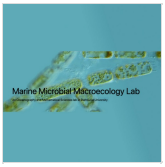

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Lipids in microalgae: The Extraction by modified Folch solvent

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

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ABSTRACT

In this protocol, lipids in miroalgae is extracted with 2 ml Folch solvent (2:1 chloroform-methanol v/v) after frozen and thawed with the addition of 100 ul water. Non-lipid substances are removed by filtration. Filtrate is then mixed with 0.88% potassium chloride solution to form a biphase system. The lower phase with extracted lipids are collected and dried under N_2 gas flow. The residue is dissolved in 5 ml chloroform and stored under -80 ºC.

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.

Liefer JD, Garg A, Fyfe MH, Irwin AJ, Benner I, Brown CM, Follows MJ, Omta AW, Finkel ZV (2019). The Macromolecular Basis of Phytoplankton C:N:P Under Nitrogen Starvation.. Frontiers in microbiology. https://doi.org/10.3389/fmicb.2019.00763

PROTOCOL CITATION

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PARENT PROTOCOLS

In steps of

Particulate phosphorus in microalgae

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

GUIDELINES

Sample Collection

1. Biomass requirement

Considering that (1) lipids are approximately 10~30% of microalgal dry mass and (2) the linear range for colorimetric lipid analysis is 5 to 80 ug, the biomass for lipid sample is around 200 ug dry mass/sample.

If the quantitaion of phosphorus in lipids is expected, then the ideal biomass is around 500~2000 ug dry mass/sample.

2. Collect lipids sample by pelleting

- Aliquot culture sample to 15 ml or 50 ml polypropylene centrifuge tube. If sample volume is more than 50 ml, use multiple tubes.
- Centrifuge sample at <5000 rpm for about 20 min under the temperature close to growth condition.
- Remove supernatant and leave around 100~300 ul of supernatant with pellet in order to avoid disturbance and sample loss.
- Freeze pellet sample in liquid nitrogen during sampling.
- Freeze-dry pellet and store at -80 ºC until extraction.
- Verify the cell lysis and loss of material to the supernatant:
  Preserve 1 ml of supernatant with 20 ul Lugol solution.
  Count cell number and perform chlorophyll-a fluorometry on supernatant.

3. Collect lipids sample by filtration

- Use precombusted GFF filter to avoid organic contamination.
- Filter at low vacuum pressure (<5 mbar) with precombusted filter towers (funnel and base).
- Fold filter in half with tweezers (rinsed by 95% ethanol and dried prior to use)
- Place into a 10 ml precombusted glass centrifuge tube (cap must be rinsed by 95% ethanol and dried)
- For blank, filter the same amount of blank media as sample.
- Freeze in liquid nitrogen during sample collection.
- Freeze-dry samples and store at -80 ºC until extraction.

MATERIALS

NAME	CATALOG #	VENDOR
Chloroform (HPLC grade)	439142-4L	Sigma Aldrich
Methanol (HPLC grade)	34860-4X2L-R	Sigma Aldrich
Potassium chloride	P3911-500G	Sigma Aldrich
95% ethanol		

MATERIALS TEXT

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

Disposable Pasteur Pipet
9 inch
VWR 14672-380

Glass Microanalysis Filter Holders
VWR® 89428-934

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

Whatman Glass Microfiber Filters, Binder Free
Grade GF/F, 25 mm
GE Healthcare 1825-025

Bottle-top dispenser
BrandTech Dispensette® S 4731330

PYREX® Media Bottles
Corning® 1395-100

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

Screw Caps for Screw-Thread Sample Storage Vials
Screw Caps, 15-425, PTFE/PE Foam Liner
Thermo Scientific B7815-15

Lifetime Red™ Graduated Cylinders
10 mL
Corning® PYREX® 3046-10

Filter forceps
blunt end, stainless steel
Millipore XX6200006P

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

Aluminum Clamp
VWR® 89428-944

VWR ANALOG VORTEX MIXER
VWR 10153-838
With tube insert

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

Reacti-Vap Evaporator
Thermo Scientific TS-18825

Safetypette
Jencons 75856-442

BT Barrier Pipet Tips
Pre-Sterile
Neptune® BT1250, BT100, BT10

Other items:
Sonicator
Latex bulb for pasteur pipets
50 ml plastic syringe connected with silicon stopper to provide positive pressure
Glass graduated cylinder with top (100 ml)
Amber bottle for the storage of Folch solvent (500 ml)

