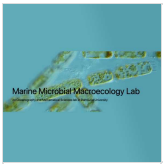

Version 2

Jul 21, 2021

Total protein in microalgae: Pierce BCA protein assay V.2

Yingyu YY Hu[1], Zoe V. Finkel[1]

[1]Dalhousie University

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


Yingyu Hu
Dalhousie University

ABSTRACT

Here we describe a protocol for extracting total protein from microalgae and quantifying total protein by Pierce BCA protein assay.

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0011430_Pierce_BCA_Protein_Asy_UG.pdf

PROTOCOL CITATION

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microalgae, total protein, Pierce BCA, protein solubilization buffer, bead mill cell disruption, microplate

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GUIDELINES

1. Working range of Pierce BCA assay is 20-2000 ug/ml protein.
2. Minimum sampling volume (mL) = 750/(Chl-a_ug/L), if protein is extracted by 500 ul extraction buffer.

Sample collection

1 Calculate the volume to obtain enough biomass for the assay:

Minimum sampling volume (mL) = 750/(Chl-a_ug/L), if protein is extracted by 500 ul extraction buffer.

2 Use 5 inches Hg to filter microalgae samples onto polycarbonate filters (pore size varies).

3 Fold filter in quarter and place into an MP biomedical bead tube.

- Use 2 ml lysing matrix tube for 25 mm filter; 15 ml Teenprep tube for 47 mm filter
- Large ceramic beads are recommended for diatoms and larger phytoplankton cells (2 mL: MP116913500/ 15 mL: MP116933050)
- Small yttrium beads are recommended for picoplankton (2 mL: MP116960100/ 15 mL: MP116975050)

LYSING TUBES
MATRIX D 2 mL/15 mL
MP BIOMEDICALS 116913500/116933050

Lysing tubes
Matrix Y 2 mL/15 mL

MP Biomedicals	**MP116960100/MP11697505 0**

4 Flash-freeze tubes in thermo flask with liquid nitrogen

5 Store at **-80 °C** until further processing

6 Freeze dry samples before processed.

Prepare protein solubilization buffer (PSB)

7

Ni G, Zimbalatti G, Murphy CD, Barnett AB, Arsenault CM, Li G, Cockshutt AM, Campbell DA (2017). Arctic Micromonas uses protein pools and non-photochemical quenching to cope with temperature restrictions on Photosystem II protein turnover.. Photosynthesis research.
https://doi.org/10.1007/s11120-016-0310-6

8 In order to obtain compatible results, prepare sufficient PSB so that the same PSB can be used for sample extraction, blank filter extraction and standard solutions
(1) Extract all samples: Each sample requires 0.25 mL PSB

(2) Extract all blank filters: Each filter requires 0.25 mL PSB
(3) Each standard solution (500 ul) requires 0.125 mL PSB

9 For each 10 g PSB

9.1 Use antistatics weighing dish to weigh the following chemicals:

Antistatic weighing dish
Fisherbrand **08-732-112**

(1) 0.136 g Tris base

Tris base **Bioshop Catalog #TRS001.500**

(2) 0.133 g Tris HCl

Tris HCl **Bioshop Catalog #TRS002.500**

(3) 0.8 g Lithium dodecyl sulphate
Lithium Dodecyl Sulphate **Bioshop Catalog #LDS701.25**

9.2 Place a plastic beaker on the top of the scale surface

9.3 Remove the cap of a 15 mL tube and sit it in the beaker

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 15 mL
Corning® **352096**

9.4 Tare the total weight of beaker and tube

9.5 Transfer all chemicals weighed in **go to step #9.1** into the tube, rinse the dish with small amount of MilliQ water to make certain all of the solutes is transferred into the tube

9.6 Use a transfer pipet to add 4 g glycerol into the tube

Glycerol **Bioshop Catalog #GLY001.500**

9.7 Add 40 ul 0.5 M EDTA into the tube

EDTA buffer solution (0.5 M) **Sigma Aldrich Catalog #4055-100ml**

9.8 Top to 10 g with MilliQ water

9.9 Mix by shaker until all solutes are completely dissolved.

Prepare this solution in advance, since it takes some time to have solutes dissolved, especially LDS.

Prepare Pefabloc solution

10

4-(2-2-aminoethyl)-benzenesulfonyl fluoride HCL (AEBSF, Pefabloc) **Bioshop Catalog #AEB602.100**

Pefabloc is a protease inhibitor, and it loses activity over 24 hours.

11 Add 20.86 mL MilliQ into 100 mg Pefabloc to obtain a final concentration of 20 mM.

12 Aliquot into 2.5 ml portions and keep frozen at **-20 °C**

13 The solution can be frozen~thawed multiple times.

Assay Day 1: Extract protein from microalgae

14 Prepare protein extraction buffer (PEB):

Each 1 mL PEB has

250 ul PSB
20 ul 20 mM Pefabloc
730 ul MilliQ water

15 Prepare ice-bath, keep all samples in the ice-bath

16 If samples and blank filters are already in bead tubes, add 1 mL PEB onto the filter directly.

Volume of PEB varies due to the actual biomass collected (see guideline)

17 If samples are in cryo tubes instead of bead tubes

17.1 Rinse forceps with 70% ethanol and air dry

Filter forceps
blunt end, stainless steel
Millipore **XX6200006P**

17.2 Label bead tubes and use clean forceps to transfer samples and blank filters into its corresponding bead tube.

17.3 Add 1 mL PEB onto the filter.
When using 15 mL Teenprep tube, horizontally shake the tube to bury filter into beads before adding PEB, which makes filter easy to be homogenized.

