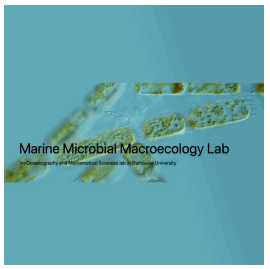
Dec 12, 2022

Chloroform-methanol protein precipitation from microalgae and Pierce BCA assay



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Ying-Yu Hu[1], Christopher Lord[1], Zoe V Finkel[1]

[1]Dalhousie University

Marine Microbial Macroecology Lab
Tech. support email: **ruby.hu@dal.ca**

Ying-Yu Hu
Dalhousie University

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We use this protocol and it's working

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Abstract

Chlorophyll, phospholipids, sucrose, glycerol and some detergent in crude protein extracted from microalgae can interfere the Pierce BCA protein assay. In order to remove these interference, bead miller extracted protein is precipitated by chloroform-methanol prior to BCA assay. The resulting precipitation is dissolved into Sarcosine-Tris solution. Low limit of detection is about 5 ug/mL.

Protocol materials

- Tris(hydroxymethyl)aminomethane hydrochloride 1M pH 8.0 RNase free **Fisher Scientific Catalog #**AAJ60080AK
- N-lauroylsacosine sodium salt solution (30%) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**61747
- Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34860
- Chloroform (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**439142-4L
- Thermo Scientific™ Pierce™ Bovine Serum Albumin Standard 2 mg/mL (50 mL) **Thermo Scientific Catalog #**Thermo Scientific™ 0023210
- Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225

Troubleshooting

Safety warnings

Use fume-hood when handling methanol and chloroform.

All waste containing methanol and chloroform shall be collected in waste container for halogenated organic solvents.

Reagent preparation

1 Tris buffer [M] 5 Mass Percent (pH 8.0)

Add 500 µL [M] 1 Mass Percent pH 8.0 Tris into 100 mL MilliQ water

Tris(hydroxymethyl)aminomethane hydrochloride 1M pH 8.0 RNase free **Fisher Scientific Catalog #**AAJ60080AK

2 20% Sarcosine

Dilute 2 part 30% N-lauroylsarcosine sodium salt with 1 part [M] 5 Mass Percent (pH 8.0) Tris buffer

N-lauroylsacosine sodium salt solution (30%) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**61747

Protein precipitation

1h 12m

3 Thaw protein extract

4 Turn on refrigerate centrifuge

Equipment	
CENTRIFUGE 5430 R	NAME
Eppendorf	BRAND
MP2231000510	SKU

5 Turn on incubator/shaker, preheat to 37 °C

Equipment

SHAKING INCUBATOR	NAME
71L	TYPE
Corning® LSE™	BRAND
6753	SKU

6 Prepare ice-bath

7 Well mix the extract and then transfer 100 µL of extract to 2 mL microtube (Abdos tubes give better precipitation results), in replicate.

Equipment

Micro Centrifuge Tubes	NAME
Abdos	BRAND
P10203	SKU

Note

If extract has debris, spin down debris by 13300 rpm, Room temperature, 00:05:00 and transfer only clear supernatant. Debris can cause overestimation of protein content.

8 In the fume hood, add 400 µL methanol

Methanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34860

9 Gently vortex for 00:00:30 by using a tube insert 30s

Equipment

VWR ANALOG VORTEX MIXER	NAME
VWR	BRAND
10153-838	SKU
With tube insert	SPECIFICATIONS

10 In the fume hood, add 100 µL chloroform

Chloroform (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**439142-4L

11 Gently vortex for 00:00:30 by using a tube insert 30s

12 In the fume hood, add 300 µL MilliQ

13 Gently vortex for 00:00:30 by using a tube insert 30s

14 Incubate On ice for 00:30:00 30m

15 20000 rcf, 4°C, 00:10:00 10m

16 In the fume hood, remove upper phase by leaving about 250 µL liquid

Note

Do not disturb the interphase

17 In the fume hood, add 300 µL methanol

18 Gently mix the liquid until bottom layer disappear and the solution is homogenous.

Note

The formation of small pellet might be observed, but might be invisible due to low protein mass.

19 20000 rcf, 4°C, 00:10:00 10m

20 In the fume hood, remove all solvent.

Note

Watch the pipet closely. Do not remove pellets with the solvent.

21 If pellet tends to be aspired with solvent, add another 300 µL methanol, gently vortex, and 20000 rcf, 4°C, 00:10:00 10m

22 In the fume hood, remove most solvent by using 1000 uL pipet tip, and then remove the rest by using 100 uL pipet tip. Do not remove pellet with solvent.

23 Dry pellet in vacuum desiccator for at least 00:30:00 at Room temperature 30m

Note

Any methanol and chloroform residue can affect the re-dissolving of pellet in BCA assay. However, do not dry protein pellet for too long, otherwise it might be difficult to re-dissolve.

BCA assay

30m

24 Add 5 µL 20% sarcosine and 95 µL [M] 5 Mass Percent (pH 8.0) Tris buffer to dry protein pellet, incubate at 37 °C for 15 to 30 min.

