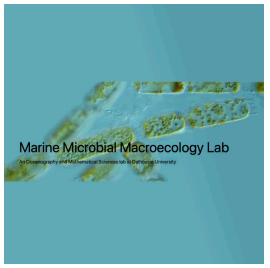
Jan 29, 2025 Version 3

# Lipids in microalgae: Quantitation by acid-dichromate method in microtiter plate V.3

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Ying-Yu Hu[1], Zoe V. Finkel[1]

[1]Dalhousie University

Marine Microbial Macroecology Lab
Tech. support email: **ruby.hu@dal.ca**

Ying-Yu Hu
Dalhousie University



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

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**Keywords:** acid-dichromate, lipids, microalgae, microtiter plate, samples with lipid concentration, µg of lipid, preliminary test for lipid, lipids in microalgae, lipid concentration, total lipids in microalgae, lipid quantitation, times higher sensitivity in lipid quantitation, quantitating total lipid, dichromate method in microtiter plate, lipid, measuring absorbance, dichromate method, dichromate, samples with concentration, used colorimetric analysis technique, colorimetric analysis technique, absorbance, unknown sample, quantitation by acid, µg range, sample, well microtiter plate, toxic reagent

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## Abstract

This protocol describes a method for quantitating total lipids in microalgae using the acid-dichromate method, a widely used colorimetric analysis technique. We present a procedure utilizing a 96-well microtiter plate for safe and efficient sample handling, enabling high throughput. Only 200 - 500 µl of 0.15% acid-dichromate is required per sample, significantly reducing the amount of corrosive and toxic reagent used.

Furthermore, we demonstrate that measuring absorbance at 348 nm provides five times higher sensitivity in lipid quantitation compared to absorbance at 440 nm.

A preliminary test for lipid-unknown samples is included to minimize uncertainty in the measurements. This test ensures that the method performs reliably within the detection range of 20 to 80 µg of lipids. Without this test, samples with lipid concentrations outside this range (either less than 20 µg or greater than 80 µg) may result in inaccurate or failed measurements. Specifically, samples with concentrations above 80 µg exhibit a linear response with an opposite slope, which could cause lipid concentrations to be underestimated if the calibration curve based on the 20 to 80 µg range is used. Accurate quantification can be achieved with as little as 20 µg, and the working detection limit is approximately 5 µg.

Citation

Pand SV, ParvinKhan R, Venkitasubramanian TA. (2026). Microdetermination of lipids and serum total fatty acids. Analytical Biochemistry..

https://doi.org/10.1016/0003-2697(63)90094-0 LINK

## Protocol materials

- Sodium sulphite
- Concentrated sulphuric acid
- Glyceryl tripalmitate
- Chloroform (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**439142-4L
- Potassium dichromate **Fisher Scientific Catalog #**P188-100

## Troubleshooting

## Safety warnings

Safety information

Any items contaminated by potassium dichromate should be disposed as hazardous waste.

## Before start

Clear vials are pre-combusted at 500 °C for 06:00:00

Reagent bottle for dichromate reagent is rinsed by 95% ethanol and air-dried.
Glass serological pipets are rinsed by 95% ethanol and air-dried.
All caps are rinsed by 95% ethanol and air-dried.
Barrel and needle of the glass syringe is rinsed by chloroform and air-dried.

## Preliminary test of sample biomass

1 For microalgae samples that have been rarely studied with no available information on lipid content, it is essential to conduct a preliminary test before processing all samples.

2 Blank

> Note
>
> The process of redissolving and subsampling is essential for purification purposes, aimed at removing water-soluble organic substances such as proteins, amino acids, sugars, RNA, and DNA, which could potentially interfere with the dichromate assay.

2.1 Add 5 mL chloroform into the dried extract

Chloroform (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**439142-4L

2.2 Transfer 4.5 mL into a new vial.

2.3 Dry this 4.5 mL extract solution for assay.

2.4 Discard the remaining extract solution into waste tank.

3 One replicate from each sample

3.1 Add 4 mL chloroform into the dried sample extract.

3.2 Transfer 2 mL extract into another new vial.

3.3 Dry the extract in the new vial for assay.

3.4 Store the remaining extract -80 °C (for phospholipids assay).

4 Preparation of standard
Glyceryl tripalmitate

4.1 Place frozen glyceryl tripalmitate (GTP) in vacuum desiccator with lose cap until it is warmed to Room temperature before making primary standard solution

4.2 Weigh and transfer GTP (around 2 mg ) to a clear vial , log the actual weight.

| | GTP (mg) |
|---|---|
| | |

4.3 Dissolve GTP by chloroform for a concentration of about 1 mg/mL, gently vortex.

| | chloroform (mL) |
|---|---|
| | |

4.4 The following steps require a 100 uL glass syringe: the syringe barrel and needle is chloroform rinsed and air-dried

Equipment

| | |
|---|---|
| Gastight® 1700 Series Syringes | NAME |
| 1710N | TYPE |
| Hamilton | BRAND |
| 81000 | SKU |

4.5 5 ug/vial:

In a 12 ml clear vial, add 5 µL GTP primary standard to each vial.