Volume of PEB varies due to the actual biomass collected (see guideline)

18 Turn on FastPrep

FastPrep-24 5G
Bead beater
MP Biomedicals **116005500**

19 Check the cap of each tube to make certain cap is tightly screwed. Organize the tubes in order, take notes of the position of each tube, in case the labels get rubbed out during extraction.

20 Run 1 min at 6.5 m/s

21 Keep tubes **On ice** for **00:01:00**

22 Check labels. Put tubes back into FastPrep.

23 Run 1 min at 6.5 m/s

24 Keep tubes **On ice** for **00:01:00**

25 Check labels. Put tubes back into FastPrep.

26 Run 1 min at 6.5 m/s

27 Keep tubes **On ice** for **00:01:00**

28 Check labels. Put tubes back into FastPrep.

29 Run 1 min at 6.5 m/s

30 Turn on refrigerated centrifuge

CENTRIFUGE 5430 R
Eppendorf **MP2231000510**

31 Keep tubes **On ice** for **00:01:00**

32 Centrifuge: 10m
2 mL Lysing Matrix tubes at **14800 x g, 4°C, 00:05:00**
15 mL Teenprep tube at **6500 x g, 4°C, 00:05:00**

33 Transfer all supernatant to a 1.7 mL microtube

For the extraction with 500 ul PEB in Teenprep tube, use 200 ul tip and go straight to the bottom from the side.

Microcentrifuge Tubes
1.7 mL/0.6 mL
Axygen Scientific MCT-175-C/MCT-060-L-C

34 Centrifuge at **14800 x g, 4°C, 00:05:00** to completely remove debris. 5m

35 Transfer supernatant to microtube, avoid any debris.

36 Freeze at **-80 °C**

Assay Day 2: Prepare Bovine serum albumin (BSA) standard solutions

37 BSA 2 mg/mL standard **Thermo Fisher Scientific Catalog #23209**

38 Thaw 20 mM pefabloc and transfer 150 ul to a 600 ul mcirotube. Put the rest of the stock back into the freezer immediately.

39 Organize eight 1.7 mL microtubes in the tube rack, label the tubes from SD1 to SD8.

40 Use 1.25 mL Finntip Stepper Tip (Thermo Fisher 9404180) to dispense 125 ul PSB into each microtube.

Finntip stepper tip
1.25 mL
Thermo Fisher 9404180

41 Use 0.5 mL Finntip Stepper Tip to dispense 10 ul pefabloc into each microtube

Finntip™ Stepper Pipette Tips
500 uL
Thermo Scientific™ **9404170**

Wipe or dab the liquid drop on the outside of the tip, avoid wiping the tip open before dispensing the liquid.

42 Add MilliQ into each microtube according to the sheet below:

Standard	Pefabloc (20 mM) (µL)	PSB (µL)	MQ (uL)	BSA (2mg/mL) (uL)	Final Conc. (mg/mL)
SD1	10	125	365	0	0
SD2	10	125	360	5	0.02
SD3	10	125	353	12	0.048
SD4	10	125	340	25	0.1
SD5	10	125	315	50	0.2
SD6	10	125	265	100	0.4
SD7	10	125	165	200	0.8
SD8	10	125	115	250	1

43 Break the ampule of BSA standard

SCIENCEWARE® Break-Safe™ Ampule Opener
Bel-Art® **89217-378**

44 Reverse pipet certain amount of BSA (2 mg/mL) into each tube according to the sheet **go to step #42**

Wipe or dab the liquid drop on the outside of the tip, avoid wiping the tip open before dispensing the liquid.

