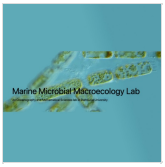

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Total protein in microalgae: Pierce BCA protein assay

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1 *Works for me* This protocol is published without a DOI.

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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

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KEYWORDS

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

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GUIDELINES

1. Considering the working range of Pierce BCA assay is 20-2000 ug/ml protein, an ideal protein biomass for each sample is around 200 ug/filter extracted by 1 ml protein extraction buffer. The minimum requirement of protein is no less than 50 ug (extracted by 500 ul protein extraction buffer).
2. Under replete condition, assuming protein is 32% in dry mass, 50 ug of protein requires around 160 ug of dry mass, which is about 60 ug total carbon for each sample.

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

Sample collection

1 Calculate the volume to obtain enough biomass for the assay (see guidelines)

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 bead tube for 25 mm filter; 15 ml bead 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	**MP116960100/MP11697505**
Biomedicals	**0**

4 Flash-freeze tubes in thermo flask with liquid nitrogen

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

Prepare protein solubilization buffer (PSB)

6

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

7 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

8 For each 10 g PSB

8.1 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**

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

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

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

8.4 Tare the total weight of beaker and tube

8.5 Transfer all chemicals weighed in **go to step #8.1** into the tube, rinse the dish with small amount of milliQ water to make certain all of the solute is tranferred into the tube

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

Glycerol **Bioshop Catalog #GLY001.500**

8.7 Add 40 ul 0.5 M EDTA into the tube
EDTA buffer solution (0.5 M) **Sigma Aldrich Catalog #4055-100ml**

8.8 Top to 10 g with milliQ water

8.9 Mix by vortex or shaker until all solute is completely dissolved.

Prepare Pefabloc solution

9 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.

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

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

12 The solution can be frozen~thawed multiple times.

Extract protein from microalgae

13 Prepare protein extraction buffer (PEB):
In each 1 mL PEB:
250 ul PSB
20 ul 20 mM Pefabloc
730 ul milliQ water

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

15 If samples and blank filters are in bead tubes, add 1 mL PEB to each tube directly.

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

16 If samples are in cryo tubes instead of bead tubes

16.1 Rinse forceps with 70% ethanol and air dry

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

16.2 Label bead tubes and add 1 mL PEB into each bead tube.

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

16.3 Use clean forceps to transfer samples and blank filters into its corresponding bead tube.

17 Turn on FastPrep

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

18 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.

19 Run 1 min at 6.5 m/s

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

21 Check labels. Put tubes back into FastPrep.

22 Run 1 min at 6.5 m/s

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

24 Check labels. Put tubes back into FastPrep.

25 Run 1 min at 6.5 m/s

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

27 Check labels. Put tubes back into FastPrep.

28 Run 1 min at 6.5 m/s

29 Turn on refrigerated centrifuge

CENTRIFUGE 5430 R
Eppendorf **MP2231000510**

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

31 Centrifuge tubes at **14800 x g, 4°C, 00:05:00**

32 Transfer 100 ul supernatant to a 600 ul microtube

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

Sample volume for Pierce BCA is 25 ul for each assay. Considering that sample is dispensed into microplate by reverse pipetting, it requires at least 100 ul to dispense 2X25 ul sample.
If triplicate is preferred, transfer 150 ul supernatant.

33 Freeze both the supernatant and the rest at **-80 °C**

Prepare Bovine serum albumin (BSA) standard solutions

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

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

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

37 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.

