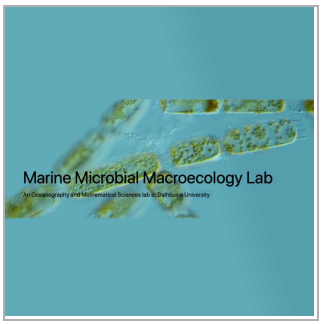

DEC 12, 2022

WORKS FOR ME 1

Chloroform-methanol protein precipitation from microalgae and Pierce BCA assay

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.yxmvm2e25g3p/v1

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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.

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PROTOCOL CITATION

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Protein , Microalgae, Chloroform-methanol precipitation, Pierce BCA assay

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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 mM (pH 8.0)

Add 500 µL [M] 1 M 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 mM (pH 8.0) Tris buffer

N-lauroylsacosine sodium salt solution (30%) **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

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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

8 In the fume hood, add 400 µL methanol

Methanol **Sigma Aldrich Catalog #34860**

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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) **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

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17 In the fume hood, add 300 µL methanol

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

Note

19 10m

20000 rcf, 4°C, 00:10:00

20 In the fume hood, remove all solvent.

Note

21 10m

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

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 30m

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

Note

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30m

BCA assay

24 Add 5 µL 20% sarcosine and 95 µL [M] 5 mM (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

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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

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

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

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.

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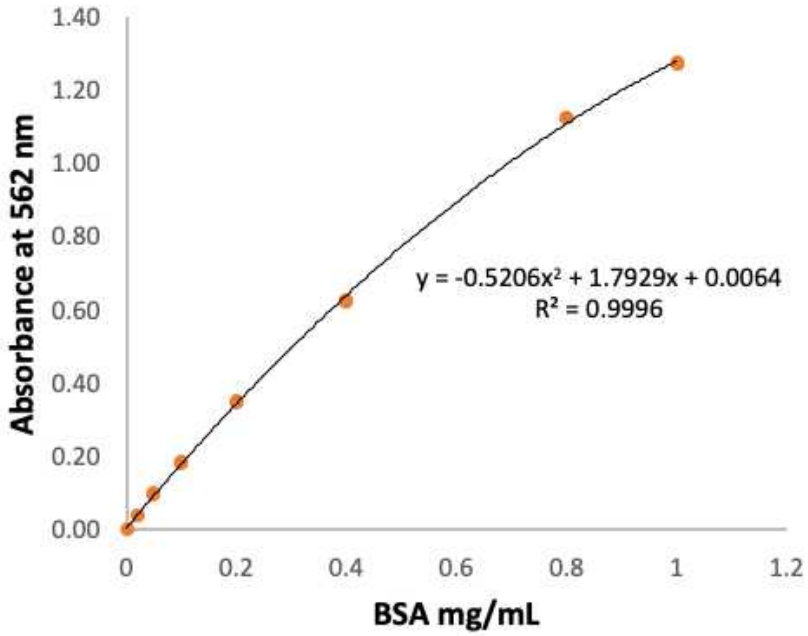

38 For the calculation convenience, plot BSA concentration (Conc) versus Corrected absorbance (Abs) to obtain a standard curve as following:
Conc_mg/mL = *a* X Abs^2 + *b X* Abs + *c*

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.

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