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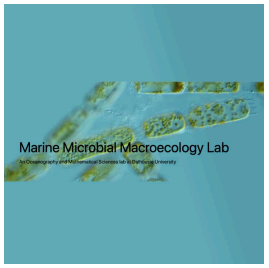
Rapid extraction of total lipids from microalgae V.5



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

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Keywords: lipids, microalgae, Folch solvent, rapid extraction of total lipid, total lipids from miroalgae, extracted lipid, rapid extraction, total lipid, extraction, microalgae, contamination from filtration system, filtration system, filtration, filter debris, folch solvent, mixture of water, centrifugation,

Abstract

In this protocol, total lipids from miroalgae is extracted with a mixture of water and Folch solvent (2:1 chloroform-methanol v/v). Filter and cell debris is commonly removed by filtration, which is laborious and time consuming. It is also the main reason to either cause sample loss and therefore underestimation; or contamination from filtration system and therefore overestimation. We now use centrifugation to remove filter debris, which stays in between the organic phase and water phase. The extracted lipids is dried under N_2 gas flow, stored under -80 ºC for further measurement.

Citation

FOLCH J, LEES M, SLOANE STANLEY GH
. A simple method for the isolation and purification of total lipides from animal tissues.
J Biol Chem, 1957, 226, 497-509.

Citation

Axelsson M, Gentili F (2014). A single-step method for rapid extraction of total lipids from green microalgae..
PloS one.

https://doi.org/10.1371/journal.pone.0089643 LINK

Guidelines

Biomass requirement

Considering that

(1) lipids are approximately 10~30% of microalgal dry mass
(2) the linear range for colorimetric lipid analysis is 4.2 to 80 µg, the low limit of quantitation is 20 µg

The minimum requirement of sample volume for total lipids is calculated as following:
V_L=20/ChI-a/(17.3/1.1)

If both total lipids and phospholipids are expected to be measured, the minimum sample volume needs to be at least doubled.

Protocol materials

- Chloroform (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**439142-4L
- Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34860
- Potassium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3911-500G

Troubleshooting

Safety warnings

Safety information

Operate chloroform in fume hood.

Safety information

Follow the disposal guidelines regarding the halogenated organic waste.

Collect microalgae samples

1 Precombust GFF filter at 450 °C for 04:00:00 4h

2 Rinse forceps with 95% ethanol, air-dry.

Equipment

Filter forceps	NAME
blunt end, stainless steel	TYPE
Millipore	BRAND
XX6200006P	SKU

Note

Wipe-dry forceps can cause carbon contamination of samples.

3 Filter microalgae in liquid media onto precombusted GFF filters, using gentle vacuum pressure (130 mm Hg).

4 Rinse sample with filtered seawater

5 Place sample filters in cryogenic vials

6 Filter blank media (without cells) through precombusted GFF filter as blank.

7 Flash freeze filters and stored at -80 °C

8 Freeze dry before measurement.

Equipment

FreeZone® 2.5 L Benchtop Freeze Dryers	NAME
Labconco®	BRAND
700202000	SKU

9 Follow <Total particulate carbohydrate from microalgae> protocol to hydrolyze the sample.
Hydrolysation treatment can improve the extraction efficiency:

1. Hydrolysis releases bound lipids into easily extractable forms.
2. Acidified water fraction can facilitate separation of the lipid fraction from extraneous protein and other material.
3. Acid can charge phospholipid to optimize extraction.

Prepare glassware

10 Precombust the centrifuge tubes at 500 °C for 06:00:00 6h

11 Precombust pasteur pipets at 500 °C for 02:00:00 2h

Equipment

Disposable Soda-Lime Glass Pasteur Pipets	NAME
5 3/4"	TYPE
Fisherbrand	BRAND
13-678-6A	SKU

12 Rinse caps with 95% ethanol and air-dry prior to use

13 Latex bulbs are required for Pasteur pipets

Prepare reagent

14 Folch solvent ($CHCl_3$: MeOH=2:1 v/v)

14.1 Mix two parts of chloroform and one part of methanol in a 1 L amber bottle. Log the volume of each solvent for double checking the ratio.

	A	B
	Chloroform (mL)	
	Methanol (mL)	

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

Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34860

14.2 Attach dispensette to the bottle, mix well.

Equipment

Bottle-top dispenser	NAME
BrandTech Dispensette® S	BRAND
4731330	SKU

14.3 Label bottle with MSDS label.

15 KCl solution ([M] 0.88 %)

Note

1.5 mL per sample

15.1 Weigh the pyrex media bottle and tare.

Equipment

PYREX® Media Bottles	NAME
Corning®	BRAND
1395-100	SKU

15.2 Directly weigh 0.44 g KCl in the bottle.

Potassium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3911-500G

15.3 Top bottle with MilliQ water to 50 g

	A	B
	KCl (g)	
	Final (g)	

Prepare for extraction

16 If lipids samples are not processed for carbohydrate, transfer freeze dried samples and blanks into muffled centrifuge tubes

Equipment

Disposable Glass Screw-Cap Centrifuge Tubes	NAME
10 mL	TYPE
Corning®	BRAND
99502-10	SKU

Equipment

Polypropylene Screw Caps	NAME
Linerless, 15-415	TYPE
Kimble Chase	BRAND
73805-15415	SKU

16.1 Add 100 µL MilliQ directly onto the sample.

16.2 Freeze at -80 °C 00:10:00 10m

16.3 Remove vials from freezer.

16.4 Purge the dispensette, fill the tubing with solvent before dispensing solvent into sample tube.

16.5 Dispense 2.0 mL Folch solvent into sample tube.

17 If lipids samples have been hydrolyzed for carbohydrate and solvent has already been added, go to the vortex step directly.

