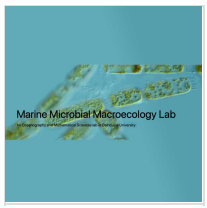

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Estimate phospholipids from microalgae

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Here we describe a protocol to estimate phospholipids from microalgae.
After the extraction and measurement of total lipids from microalgae, the rest of lipids extract is dried by nitrogen flow and then dried with magnesium sulphate at 90 °C. A 650 °C dry combustion is used to decompose 80% phospholipids. The ash is digested by 0.5 mL 0.2 M HCl for 30 minutes at 90°C. The resulting orthophosphate is detected by mixing the digested sample with a mixture of molybdate and ascorbic acid to produce molybdenum blue (Chen 1956).

P.S. Chen, T.Y. Toribara and Huber Warner. Microdetermination of Phosphorus. Anal. Chem..
https://doi.org/10.1021/ac60119a033

Yingyu Hu 2021. Estimate phospholipids from microalgae. **protocols.io** https://protocols.io/view/estimate-phospholipids-from-microalgae-b2yxqfxn

phospholipids, high temperature dry combustion

protocol ,

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Prepare phospholipids sample

1 Dry remaining organic phase extract of total lipids at **37 °C** under a stream of N_2 gas (<2 psi)

High temperature dry combust 9h

2 Use diamond pen to engrave the sample vials with numbers. Log number and sample code.

3 **0.17 M** $MgSO_4$ reagent:

Dissolve **1.023 g** $MgSO_4$ in 50 mL MilliQ water

Magnesium sulfate anhydrous **Fisher Scientific Catalog #M65500**

4 Add **200 µL** **0.17 M** $MgSO_4$ to the dry extract.

Sing-use pipet tip to avoid cross-contamination.

5 Cover the uncapped vials with foil and place in the oven at **90 °C** until samples are completely dry.

Forced air oven

VWR 89511-410

Remove samples out of the oven as soon as they are dried. If muffle furnace is not available, keep samples in vacuum desiccator.

9h

6 Combust dried samples at **650 °C** for **09:00:00**

Muffle furnace
F30428C
Thermo **10-505-13**

Only place glass vials in the muffle furnace. Foil can be combusted under **650 °C**

7 Allow samples to gradually cool down in the muffle furnace.

Digestion

8 **0.2 M** HCl reagent:

In a reagent bottle, dissolve one part of **12 N** HCl in 59 parts of MilliQ water

12 N Hydrochloric acid **Contributed by users**

Volume of HCl_0.2M_mL = (0.5_mL) X (#Sample + #Blank)

9 Preheat oven to **90 °C**

10 Add **0.5 mL** **0.2 M** HCl to each vial.

11 Tightly cap the vial and vortex.

12 Place vials in the oven for **00:30:00** 30m

13 Cool samples down to **Room temperature**

Preparing standard working solutions 2h

14 Standard working solutions and reagents can be prepared during sample digestion.

15 KH_2PO_4 primary standard stock solution (≈ 1 mM)

Potassium dihydrogen orthophosphate **ACP**

Chemicals Catalog #P-4550

15.1 Transfer about 1 g KH_2PO_4 into a beaker, cover the beaker with foil

15.2 Place the beaker into an oven, dry KH_2PO_4 at **110 °C** for at least **02:00:00** 2h

15.3 Move KH_2PO_4 into a vacuum desiccator, allow KH_2PO_4 to cool to room temperature

15.4 Dissolve around **0.136 g** dried KH_2PO_4 in **1 L** MilliQ water.

- Use 1 L volumetric flask
- Take notes of the actual weight of KH_2PO_4 for final concentration of standard stock solution

15.5 Transfer standard stock solution into a 1 L bottle and store in the fridge.

This stock solution lasts quite a long time, unless there is evidence for growth of algae or other extraneous biotic material.

16 Standard working solution

KH2PO4	Primary (ul)	MilliQ (ul)
S1	0	1000
S2	5	995
S3	10	990
S4	20	980
S5	50	950
S6	100	900
S7	150	850
S8	200	800

17 Transfer **500 µL** of each standard working solution to 2 mL microtube.

Preparing working reagents

18

All reagents are freshly prepared before colorimetric measurement.

19 [M]**6 N** (3 M) sulfuric acid reagent:

Carefully add 1 part [M]**18 M** concentrated sulfuric acid into 5 part MilliQ water

18M sulfuric acid **Contributed by users**

20 [M]**2.5 %** ammonium molybdate reagent:

Weigh **0.25 g** ammonium molybdate in a Falcon tube and top to **10 g** with MilliQ water.
Cap and shake until totally dissolved.

Ammonium molybdate **Sigma**
Aldrich Catalog #09878-100G

21 [M]**10 %** ascorbic acid reagent:

Weigh **1 g** ascorbic acid in a Falcon tube and top to **10 g** with MilliQ water;
Cap and shake until all dissolved.

Ascorbic acid **Sigma**
Aldrich Catalog #A5960-100G

Wrap the tube with foil if the reagent is not used right after prepared.