38 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**

39 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

40 Break the ampule of BSA standard

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

41 Reverse pipet certain amount of BSA (2 mg/mL) into each tube according to the sheet **go to step #39**

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

42 Vortex each tube.

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

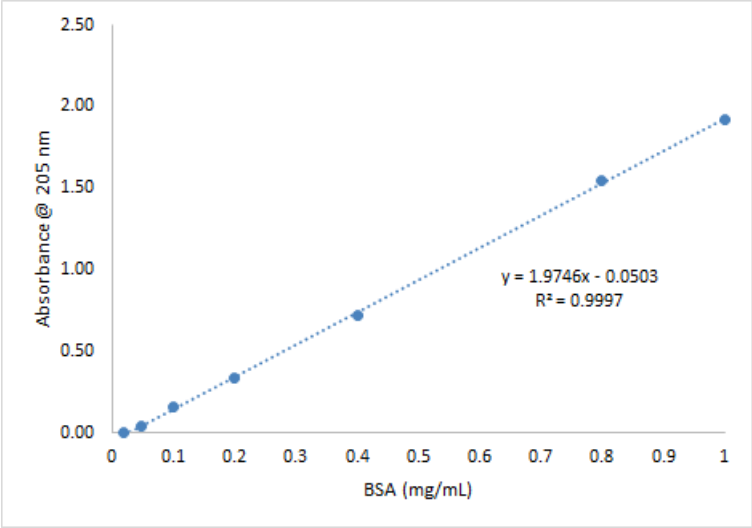
µDrop™ Plates
Thermo Scientific **N12391**

44 Read absorbance of eight standard solutions at 205 nm

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

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

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



47 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.

Prepare BCA working reagent (WR)

48 Use the following formula to determine the total volume of WR required. Consider 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

49 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**

Pierce BCA assay 2h

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

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

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

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

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

52

	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										

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

54 Use 2.5 mL Finntip stepper tip to load 200 ul WR to each well.

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.

55 Cover plate with lid.

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

57 Read absorbance at 562 nm

Varioskan LUX Multimode Microplate Reader

Thermo Fisher **VL0L00D0**

58 Put plate back into incubator. Shake and incubate for another **01:30:00**

59 Read absorbance at 562 nm

Calculate protein content per filter

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

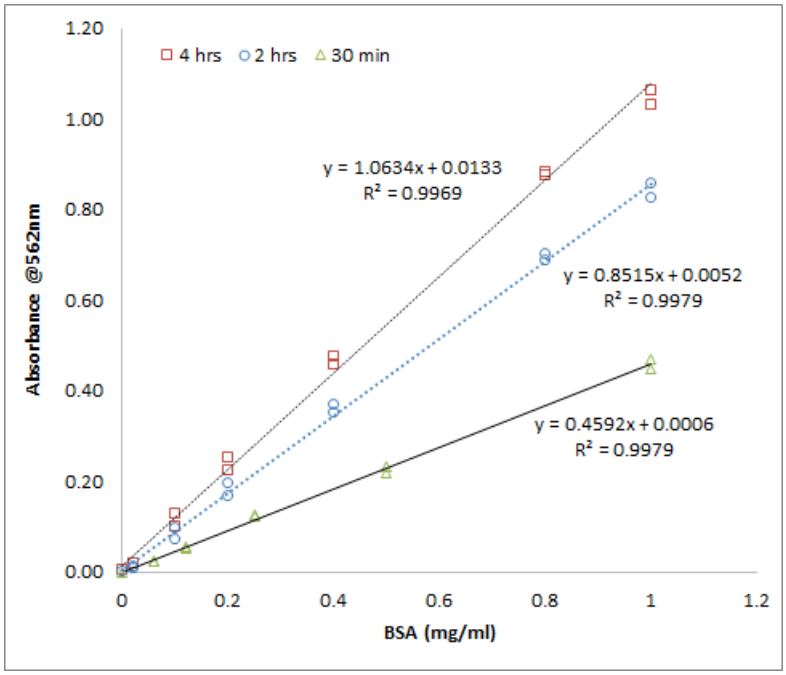
61 Subtract the average 562 nm absorbance measurement of the blank sample (filter) replicates from the 562 nm measurements of all other individual **sample**.

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

If using cruve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit.

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

64



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

65 Protein_mg/filter = Protein_mg/mL X PEB_mL