25 Use tube insert, vortex all tubes for 15 to 30 min until pellet is completely re-dissolved.

26 BSA standard solutions

Thermo Scientific™ Pierce™ Bovine Serum Albumin Standard 2 mg/mL (50 mL) **Thermo Scientific Catalog #**Thermo Scientific™ 0023210

	Standard	20% sarcosine (uL)	5 mM Tris (uL)	2 mg/mL BSA (uL)	Final Conc. (mg/mL)
	SD1	5	95	0	0
	SD2	25	470	5	0.02
	SD3	25	463	12	0.048
	SD4	25	450	25	0.1
	SD5	25	425	50	0.2
	SD6	25	375	100	0.4
	SD7	25	275	200	0.8
	SD8	25	225	250	1

27 Vortex and then use reverse pipetting: transfer 100 µL standard solutions into the corresponding tubes, except for SD1 (it has already been 100 uL).

28 Use the following formula to determine the total volume of working reagent (WR) required. Consider leaving several mL of extra volume:

(# standards + # samples) X (800 µL) = total volume WR required

29 Prepare WR by mixing 50 parts of BCA reagent A with 1 part of BCA Reagent B in a 50 mL falcon tube

Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225

30 Use one tip and reverse pipetting: Add 800 µL WR into each tube, make sure that the tip doesn't have contact with the solution, so that samples are not cross-contaminated.

Note

Since BCA assay is sensitive to duration, although reagent is aqueous, it is more efficient to use reverse pipetting and quickly dispense reagent into all tubes, therefore the duration difference amongst standards and samples can be minimized.

31 Vortex each tube, shake and incubate at 37 °C for 00:30:00 30m

32 Remove samples from the incubator.

33 Load samples into microplate in duplicate:

Note

1. Reverse pipetting: aspire 200 µL sample from the middle of solution
2. Tip gently touches the side of the well, avoid bending. Dispense 200 uL into the microplate
3. Dispose the tip
4. Use a new tip, reverse pipet another 200 µL as replicate
5. Tip gently touches the side of the well, avoid bending. Dispense 200 uL into the microplate

Equipment

NAME	TYPE	BRAND	SKU
96-Well Microplates	Polystyrene, Clear,	Greiner Bio-One	82050-760

34 Shake for 5 s at 600 rpm in a continuous and high force mode
Read endpoint 562 nm with a measurement time 100 ms

Equipment

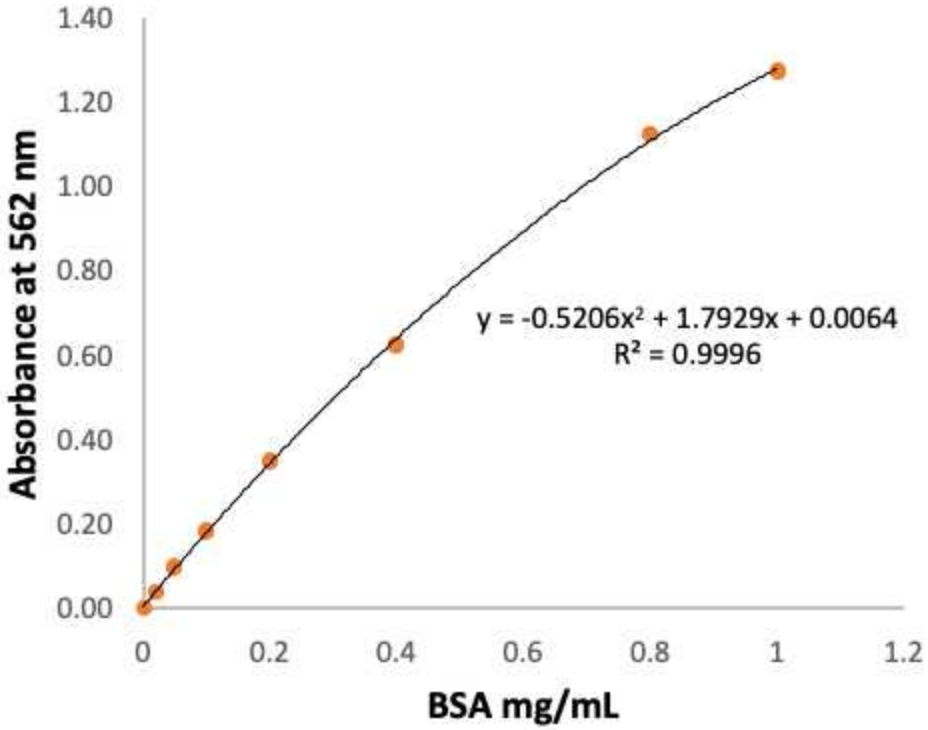
Varioskan LUX Multimode Microplate Reader	NAME
Thermo Fisher	BRAND
VL0L00D0	SKU

Calculation

35 Subtract the average 562 nm absorbance measurement of the blank standard replicates from the 562 nm measurements of all other individual **standard**.

36 Subtract the average 562 nm absorbance measurement of the blank sample (filter) replicates from the 562 nm measurements of all other individual **sample**.

37 Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard versus its concentration in mg/ml. The standard curve is quadratic.



38 For the calculation convenience, plot BSA concentration (Conc) versus Corrected absorbance (Abs) to obtain a standard curve as following:
Conc_mg/mL = *a* X Abs^2 + *b X* Abs + *c*

Use the corrected measured absorbance of samples (Abs) to calculate the total protein concentration (Conc_mg/mL) from each sample.

39 Protein_mg/filter = Conc_mg/mL X PEB_mL
Where PEB is the volume of protein extraction buffer used to extract protein from microalgae sample.