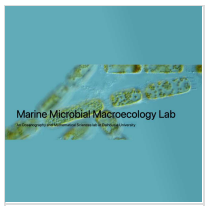

Version 5

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Rapid extraction of total lipids from microalgae V.5

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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.

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.

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

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PROTOCOL CITATION

Yingyu Hu, Zoe V Finkel 2022. Rapid extraction of total lipids from microalgae. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.dm6gpr9jdvzp/v5
Version created by Yingyu Hu

WHAT'S NEW

Find a solution for filter pieces turning into slurry after vortexing.

KEYWORDS

lipids, microalgae, Folch solvent

LICENSE

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66101

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/Chl-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.

SAFETY WARNINGS

Operate chloroform in fume hood.

Follow the disposal guidelines regarding the halogenated organic waste.

Collect microalgae samples

4h

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

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

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Filter forceps
blunt end, stainless steel
Millipore **XX6200006P**

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.

FreeZone® 2.5 L Benchtop Freeze Dryers
Labconco® **700202000**

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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

Disposable Soda-Lime Glass Pasteur Pipets
5 3/4"
Fisherbrand **13-678-6A**

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)

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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) **Sigma Aldrich Catalog #439142-4L**

Methanol **Sigma Aldrich Catalog #34860**

14.2 Attach dispensette to the bottle, mix well.

Bottle-top dispenser
BrandTech Dispensette® S 4731330

14.3 Label bottle with MSDS label.

15 KCl solution ([M] **0.88 %**)

1.5 mL per sample

15.1 Weigh the pyrex media bottle and tare.

PYREX® Media Bottles
Corning® **1395-100**

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15.2 Directly weigh **0.44 g** KCl in the bottle.

Potassium chloride **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

Disposable Glass Screw-Cap Centrifuge Tubes
10 mL
Corning® **99502-10**

Polypropylene Screw Caps
Linerless, 15-415
Kimble Chase **73805-15415**

16.1 Add **100 µL** MilliQ directly onto the sample.

16.2 Freeze at **-80 °C** **00:10:00** 10m

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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

28m

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

VWR ANALOG VORTEX MIXER

VWR **10153-838**

With tube insert

19 Sonicate **00:02:00**

30m

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

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

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Disposable Glass Screw-Cap Centrifuge Tubes
10 mL
Corning® **99502-10**

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

Disposable Pasteur Pipet
9 inch
VWR **14672-380**

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

Disposable Glass Screw-Cap Centrifuge Tubes
10 mL
Corning® **99502-10**

Polypropylene Screw Caps
Linerless, 15-415
Kimble Chase **73805-15415**

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

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General-purpose benchtop centrifuge
IEC CENTRA CL2
Thermo **00427 0F**

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

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**

General-purpose benchtop centrifuge
IEC CENTRA CL2
Thermo **00427 0F**

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

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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**

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

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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

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.

LSE digital dry bath heater

Corning	**6885-DB**

Blocks for Corning® LSE Digital Dry Bath Heaters

Corning	**480124**

43 Place tubes (#T2) in the heater.

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

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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.

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

Glass Vials PTFE/SILiCone SEPTA Clear
16 mL
Thermo Scientific **B7990-4**

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

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		

Reacti-Vap Evaporator
Thermo Scientific **TS-18825**

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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.

Safetypette

Jencons **75856-442**

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
Start		
End		

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

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