4.6 10 ug/vial:
In a 12 ml clear vial, add 10 µL GTP primary standard to each vial.

4.7 20 ug/vial:
In a 12 ml clear vial, add 20 µL GTP primary standard to each vial.

4.8 40 ug/vial:
In a 12 ml clear vial, add 40 µL GTP primary standard to each vial.

4.9 80 ug/vial
In a 12 ml clear vial, add 80 µL GTP primary standard to each vial.

4.10 100 ug/vial
In a 12 ml clear vial, add 100 µL GTP primary standard to each vial.

4.11 120 ug/vial
In a 12 ml clear vial, add 2X 60 µL GTP primary standard to each vial.

4.12 160 ug/vial
In a 12 ml clear vial, add 2X 80 µL GTP primary standard to each vial.

4.13 200 ug/vial
In a 12 ml clear vial, add 2X 100 µL GTP primary standard to each vial.

4.14 240 ug/vial
In a 12 ml clear vial, add 3X 80 µL GTP primary standard to each vial.

4.15 300 ug/vial
In a 12 ml clear vial, add 3X 100 µL GTP primary standard to each vial.

5 Dry working standards at Room temperature under $N_2$ gas stream (<2 psi).

6 Estimate the total volume of potassium dichromate required:
Number of standards and standard blanks: 12+2
Number of samples and sample blanks: N

$$V = 0.5 * 14 + 0.2 * N$$

7 Transfer concentrated sulfuric acid from the original package to a 95% ethanol rinsed and air-dried glass reagent bottle for temporary storage.
Concentrated sulphuric acid

8 Weigh a 95% ethanol rinsed and air-dried glass reagent bottle, and tare the balance

9 Use 5 ml serological pipet to measure and transfer concentrated sulfuric acid to this vial. The volume of sulfuric acid is several milliliter more than estimated in go to step #6 .
Write down the weight of sulfuric acid.

| | Sulfuric acid (mL) | Sulfuric acid (g) |
|---|---|---|
| | | |

10 The weight of dichromate required for the 0.15% (w/w) acid-dichromate reagent equals the weight of sulfuric acid multiplied by (0.15/99.85).
Potassium dichromate **Fisher Scientific Catalog #**P188-100

| | Dichromate (g) |
|---|---|
| | |

11 Weigh dichromate and dissolve it into concentrated sulfuric acid. Cap the vial and vortex until complete dissolve.

12 Label two 12 mL vials with “+ Blank” and “- Blank”.
"-Blank" is 0 ug GTP.
"+Blank" is the reference of absorbance.

13 Prepare boiling water bath on hot plate, place a vial rack in the water bath

14 Add 0.5 mL of acid-dichromate reagent to `\textcolor{red}{standards, +Blank, -Blank}`. Cap and vortex right after.

Note

Use 5 mL glass serological pipet, fill to "4 mL", dispense 0.5 mL .

15 Add 0.2 mL of acid-dichromate reagent to \textcolor{red}{samples, sample} \textcolor{red}{blanks} . Cap and vortex right after.

> Note
>
> Use 5 mL glass serological pipet, fill to "4 mL", dispense 0.2 mL .

16 Keep reaction vials in boiling water for 00:15:00 .

17 Cool vials to Room temperature in the fumehood

18 Prepare [M] 0.2 g/ml sodium sulphite solution
Sodium sulphite
Weigh 0.2 g sodium sulphite in a 2 ml microtube.
Add 1 mL MilliQ water into the tube.
Vortex

19 Add 1.125 mL MilliQ (1 mL + 125 uL by pipet) to \textcolor{red}{standards, +Blank, -Blank}. Cap immediately and vortex.