EQUIPMENT

NAME	CATALOG #	VENDOR
Lifetime Red™ Graduated Cylinders	3046-10	
Safetypette	75856-442	VWR international Ltd
Storage Vials and Closures	B7800-12A	VWR international Ltd
Whatman Glass Microfiber Filters, Binder Free	1825-025	VWR international Ltd
Bottle-top dispenser	4731330	VWR international Ltd
Polypropylene Screw Caps	73805-15415	VWR international Ltd
Filter forceps	XX6200006P	Emdmillipore
VWR® Vials, Borosilicate Glass, with Phenolic Screw Cap	66012-044	
PYREX® Media Bottles	1395-100	VWR international Ltd
VWR® Volumetric Pipets, Reusable, Color Coded, Class A	10546-004 and 10546-014	
Aluminum Clamp	89428-944	VWR international Ltd
VWR ANALOG VORTEX MIXER	10153-838	VWR international Ltd
General-purpose benchtop centrifuge	00427 0F	
BT Barrier Pipet Tips	BT1250, BT100, BT10	VWR international Ltd

NAME	CATALOG #	VENDOR
Disposable Glass Screw-Cap Centrifuge Tubes	99502-10	VWR international Ltd
Disposable Pasteur Pipet	14672-380	VWR international Ltd
Reacti-Vap Evaporator	TS-18825	VWR international Ltd
Glass Microanalysis Filter Holders	89428-934	VWR international Ltd
Screw Caps for Screw-Thread Sample Storage Vials	B7815-15	VWR international Ltd

SAFETY WARNINGS

Operate chloroform in fumehood.

Follow the disposal guidelines regarding the halogenated organic waste.

BEFORE STARTING

Precombust the glassware at **500 °C** for no less than **06:00:00**

Precombust GFF filter at **450 °C** for no longer than **04:00:00**

Rinse the uncombustable items with 95% ethanol and dry prior to use:

Prepare reagent

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

1.1 Mix two parts of chloroform and one part of methanol in a 500 ml amber bottle

1.2 Attach dispensette to the bottle

1.3 Label bottle with MSDS label.

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

2.1 Weigh the pyrex media bottle and press the tare button

2.2 Directly weigh **0.44 g** KCl in the bottle

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

Extraction

3 Transfer sample into muffled centrifuge tube, use precombusted filter as blank.

4 Add **100 µl** MilliQ directly onto the sample.

5 Freeze at **-80 °C** **00:10:00**

6 Remove vials from freezer.

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

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

9 Vortex **00:02:00**

10 Sonicate **00:02:00**

11 Keep sample in the dark at **Room temperature** **01:00:00**

12 Centrifuge extract **32000 rpm, Room temperature 00:05:00**

13 Remove supernatant to a clean glass centrifuge tube by pasteur pipet.
Save the pasteur pipet for later.

14 Add **50 µl** MilliQ and **1 mL** Folch solvent to the residue.

15 Vortex **00:02:00**

16 Sonicate **00:02:00**

17 Remove supernatant with the pasteur pipet used in #13.
Combine with the supernatant obtained in #13.
Save the pasteur pipet for later.

18 Add **50 µl** MilliQ and **1 mL** Folch solvent to the residue.

19 Vortex **00:02:00**

20 Sonicate **00:02:00**

21 Remove supernatant with the pasteur pipet used in #13.
Combine with the supernatant obtained in #13.
Save the pasteur pipet for later.

