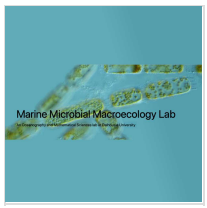

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Rapid extraction of total lipids from microalgae V.4

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In this protocol, total lipids from miroalgae is extracted with Folch solvent (2:1 chloroform-methanol v/v) and the addition of 5% water. 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. The extract is then mixed with 0.88% potassium chloride solution to form a biphasic system, where in between the two phases is the thin distinct cell debris layer. The lower phase with extracted lipids is collected and dried under N_2 gas flow. The residue is 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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lipids, microalgae, Folch solvent

protocol ,

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63296

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.

Operate chloroform in fumehood.

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.

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 (5 inches 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**

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8 Freeze dry before measurement.

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

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, scintillation vials and storage vials 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

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13 Rinse serological pipets and the reagent bottle for dichromate reagent with 95% ethanol until there is not stain and with chloroform for the final rinse. Air-dry.

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

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

14 Latex bulbs are required for Pasteur pipets

Prepare reagent

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

15.1 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**

15.2 Attach dispensette to the bottle, mix well.

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Bottle-top dispenser

BrandTech Dispensette® S 4731330

15.3 Label bottle with MSDS label.

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

16.1 Weigh the pyrex media bottle and tare.

PYREX® Media Bottles

Corning® **1395-100**

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

Potassium chloride **Sigma Aldrich Catalog #P3911-500G**

16.3 Top bottle with MilliQ water to **50 g**

A	B
KCl (g)	
Final (g)	

Extraction

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

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It takes about 7 to 8 hours to process 16 samples.

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

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

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

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

17.3 Remove vials from freezer.

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

17.5 Dispense **2.0 mL** Folch solvent into sample tube.

18 If lipids samples have been hydrolyzed for carbohydrate and solvent has already been added,

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go to the vortex step directly.

19 Vortex **00:28:00** by using a tube insert. 28m

VWR ANALOG VORTEX MIXER
VWR **10153-838**
With tube insert

20 Sonicate **00:02:00**

21 Vortex **00:30:00** by using a tube insert. 30m

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

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

23 Place one pasteur pipet (#P1) into each tube

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

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24 Prepare another set of precombusted tubes (#T2) for supernatant. 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**

25 Work on eight samples first. Use the pasteur pipet (#P1) to gently lift the filter upwards and transfer liquid (as much as possible) to the centrifuge tube. Keep the pasteur pipet (#P1) in its corresponding empty tube (#T1).

26 Add **50 µL** MilliQ and **1 mL** Folch solvent to the residue.

27 Vortex the eight samples at the highest speed to loosen the filter.

28 Vortex the eight samples by using a tube insert while transferring supernatant from another eight samples to centrifuge tubes. 15m

29 Sonicate **00:02:00**

30 Vortex the second eight samples by using a tube insert while transferring supernatant from

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the first eight samples to centrifuge tubes.

Same alternate routine for the following steps.

31 Use the pasteur pipet (#P1) to gently lift the filter upwards and transfer all liquid to the centrifuge tube (#T1). Keep the pasteur pipet (#P1) in its corresponding empty tube (#T1).

32 Add **50 µL** MilliQ and **1 mL** Folch solvent to the residue.

33 Vortex while transferring supernatant from another set of samples to centrifuge tubes.

34 Sonicate **00:02:00**

35 Use the pasteur pipet (#P1) to gently lift the filter upwards and transfer all liquid to the centrifuge tube (#T1). Keep the pasteur pipet (#P1) in its corresponding empty tube (#T1).

36 Add **50 µL** MilliQ and **1 mL** Folch solvent to the residue.

37 Vortex while transferring supernatant from another set of samples to centrifuge tubes.

38 Sonicate **00:02:00**

39 Use the pasteur pipet (#P1) to gently lift the filter upwards and transfer all liquid to the centrifuge tube (#T1).

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40 Leave the pasteur pipet (#P1) in the tube with filter for a while, the tip gradually sucks liquid dripping from filter.

41 Pipette the pasteur pipet in water layer up and down to rinse off organic extract. Put the pipet back in the tube with filter.

Separation 1h 30m

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

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

43 Filter and cell debris stay between the two layer.

44 Use pasteur pipet (#P1) to remove supernatant as much as possible. Do not disturb the debris.

45 Return pipet (#P1) back to the tube with filter.

46 Add 500 ul KCl solution, vortex and then centrifuge at **3200 rpm, Room temperature, 00:05:00** 5m

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

47 Use #P1 to remove supernatant as much as possible. Do not disturb the debris.

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48 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

49 Place tubes in the heater.

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

50 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.

If it has already been the end of the day, keep samples at -80 °C

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

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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**

30m

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

A	B	C
	Time	Gas cylinder pressure
Start		
End		

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

Purification 30m

52 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,

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<100 ug) into a new vial. Log the actual volume transferred.

Safetypette

Jencons **75856-442**

53 Dry extract at **37 °C** under a stream of N_2 gas (<2 psi) for about **00:30:00** (Generally 2 mL/5 min). 30m

A	B	C
	Time	Gas cylinder pressure
Start		
End		

54 Freeze dried extract and excess extract (in chloroform) at **-80 °C** .

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