20 Add 450 µL MilliQ to \textcolor{red}{samples, sample} \textcolor{red}{blanks}. Cap immediately and vortex.

21 Cool vials to room temperature.

22 Add 25 µL [M] 0.2 g/ml sodium sulphite solution to the “+Blank” vial. Vortex.

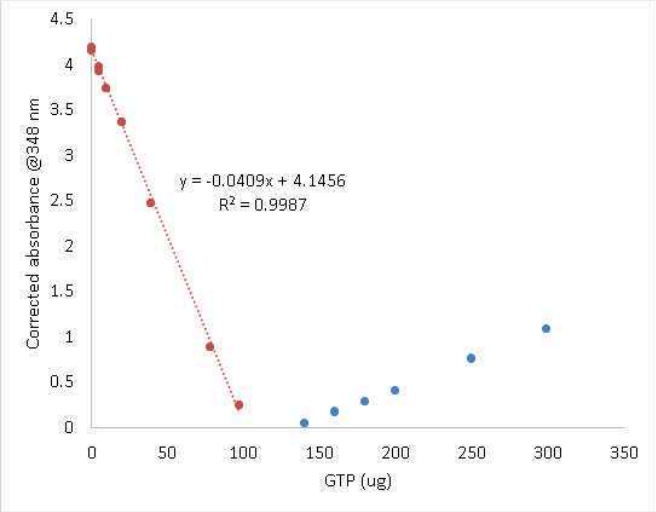
23 Reverse pipetting, load 200 µL reactant into microtiter plate.

24 Read absorbance at 348 nm

25 Subtract absorbance of "+Blank" from the absorbance of standards.

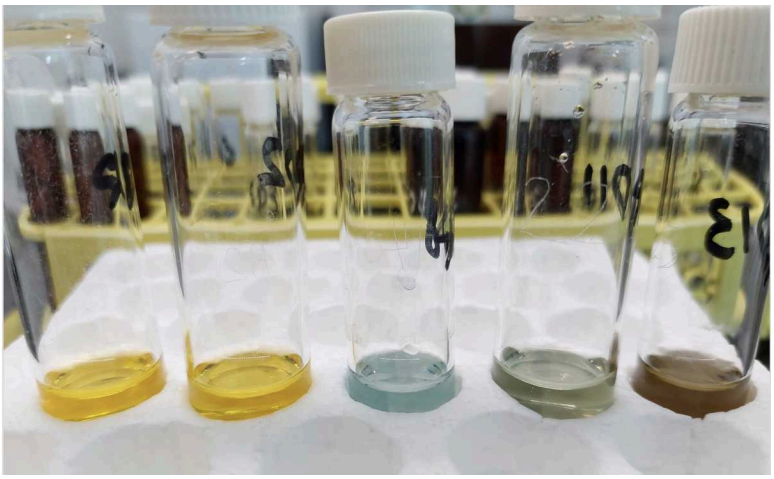
26 Plot the resulted absorbance versus mass of GTP (ug).

Expected result




Note

Take notes of the color for using the corresponding curve.



Concentration of lipids (left to right): 5 ug, 10 ug, 100 ug, 200 ug and 300 ug

27 Calculate the mass of lipids by using the standard curve and the absorbance, including sample blanks and samples.
$GTP_{ug} = (Abs - intercept)/slope$

28 $Lipids_{ug} = GTP_{ug} * (0.5/0.2)$

29 For samples: $Lipids_{ug/filter} = Lipids_{ug} * 4/2$
For blanks: $Lipids_{ug/filter} = Lipids_{ug} * 5/4.5$

30 $Lipids_{corrected} = Lipids_{sample} - Lipids_{blank}$

31 Determine the subsampling method for the remaining replicates

31.1 Preliminary result of $GTP_{ug}$ is between 20 to 80 ug
Use the same method to subsample and assay

31.2 Preliminary result of $GTP_{ug}$ is above 80 ug
Use smaller portion from the extract or use 0.5 mL dichromate for an estimated $GTP_{ug}$ value between 20 to 80 ug

31.3 Preliminary result of $GTP_{ug}$ is lower than 20 ug
Use larger portion from the extract and use 0.2 mL dichromate for an estimated $GTP_{ug}$ value between 20 to 80 ug

## Subsample for the assay

32 For sample blanks, add 5 mL chloroform and transfer 4.5 mL to a new vial. Discard the remaining extract.

33 For samples, subsample following the preliminary test.

34 Dry the extract in the new vial for assay.

35 Store the remaining extract -80 °C (for phospholipids assay).

## Assay for the samples

36 Prepare glyceryl tripalmitate (GTP) primary standard solution (around 1 mg/ml)

36.1 Place frozen GTP in vacuum desiccator with lose cap until it is warmed to Room temperature before making primary standard solution

36.2 Weigh and transfer GTP (around 1 mg ) to a clear vial , log the actual weight.

| | GTP (mg) |
|---|---|
| | |

36.3 Dissolve GTP by chloroform for a concentration of about 1 mg/mL, gently vortex.

| | chloroform (mL) |
|---|---|
| | |

37 Prepare working standards:

37.1 5 ug/vial:

In a 12 ml clear vial, add 5 µL GTP primary standard to each vial.