22 Add **50 µl** MilliQ and **1 mL** Folch solvent to the residue.

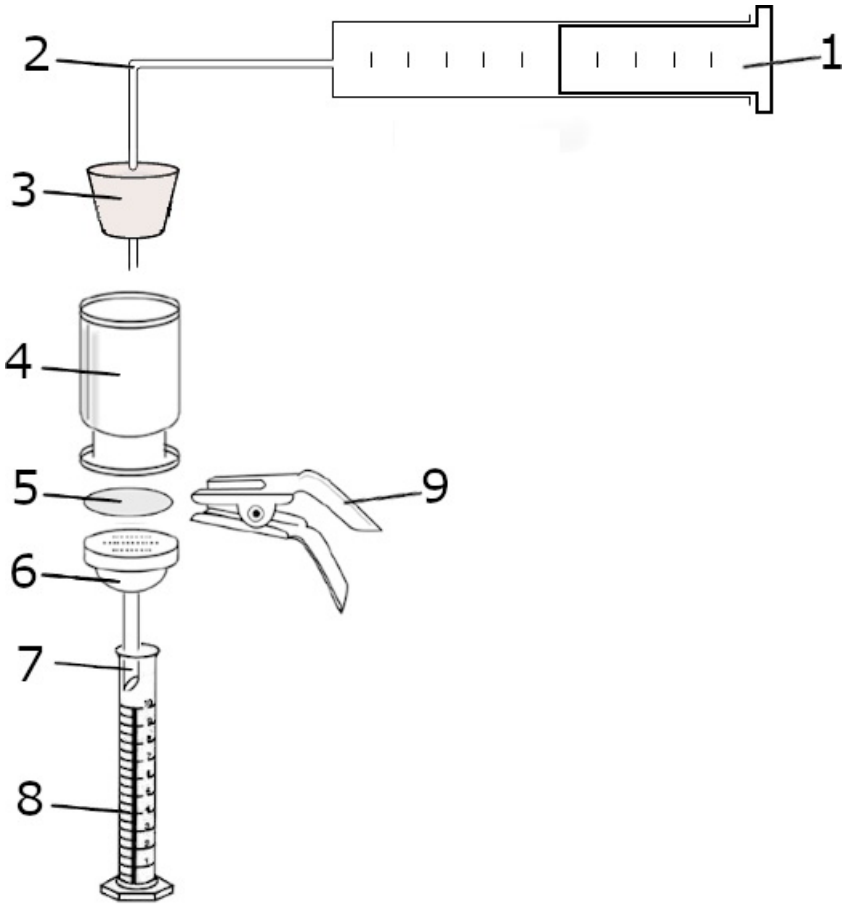
23 Vortex **00:02:00**

24 Sonicate **00:02:00**

25 Remove supernatant with the pasteur pipet used in #13.
Combine with the supernatant obtained in #13.
Save the pasteur pipet for later.

Filtration

26



How to setup the filtering system

26.1 In the figure:
(1) 50 ml syringe
(2) silicon tube
(3) silicon stopper
(4) filter tunnel
(5) GFF filter
(6) base
(7) neck of the base
(8) 10 ml graduated cylinder
(9) clamp

26.2 In order to avoid the loss of sample, before transfering extract into the funnel, check if the funnel is well assembled with the base by clamp.

26.3 The neck of the base must touch the inner side of the graduated cylinder, so that the filtrate can be all collected in the cylinder. In order to maintain the balance of air pressure, leave gap between the neck and the cylider.

27 Transfer extract into the funnel with the pasteur pipet used in #25.
Save the pasteur pipet for later.

28 Pull plunger back and push the bottom of stopper into the open top of funnel.

29 Slowly and steadily push the plunger to force the filtrate into the graduated cylinder.

30 Add **1 mL** Folch solvent and **50 µl** MilliQ into the centrifuge tube.

31 Rinse the tube.

32 Transfer the folch solvent/MilliQ mixture into the funnel with the pasteur pipet used in #27

33 Use positive pressure to force the filtrate into the same graduated cylinder.

34 Add **1 mL** Folch solvent and **50 µl** MilliQ into the centrifuge tube.

35 Rinse the tube.

36 Transfer the folch solvent/MilliQ mixture into the funnel with the pasteur pipet used in #27

37 Use positive pressure to force the filtrate into the same graduated cylinder.

38 Record the volume (V) of the filtrate in the cylinder to an accuracy of 0.1 ml.

39 Use a clean pasteur pipet to transfer all filtrate into a clean glass centrifuge tube.

40 Add **1 mL** Folch solvent and **50 µl** MilliQ into the graduated cylinder.

41 Rinse and transfer the Folch solvent/MilliQ mixture into the same glass centrifuge tube as in #39 by the pasteur pipet used in #39.

42 Add **1 mL** Folch solvent and **50 µl** MilliQ into the graduated cylinder.

43 Rinse and transfer the Folch solvent/MilliQ mixture into the same glass centrifuge tube as in #39 by the pasteur pipet used in #39.

44 Estimate the final volume of filtrate as (V+2)

Separation

45 Calculate the volume of 0.88% KCl by multiplying (V+2) by 4/21.

In order to obtain a biphase system to separate the extract from water, the final composition of $CHCl_3$:MeOH:H_2O is 8:4:3 (v/v)

46 Vortex the centrifuge tube for **00:01:00**

47 Centrifuge at **2000 rpm, Room temperature 00:05:00** or until biphase layers separate completely.

48 Remove most of upper aqueous phase with the pasteur pipet used in #39.

49 Use a clean pasteur pipet to transfer the lower organic phase to a 12 ml amber vial.

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

51 Add **5 mL** $CHCl_3$ by using serological pipet to the dry residue.

52 Freeze at **-80 °C** .