Extract lipids

18 Vortex 00:28:00 by using a tube insert. 28m

Equipment	
VWR ANALOG VORTEX MIXER	NAME
VWR	BRAND
10153-838	SKU
With tube insert	SPECIFICATIONS

19 Sonicate 00:02:00

20 Vortex 00:30:00 by using a tube insert. 30m

21 Prepare one set of precombusted tubes (#T1), label the tubes, cap is not required.

Equipment

Disposable Glass Screw-Cap Centrifuge Tubes	NAME
10 mL	TYPE
Corning®	BRAND
99502-10	SKU

22 Place one pasteur pipet into each tube (#T1)

Equipment

Disposable Pasteur Pipet	NAME
9 inch	TYPE
VWR	BRAND
14672-380	SKU

23 Prepare another set of precombusted tubes (#T2) for extract. Cap the tube to avoid contamination.

Equipment

Disposable Glass Screw-Cap Centrifuge Tubes	NAME
10 mL	TYPE
Corning®	BRAND
99502-10	SKU

Equipment

Polypropylene Screw Caps	NAME
Linerless, 15-415	TYPE
Kimble Chase	BRAND
73805-15415	SKU

24 Centrifuge at 3200 rpm, Room temperature, 00:05:00 5m

Equipment

NAME	TYPE	BRAND	SKU
General-purpose benchtop centrifuge	IEC CENTRA CL2	Thermo	00427 0F

25 Use the pasteur pipet to transfer organic layer to #T2.

Note

If some debris precipitate at the bottom, gentle blow air bubble through pasteur pipet to relocate it to water layer. Avoid transferring water layer and debris into #T2

26 Add 1 mL Folch solvent to the residue.

27 Sonicate 00:02:00

28 Vortex by using a tube insert for about 00:10:00 10m

29 Centrifuge at 3200 rpm, Room temperature, 00:05:00

Equipment

NAME	TYPE	BRAND	SKU
General-purpose benchtop centrifuge	IEC CENTRA CL2	Thermo	00427 0F

30 Use the pasteur pipet to transfer organic layer to #T2.

31 Add 1 mL Folch solvent to the residue.

32 Sonicate 00:02:00

33 Vortex by using a tube insert for about 00:10:00

34 Centrifuge at 3200 rpm, Room temperature, 00:05:00

Equipment

NAME	TYPE	BRAND	SKU
General-purpose benchtop centrifuge	IEC CENTRA CL2	Thermo	00427 0F

35 Use the pasteur pipet to transfer organic layer to #T2.

36 Add 1 mL Folch solvent to the residue.

37 Sonicate 00:02:00

38 Vortex by using a tube insert for about 00:10:00

Collect lipids extract

1h 30m

39 Centrifuge at 3200 rpm, Room temperature, 00:05:00 5m

40 Use the pasteur pipet to transfer organic layer to #T2.

41 Add 1.5 mL KCl solution to T2, vortex and then centrifuge at 3200 rpm, Room temperature, 00:05:00 5m

Note

Volume of Folch solvent to KCl is about 4 to 1.

42 Turn on heat block to 37 °C , use a thermometer to monitor the actual temperature.

Equipment

LSE digital dry bath heater NAME

Corning BRAND

6885-DB SKU

Equipment

Blocks for Corning® LSE Digital Dry Bath Heaters NAME

Corning BRAND

480124 SKU

43 Place tubes (#T2) in the heater.

Note

Organic layer turns foggy when temperature is lower than 37 °C

44 Use a new pasteur pipet to transfer the lower organic phase to a clear 12 mL storage vial. Do not disturb the cell debris in between the two phases.

Note

Clear vial helps to check if there is water drops or impurities in lipids extract after dried.

Equipment

Glass Vials PTFE/SILiCone SEPTA Clear	NAME
16 mL	TYPE
Thermo Scientific	BRAND
B7990-4	SKU

Equipment

Screw Vial Convenience Kit, 12mL solid top PTFE cap	NAME
Thermo Scientific	BRAND
B7800-12A	SKU

45 Dry organic phase extract at 37 °C under a stream of N_2 gas (<2 psi) for about 00:30:00 . 30m

	A	B	C
		Time	Gas cylinder pressure
	Start		
	End		

Equipment

Reacti-Vap Evaporator	NAME
Thermo Scientific	BRAND
TS-18825	SKU

Purify lipids extract

30m

46 The lipids extract might still have water residue (which can't be dried by nitrogen gas) or water soluble impurities.

Redissolve it with 5 mL chloroform by using glass serological pipet, transfer certain amount of chloroform dissolved extract for lipids measurement (based on the estimation, 20 to 100 ug) into a new vial. Log the actual volume transferred.

Equipment

Safetypette	NAME
Jencons	BRAND
75856-442	SKU

47 Dry extract at 37 °C under a stream of N_2 gas (<2 psi) (Generally 2 mL/5 min).

	A	B	C
		Time	Gas cylinder pressure

	A	B	C
	Start		
	End		

48 Store dried extract and excess extract (in chloroform) at -80 °C .

Citations

FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues

Axelsson M, Gentili F. A single-step method for rapid extraction of total lipids from green microalgae. **https://doi.org/10.1371/journal.pone.0089643**