 grams magnesium carbonate to 1000 mL of MilliQ water.

Magnesium carbonate **VWR International Catalog #**M7179-500G

1.2 Settle the solution for a minimum of 24 hours.

2 90% buffered acetone

2.1 100 mL clear “powder free” magnesium carbonate solution

2.2 900 mL HPLC grade acetone

Acetone **VWR International Catalog #**270725-4X2L

2.3 Mix in an amber reagent bottle.

3 Acetone/DMSO extraction solvent

3.1 Mix three parts 90% acetone and two parts of HPLC grade DMSO (Shoaf and Lium 1975)

DMSO (CAS grade) **VWR International**

CITATION

Shoaf WT, Lium BW. Improved extraction of chl and b from algae using dimethyl sulfoxide. Limnology and Oceanography.

LINK

https://doi.org/10.4319/lo.1976.21.6.0926

4 0.1 N HCl

4.1 Dissolve one part of [M] 12 N HCl in 119 parts of MilliQ water

12 N Hydrochloric acid **VWR International**

Prepare Chlorophyll-a standard

5 Primary stock: ≅10 mg/L

5.1 Dissolve 1 mg Chlorophyll-a in a 100 mL glass volumetric flask by 90% acetone, top to 100 mL.

Chlorophyll-a from spinach **VWR International Catalog #**C5753-1MG

5.2 Fill cuvette and place it in udrop plate, measure absorbance at 664 nm

Equipment

NAME	TYPE	BRAND	SKU
Spectrophotometer Cells	12.5W x 12.5L x 48H mm (pathlength 10 mm)	VWR® Spectrosil	414004-078

Equipment

NAME	BRAND	SKU
µDrop™ Plates	Thermo Scientific	N12391

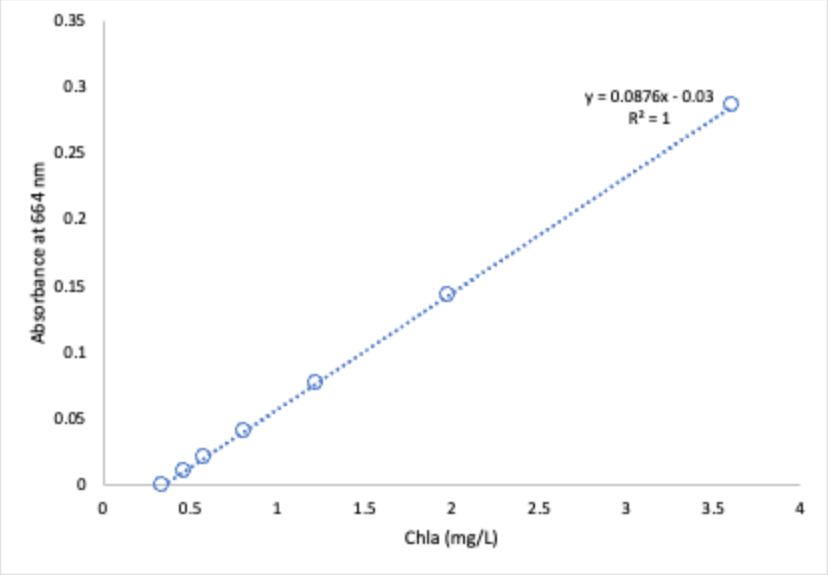
Equipment

Varioskan LUX Multimode Microplate Reader	NAME
Thermo Fisher	BRAND
VL0L00D0	SKU

5.3 Chlorophyll-a_mg/L = 11.42 X Absorbance at 664 nm, where 11.42 is the extinction coefficient of chlorophyll-a at 664 nm.

Note

Detection limit of spectrophotometrical measurement is about 350 ug/L



5.4 Label Amber vials with the actual concentration of chlorophyll-a stock solution

Equipment

Storage Vials and Closures	NAME
12 mL amber	TYPE
Thermo Scientific	BRAND
B7800-12A	SKU
VWR 66030-686	SPECIFICATIONS

5.5 Aliquote 5 mL to Amber vial with glass serological pipet (5 mL)

5.6 Store at -80 °C to -50 °C

Note

Flashpoint of acetone is -20 °C

6 Second calibration standard: (≅400 ug/L)

6.1 Warm up primary stock to room temperature and transfer 4 mL primary stock to a 100 mL glass volumetric flask

Note

Adjust the volume of primary stock according to the actual concentration of second calibration standard

6.2 Top with 90% acetone to 100 mL

6.3 Measure absorbance at 664 nm.

6.4 Chlorophyll-a_mg/L = 11.42 X Absorbance at 664 nm, where 11.42 is the extinction coefficient of chlorophyll-a at 664 nm.

Note

Detection limit of spectrophotometrical measurement is about 350 ug/L

6.5 Aliquote 5 mL to amber vials, label cryobox with actual concentration and store at -80 °C to -50 °C .

7 Working calibration standard (≅16 ug/L)

Note

Maximum concentration of detection range is 15~20 ug/L

7.1 Warm up second calibration standard and transfer 2 mL solution to a 50 mL glass volumetric flask

Note

Adjust the volume of second calibration standard according to the actual concentration of second calibration standard