22 Calculate the volume of molybdate-ascorbic reagent:
Total volume of reagent_mL = (0.5 mL) X (#standard working solution + #samples + #blanks)

23 Mix the reagents into Falcon tube:

A	B
Reagent	Part(s) as in volume
MilliQ	2
6N sulphuric acid	1
2.5% ammonium molybdate	1
10% ascorbic acid	1

Colorimetric measurement 3h

24 Preheat incubator/shaker to **37 °C**

SHAKING INCUBATOR
71L
Corning® LSE™ **6753**

25 Add **500 µL** reagent to each standard, sample (in the vial) and blank, starting from blanks, including blank for standards and blank for samples.

Finntip Stepper Tips
5 mL
Thermo Scientific **9404200**

Before dispensing the reagent, wipe or dab the liquid drop on the outside of the tip, avoid wiping the open tip.

26 Vortex.

27 Incubate at **37 °C** for **03:00:00** while shaking at 150 rpm 3h

28 Load microplate with 250 ul reactant from each tube, duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	Samples and sample blanks: 40 with duplicate									
B	S2	S2										
C	S3	S3										
D	S4	S4										
E	S5	S5										
F	S6	S6										
G	S7	S7										
H	S8	S8										

Example of loading the microplate

96-Well Microplates, Polystyrene, Clear,

Greiner Bio-One **655101**

29 Read plate in microplate reader

A	B
Shake duration	00:00:05
Shaking type	Continuous
Shaking force	High
Shaking speed [rpm]	600
Wavelength [nm]	820
Use transmittance	No
Pathlength correction	No
Measurement Time [ms]	100

Varioskan LUX Multimode Microplate Reader

Thermo Fisher **VL0L00D0**

Calculation 3h

30 Subtract the average absorbance at 820 nm of the blank standard replicates from the absorbance at 820 nm of all other standard working solutions.

31 Subtract the average absorbance at 820 nm of the blank sample (i.e. blank filter) replicates from the absorbance at 820 nm of all other individual samples.

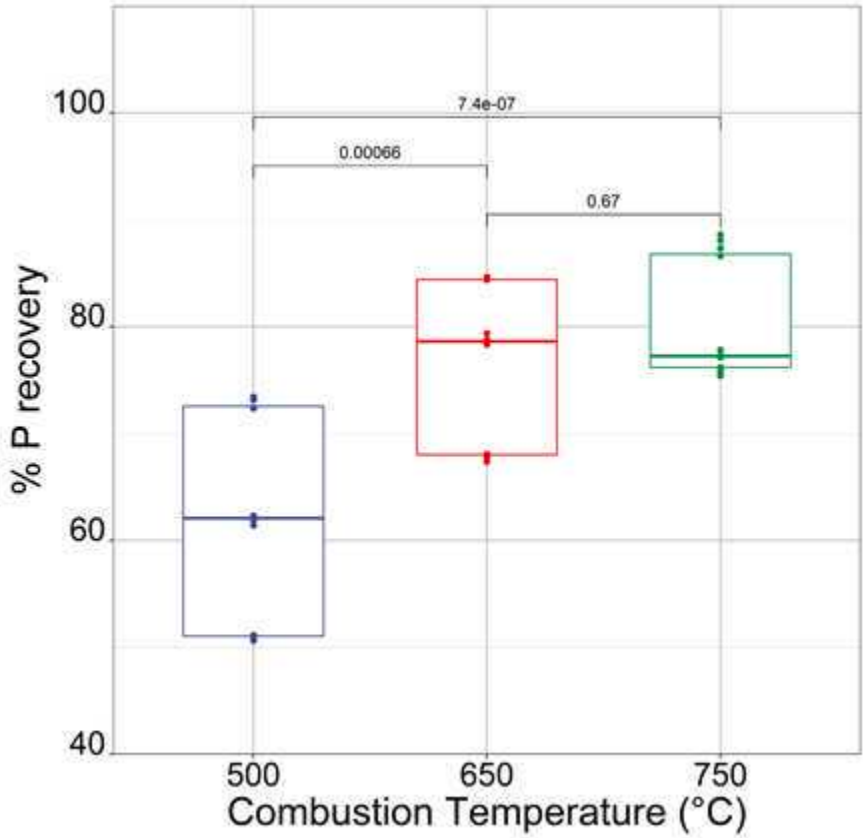
32 Prepare a standard curve by plotting the average blank-corrected 820 nm absorbance for each standard working solution versus its concentration in uM.

33 Use the standard curve to determine the orthophosphate concentration of each unknown sample by using its blank-corrected 820 nm absorbance.

34 $(P_{measured})\_ug/sample = (orthophosphate)\_uM \text{ X } (V\_HCl)\_mL \text{ X } (0.001) \text{ X } (30.97)$

$(P_{corrected})\_ug/sample = (P_{measured}) / 0.8$

Where, 0.8 is the average recovery of phospholipids after a high temperature dry combustion at **650 °C** .



35 (Phospholipids)_ug/sample = ($P_{corrected}$)/(0.01X4.3)