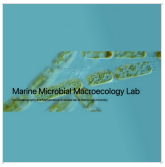

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Lipids in microalgae: Quantitation by acid-dichromate method in microtiter plate

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This is a protocol for quantitating total lipids in microalgae.

The acid-dichromate method is widely used to perform colorimetric analysis of extracted lipids. Here we present a protocol using 96-well microtiter plate for safe and efficient sample handling with high throughput. Only 500 ul of 0.15% acid-dichromate is required for each sample, which greatly reduces the amount of corrosive and toxic reagent.

In addition, comparing with the absorbance at 440 nm, the absorbance at 348 nm yields five-time higher sensitivity in lipids quantitation.

Total lipids in the samples should not excess 80 ug. Accurate quantitation can be achieved with as little as 20 ug. A working detection limit is about 5 ug.

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

PROTOCOL CITATION

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KEYWORDS

acid-dichromate, lipids, microalgae, microtiter plate

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GUIDELINES

Lipids of microalgae is extracted by Folch solvent. The extract is dissolved in chloroform and stored at -80 degree ULT freezer.

Lipids in microalgae: The Extraction by modified Folch solvent

by Yingyu Hu,

Dalhousie University

PREVIEW RUN

Estimate the mass of extracted lipids as 10~30% of total cell mass.

The quantitative range of this method is 0~80 ug.

MATERIALS

NAME	CATALOG #	VENDOR
Potassium dichromate	P188-100	Fisher Scientific
Concentrated sulphuric acid		
Glyceryl tripalmitate		
Sodium sulphite		
Chloroform (HPLC grade)	439142-4L	Sigma Aldrich

MATERIALS TEXT

Storage Vials and Closures
12 mL amber
Thermo Scientific B7800-12A
VWR 66030-686

VWR® Vials, Borosilicate Glass, with Phenolic Screw Cap
22.18 mL
VWR 66012-044
24-400 cap: VWR 89076-764

Boil-Proof Microcentrifuge Tubes
Microtube
Axygen Scientific MCT-200-C

VWR® Volumetric Pipets, Reusable, Color Coded, Class A
0.5 mL and 5 mL
VWR 10546-004 and 10546-014

Safetypette
Jencons 75856-442

Gastight® 1700 Series Syringes
1710N
Hamilton 81000

Microbalance
Cubis series
Sartorius MSE6.6S-000-DM

Reacti-Vap Evaporator
Thermo Scientific TS-18825

VWR ANALOG VORTEX MIXER
VWR 10153-838
With tube insert

VWR® Advanced Hot Plates
VWR 97042-658

Varioskan LUX Multimode Microplate Reader

Thermo Fisher VL0L00D0

96-Well Microplates, Polystyrene, Clear,

Greiner Bio-One 655101

EQUIPMENT

NAME	CATALOG #	VENDOR
Safetypette	75856-442	VWR international Ltd
Gastight® 1700 Series Syringes	81000	VWR international Ltd
Reacti-Vap Evaporator	TS-18825	VWR international Ltd
VWR ANALOG VORTEX MIXER	10153-838	VWR international Ltd
VWR® Advanced Hot Plates	97042-658	VWR international Ltd
Storage Vials and Closures	B7800-12A	VWR international Ltd
Varioskan LUX Multimode Microplate Reader	VL0L00D0	
96-Well Microplates, Polystyrene, Clear,	655101	VWR international Ltd
Microbalance	MSE6.6S-000-DM	Fisher Scientific
VWR® Vials, Borosilicate Glass, with Phenolic Screw Cap	66012-044	
Boil-Proof Microcentrifuge Tubes	MCT-200-C	VWR international Ltd
VWR® Volumetric Pipets, Reusable, Color Coded, Class A	10546-004 and 10546-014	

SAFETY WARNINGS

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

BEFORE STARTING

Pre-combust 12 ml amber vials (one for each sample/sample blank/standard/standard blank) and glass vials (for MilliQ water, concentrated sulphuric acid, acid-dichromate reagent).

Rinse vial caps with 95% ethanol and dry.