37.2 10 ug/vial:
In a 12 ml clear vial, add 10 µL GTP primary standard to each vial.

37.3 20 ug/vial:
In a 12 ml clear vial, add 20 µL GTP primary standard to each vial.

37.4 40 ug/vial:
In two 12 ml amber vials, add 40 µL GTP primary standard to each vial.

37.5 80 ug/vial
In a 12 ml clear vial, add 80 µL GTP primary standard to each vial.

38 Dry working standards at Room temperature under $N_2$ gas stream (<2 psi).

39 Estimate the total volume of potassium dichromate required:
Number of standards and standard blanks: 6
Number of sample blanks and samples using 0.2 mL dichromate: N
Number of samples using 0.5 mL dichromate: M

$$V = 0.5 * (6 + M) + 0.2 * N$$

40 Transfer concentrated sulfuric acid from the original package to a 95% ethanol rinsed and air-dried glass reagent bottle for temporary storage.

41 Weigh a 95% ethanol rinsed and air-dried glass reagent bottle, and tare the balance

42 Use 5 ml serological pipet to measure and transfer concentrated sulfuric acid to this vial.
The volume of sulfuric acid is several milliliter more than estimated in go to step #6.
Write down the weight of sulfuric acid.

| | Sulfuric acid (mL) | Sulfuric acid (g) |
|---|---|---|
| | | |

43 The weight of dichromate required for the 0.15% (w/w) acid-dichromate reagent equals the weight of sulfuric acid multiplied by (0.15/99.85).

44 Weigh dichromate and dissolve it into concentrated sulfuric acid. Cap the vial and vortex until complete dissolve.

| | Dichromate (g) |
|---|---|
| | |

45 Label two 12 mL vials with “+ Blank” and “- Blank”.
"-Blank" is 0 ug GTP.
"+Blank" is the reference of absorbance.

46 Prepare boiling water bath on hot plate, place a vial rack in the water bath

47 Add 0.5 mL of acid-dichromate reagent to `\textcolor{red}{standards, +Blank, -Blank}` **and some of the samples**. Cap and vortex right after.

> Note
>
> Use 5 mL glass serological pipet, fill to "4 mL", dispense 0.5 mL .

48 Add 0.2 mL of acid-dichromate reagent to (1) `\textcolor{red}{sample} \textcolor{red}{blanks}`, (2) **some of the** `\textcolor{red}{samples}` .
Cap and vortex right after.

> Note
>
> Use 5 mL glass serological pipet, fill to "4 mL", dispense 0.2 mL .

49 Keep reaction vials in boiling water for 00:15:00 .

50 Cool vials to Room temperature in the fumehood

51 Prepare [M] 0.2 g/ml sodium sulphite solution

Weigh 0.2 g sodium sulphite in a 2 ml microtube.

Add 1 mL MilliQ water into the tube.

Vortex

52 Prepare two vial racks, one is for those with 0.5 mL dichromate, the other is for those with 0.2 mL dichromate.
Label the racks to avoid adding MilliQ in wrong volume.

53 Add 1.125 mL MilliQ (1 mL + 125 uL by pipet) to those reacted with 0.5 mL dichromate.
Cap immediately and vortex.

54 Add 450 µL MilliQ to those reacted with 0.2 mL dichromate.
Cap immediately and vortex.

55 Cool vials to room temperature.

56 Prepare sodium sulphite [M] 0.2 g/ml

In a 2 mL microtube, add 0.4 g sodium sulphite and 2 mL MilliQ, vortex.

57 Add 25 µL [M] 0.2 g/ml sodium sulphite solution to the “+Blank” vial. Vortex.

58 Reverse pipetting, load 200 µL reactant into microtiter plate.

59 Read absorbance at 348 nm

60 Subtract absorbance of "+Blank" from the absorbance of standards.

61 Plot the resulted absorbance versus mass of GTP (ug).

62 Calculate the mass of lipids by using the standard curve and the absorbance, including sample blanks and samples.
$GTP_{ug} = (Abs - intercept)/slope$

63 $Lipids_{ug} = GTP_{ug} * (0.5/V_{dichromate})$ , where $V_{dichromate}$ is the actual volume of dichromate reagent added.

64 $Lipids_{ug/filter} = (V_1/V_2) * Lipids_{ug}$ , where $V_1$ is the volume of chloroform added into the dried extract, $V_2$ is the volume of extract used in the assay.

65 $Lipids_{corrected} = Lipids_{sample} - Lipids_{blank}$

## Citations

Pand SV, ParvinKhan R, Venkitasubramanian TA.. Microdetermination of lipids and serum total fatty acids. **https://doi.org/10.1016/0003-2697(63)90094-0**