45 Vortex each tube.

46 Dispense 4 uL of each standard solution onto microdrop plate.

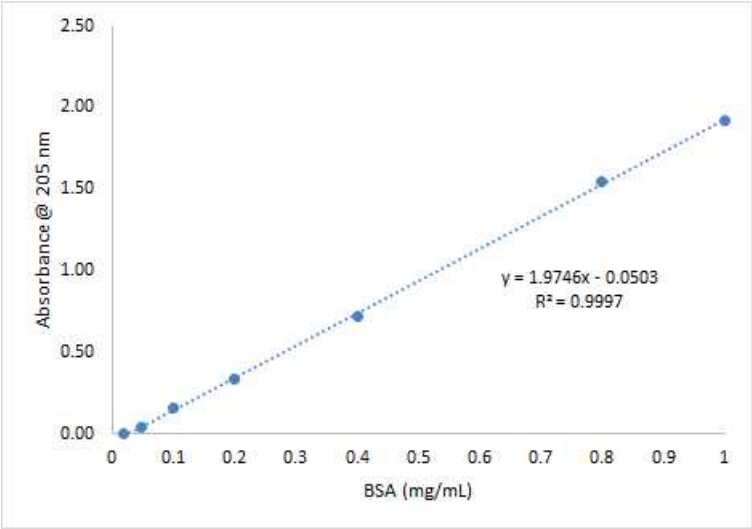
μDrop™ Plates
Thermo Scientific N12391

47 Read absorbance of eight standard solutions at 205 nm

Varioskan LUX Multimode Microplate Reader
Thermo Fisher VL0L00D0

48 Subtract absorbance at 205 nm of blank standard from the 205 nm measurements of all other standard solutions

49 Plot the blank-corrected 205 nm measurement for each standard solution versus its concentration in mg/ml.



Example of BSA standard curve: Absorbance read at 205 nm versus concentration (mg/mL)

50 If the standard curve has good Coefficient of Determination, i.e. R^2>0.99, the standard solutions are in good quality; otherwise, prepare a new series of standard solutions until the quality of standard solutions passes the screen.

51 Standard solutions can be kept at **Room temperature** .

Assay Day 2: Prepare BCA working reagent (WR)

52 Use the following formula to determine the total volume of WR required. Consider leaving several mL of extra volume since Finntip stepper is unable to expel the entire volume from the tip:

(# standards + # samples + # blank filters) X (# replicates) X (200 ul) = total volume WR required

53 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**

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 50 mL
Corning® **352070**

Assay Day 2: Pierce BCA assay 2h

54 Turn on incubator and preheat to **37 °C**

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

55 Transfer extracted samples from **-80 °C** to **On ice**

56 Prepare 96-well microplate and lid:
Each microplate can load eight standard solutions and forty samples+blanks, duplicated

Pipet tip is for single use only.

96-Well Microplates
Polystyrene, Clear,
Greiner Bio-One **82050-760**

Microplate Lids
Polystyrene
Greiner Bio-One **07000288**

57

	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 organizing samples on the microplate.

58 Reverse pipette 25 ul of each standard or sample/blank replicate into the microplate well.

59 Use 2.5 mL Finntip stepper tip to load 200 ul WR, starting from blank, including blank of standard solution and blank of the samples.

Finntip™ Stepper Pipette Tips
2.5 mL
Thermo Fisher **9404190**

Wipe or dab the liquid drop on the outside of the tip, avoid wiping the tip open before dispensing the liquid.

Dispense WR column by column.
Refill the stepper in between two columns of wells.

60 Cover plate with lid.

61 Shake and incubate at **37 °C** for **00:30:00**

62 Read absorbance at 562 nm

Varioskan LUX Multimode Microplate Reader
Thermo Fisher **VL0L00D0**

Calculate protein content per filter

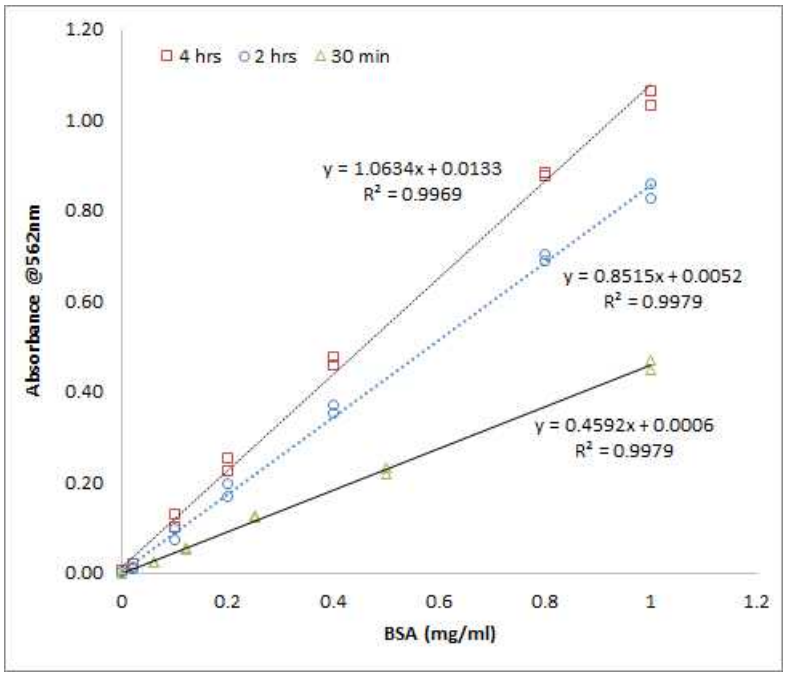
63 Subtract the average 562 nm absorbance measurement of the blank standard replicates from the 562 nm measurements of all other individual **standard**.

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

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

66 Use the standard curve to determine the protein concentration of each unknown sample by using its blank-corrected 562 absorbance.

67



Increasing the incubation time can increase the net 562 nm measurement for each well and lower the minimum detection of the assay.

68 Protein_mg/filter = Protein_mg/mL X PEB_mL