Rinse serological pipet (0.5 and 5 mL) with chloroform. The pipets are used to measure and transfer extract, concentrated sulphuric acid and acid-dichromate reagent.

Rinse syringe with chloroform and dry.

Preparation of Standard

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

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

1.2 Weigh around **1 mg** GTP, take note of the actual weight.

1.3 Dissolve GTP by **1 mL** chloroform in amber vial, gently vortex.

2 Prepare working standards:

2.1 5 ug/vial:
In two 12 ml amber vials, add **5 µl** GTP primary standard to each vial. Cap the vial to avoid contamination.

2.2 10 ug/vial:
In two 12 ml amber vials, add **10 µl** GTP primary standard to each vial. Cap the vial to avoid contamination.

2.3 20 ug/vial:
In two 12 ml amber vials, add **20 µl** GTP primary standard to each vial. Cap the vial to avoid contamination.

2.4 40 ug/vial:
In two 12 ml amber vials, add **40 µl** GTP primary standard to each vial. Cap the vial to avoid contamination.

2.5 80 ug/vial
In two 12 ml amber vials, add **80 µl** GTP primary standard to each vial. Cap the vial to avoid contamination.

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

Preparation of acid-dichromate reagent

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

V=0.5x(N+12) ml

5 Transfer concentrated sulphuric acid to a glass vial for temporary storage

6 Weigh a glass vial, and tare the balance

7 Use 5 ml serological pipet to measure and transfer concentrated sulphuric acid to this vial. The volume of sulphuric acid is several milliliter more than estimated in Step 4. Write down the weight of sulphuric acid.

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

9 Weigh dichromate and dissolve it into concentrated sulphuric acid. Cap the vial and vortex gently.

Reaction of lipids and acid-dichromate reagent

10 Allow frozen extract warm up to **Room temperature** .

11 Gently vortex the extract.

12 Transfer less than 80 ug extracted lipids to 12 ml vial by glass serological pipet.
Take notes of the volume and calculate the fraction of extract used in the analysis.

13 Freeze the rest of the extract in ULT freezer **-80 °C** .

14 Dry extract at **Room temperature** under a stream of N_2 gas (<2 psi)

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

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

17 Add **0.5 mL** of acid-dichromate reagent to each vial (standards, +Blank and –Blank, samples and sample blanks).
Cap and vortex right after.

18 Keep reaction vials in boiling water for **00:15:00** .

19 Cool vials to **Room temperature** in the fumehood

20 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

21 Add **1.125 mL** MilliQ (1 mL + 125 uL by pipet) to each vial. Cap immediately and vortex.

22 Cool vials to room temperature.

23 Add **25 µl** [M]**0.2 g/ml** sodium sulphite solution to the "+Blank" vial. Vortex.

Colorimetric analysis

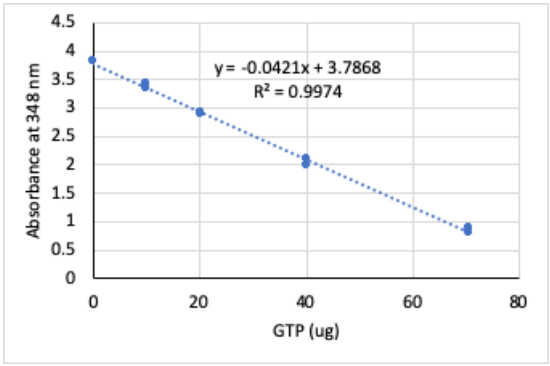
24 Vortex each vial and load **250 µl** reactant into microtiter plate.

25 Read absorbance at 348 nm

Calculation

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

27 Plot the resulted absorbance versus mass of GTP (ug). Use this as standard curve.



Standard curve of absorbance at 348 nm versus quantity of GTP standards

28 Subtract absorbance of "+Blank" from the absorbance of samples and sample blanks.

29 Calculate the mass of lipids by using the standard curve and the resulted absorbance.

30 Subtract the lipids mass of the blank filter from the lipids mass of the samples.

31 Convert the resulted mass to total extracted lipids based on the fraction of extract used in the reaction.