7.2 Top with 90% acetone to 50 mL

Extract chlorophyll sample and blank

8 Extract samples on the filter

8.1 Label 20 mL scintillation vials and place them in scintillation vial rack.

Equipment	
Vial rack	NAME
Wheaton	BRAND
Z252425	SKU

8.2 Transfer sample and blank filter into scintillation vial

8.3 Add 5 mL Acetone/DMSO extraction solvent

8.4 Cap the vial, and vortex.

8.5 Cover the scintillation vials with another scintillation rack.

8.6 Let samples be extracted for 00:20:00 at Room temperature 20m

8.7 Vortex samples and allow samples to be extracted for another 00:10:00 10m

9 Extract liquid samples

9.1 Freeze liquid sample and blank

9.2 Add extraction solvent into frozen sample

Note

(V_sample) : (V_solvent)= 1:25 ~ 1:50

9.3 Cap the vial, vortex and place sample in the dark at room temperature for 00:20:00 20m

9.4 Vortex and place sample in the dark for another 00:10:00 10m

Daily calibrate

10 Allow Turner to warm-up for at least 15 minutes.

11 Place the solid secondary standard in Turner, wait 15 seconds for the reading to stabilize.

12 Record the reading on the solid standard calibration record sheet.

13 Reading should be less than 10% of the previously determined post calibration value.
Otherwise, calibrate Turner by liquid standard before measuring samples (Go to the last step: Calibrate Turner)

Measure chlorophyll-a and pheophytin-a of standard

14 Use polypropylene pipet tip to transfer 5 mL 90% acetone to disposable glass tube, wipe the outside of the tube dry with kimwipe and place in the instrument. Replace the light cap.

Equipment

Culture Tubes,	NAME
Disposable, Borosilicate Glass	TYPE
VWR	BRAND
47729-572	SKU

15 Wait about 15 seconds for the reading to stabilize, and zero the instrument on the sensitivity setting.

16 Transfer 5 mL working calibration standard (prepared in) to a disposable glass tube, measure the fluorescence. Log the reading as "standard before acidification (R_b)".

17 Add 150 ul 0.1N HCl to working calibration standard.

18 Carefully mix solution by vortexing for 10 seconds and measure the fluorescence again. Log the reading as "standard after acidification (R_a)".

19 Calculate the ratio, r, as follows:
$r = Rb/Ra = 7.66/3.70 = 2.07$

Measure chlorophyll-a and pheophytin-a of samples

20 Use extraction of blank filter to zero the instrument on the sensitivity setting that will be used for sample analysis.
Or simply log the reading of blank filter extraction and subtract the reading from reading of sample extraction.

21 Transfer sample extract to disposable glass tube.

22 If the display reads OVER, dilute the sample and reread. Log dilution factor (DF) and reading (R_b).

Note

Always bring total volume of diluted to 5 mL, so that the volume of 0.1 N HCl doesn't need to be changed.

23 Add 150 ul 0.1N HCl. Carefully mix solution by vortexing for 10 seconds and measure the fluorescence again. Log the reading (R_a).

Calculation

24 $$Chlorophyll - a[ug/L] = r * (R_b - R_a) * DF/(r - 1)$$

25 $$pheophytin - a[ug/L] = r * (rR_a - R_b) * DF/(r - 1)$$

Calibrate Turner

26 Standards Room temperature

26.1 Primary Chlorophyll a standards

Chlorophyll-a standard **VWR International Catalog #** E-541-0-850

26.2 Come as a set of one low (15 ~ 20 ug/L) and one high (140 ~ 160 ug/L) concentration in foil wrapped ampules. Use low concentration for the calibration only.

> **Note**
>
> Actual concentration varies by LOT, and is listed on a certificate

26.3 Break the ampule with breaker and pour directly into the test tube

27 Blank: 90% buffered acetone Room temperature

28 Press <ENT> to reach Main Menu screen.

29 Press <2> to reach the Calibration Menu screen. Set the concentration range control to AUTO.

30 From screen 2.0, press <4> to bring up screen 2.4, then <3> to bring up screen 2.43 (set conc. range control), and press <ENT> to toggle.

31 Press <Esc> to screen 2.0, press <2> to access screen 2.2 (standard solution concentration). Enter the actual concentration of the primary liquid calibration standard (e.g. the value from the certificate).

32 Press <Esc> to return to screen 2.0.

33 Press <1> to access screen 2.1 and confirm that option #2 on screen 2.1 reads YES.

34 Press <Esc> to return to HOME screen.

35 While on the HOME screen, fill a clean 13-mm culture tube with 90% acetone (the blank). Put the tube in the sample chamber and replace the light cap.

Note

Because temperature affects fluorescence, do not allow the blank to remain in the instrument any longer than necessary for a stable reading.

36 Press <ENT> , <2>, <1>, and <1> to access 2.11 After the Blank % reading is stable (“TC” on screen 2.11 cycles from 1 to 8 seconds) and assuming the Blank % is less than 200%, press <0>.

37 When “FINISHED” appears, press <ESC> all the way to HOME.

38 Remove the blank. Set tube aside.

39 While on the HOME screen, place the tube with the LOW primary chlorophyll a standard in the sample chamber and replace the light cap.

Note

Because temperature affects fluorescence, do not allow the blank to remain in the instrument any longer than necessary for a stable reading.

40 Pressing <ENT>, <2>, and <3> to access 2.3

41 Using UP and DOWN arrows to adjust Span% until the FS reading is the closest to actual value of primary standard.

42 Wait until reading is stable (“TC” on screen cycles from 1 to 8 sec.), then press <*>.

43 When “FINISHED” appears, press <ESC> all the way to HOME screen.

44 Read the primary liquid standard on the home screen by pressing <*>, to confirm that the calibration was properly set and record the calibrant and reading on the calibration log sheet.

45 Place the solid secondary standard in the instrument, read the LOW value. Record the reading on the solid standard calibration record sheet as the reference of daily check.

Equipment

10AU Solid secondary standards	NAME
Turner Designs Digital Fluorometer	TYPE
Turner designs	BRAND
10-AU-904	SKU