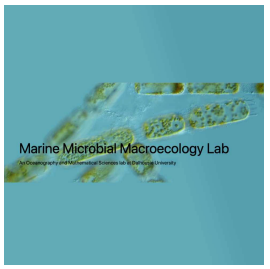
Aug 10, 2020 Version 1

Lipids in microalgae: The Extraction by modified Folch solvent V.1



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

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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.

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

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.

LINK

https://doi.org/10.3389/fmicb.2019.00763

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

MATERIALS

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

Equipment

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

Equipment

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

Equipment

Glass Microanalysis Filter Holders	NAME
VWR®	BRAND
89428-934	SKU

Equipment

Storage Vials and Closures	NAME
12 mL amber	TYPE
Thermo Scientific	BRAND
B7800-12A	SKU
VWR 66030-686	SPECIFICATIONS

Equipment

VWR® Vials, Borosilicate Glass, with Phenolic Screw Cap NAME

22.18 mL TYPE

VWR BRAND

66012-044 SKU

https://ca.vwr.com/store/product/en/4618574/vwr-vials-borosilicate-glass-with-phenolic-screw-cap LINK

24-400 cap: VWR 89076-764 SPECIFICATIONS

Equipment

Whatman Glass Microfiber Filters, Binder Free NAME

Grade GF/F, 25 mm TYPE

GE Healthcare BRAND

1825-025 SKU

Equipment

Bottle-top dispenser NAME

BrandTech Dispensette® S BRAND

4731330 SKU

Equipment

PYREX® Media Bottles

NAME

Corning®

BRAND

1395-100

SKU

Equipment

Polypropylene Screw Caps

NAME

Linerless, 15-415

TYPE

Kimble Chase

BRAND

73805-15415

SKU

Equipment

Screw Caps for Screw-Thread Sample Storage Vials

NAME

Screw Caps, 15-425, PTFE/PE Foam Liner

TYPE

Thermo Scientific

BRAND

B7815-15

SKU

Equipment

Lifetime Red™ Graduated Cylinders	NAME
10 mL	TYPE
Corning® PYREX®	BRAND
3046-10	SKU

Equipment

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

Equipment

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

Equipment

Aluminum Clamp NAME

VWR® BRAND

89428-944 SKU

Equipment

VWR ANALOG VORTEX MIXER NAME

VWR BRAND

10153-838 SKU

With tube insert SPECIFICATIONS

Equipment

General-purpose benchtop centrifuge NAME

IEC CENTRA CL2 TYPE

Thermo BRAND

00427 0F SKU

Equipment

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

Equipment

NAME	BRAND	SKU
Safetypette	Jencons	75856-442

Equipment

NAME	TYPE	BRAND	SKU
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)

Safety warnings

Safety information

Operate chloroform in fumehood.

Safety information

Follow the disposal guidelines regarding the halogenated organic waste.

Before start

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.

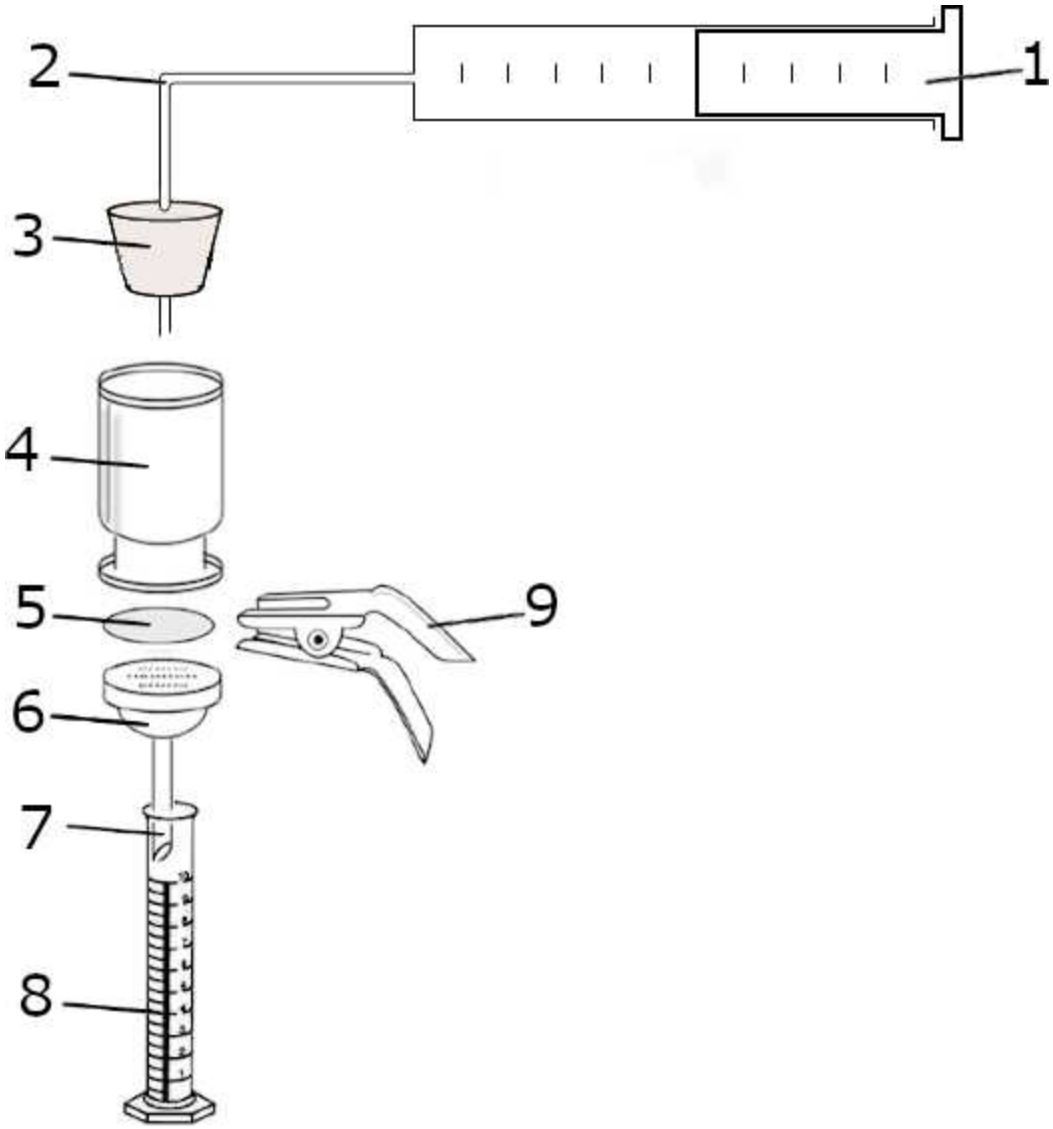
23 Vortex 00:02:00

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.

Note

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 .

Citations

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

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