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Protocol status: Working
We use this protocol and it's
working

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Comprehensive Protocol for Total Protein Extraction, Precipitation, and BCA Assay Measurement from Plankton Samples V.1

 Forked from [Total crude protein in plankton: Pierce BCA protein assay \(including the enhanced assay for low biomass\)](#)

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ABSTRACT

This protocol outlines an optimized method for extracting total protein from plankton samples, encompassing both phytoplankton and zooplankton. The procedure involves the use of lysing matrix tubes with beads of varying sizes (0.1 mm, 1.4 mm, and 4 mm), eight homogenizing cycles, and a protein extraction buffer composed of 2% SDS, 10% glycerol, 5 mM EDTA in 100 mM Tris (pH 7). The extracted crude protein is quantified using the Pierce BCA assay, either incubation at 37°C for 20 to 2000 ug/mL or at 60°C for 5 to 200 ug/mL, using bovine serum albumin (BSA) as the standard.

To enhance accuracy, the extracted protein undergoes precipitation using a chloroform and methanol solvent mixture, followed by redissolution in re-solubilization buffer composed of 1% sarcosine in 50 mM Tris (pH 7). BCA assay is then used to quantify the precipitated protein, with BSA as the standard. The absence of glycerol and the use of sarcosine instead of SDS enable a linear detection range of 5 to 2000 µg/mL in this BCA assay at 37°C incubation.

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

Keywords: microalgae, total protein, Pierce BCA, bead mill cell disruption, microplate, zooplankton, chloroform-methanol precipitation, protein extraction buffer

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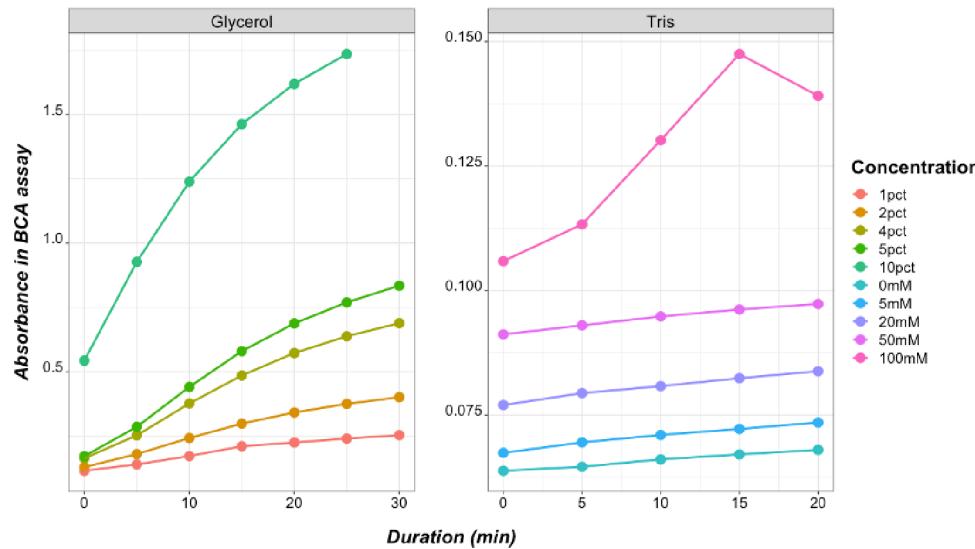
Grant ID: 549937

GUIDELINES

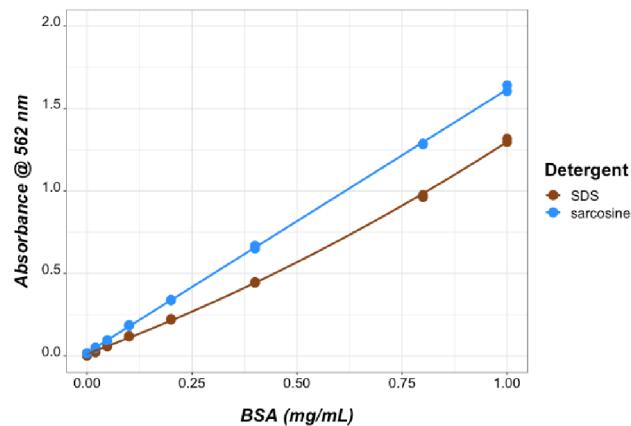
- Comparison of protein yield between the [previous method](#) and the improved method. The "Ratio" column represents the protein obtained from the improved method divided by that from the previous method.

Species	Condition	Ratio
Synechococcus (MITS1220)	Exponential	3.29 (0.45)
L. fissa (CCMP2935)	Stationary	2.87 (0.33)
L. fissa (CCMP2935)	Decline	2.23 (0.18)
Zooplankton (size: 500 - 2k um)	field sample	1.95 (0.06)
T. pseudonana (CCMP1335)	Exponential	1.25 (0.17)
P. calceolata (RCC100)	Exponential	1.23 (0.08)
P. calceolata (RCC100)	Stationary	0.98 (0.11)

- Impact of glycerol and Tris buffer concentration on BCA assay, where pct denotes percentage.



- Comparison of BCA assay response in precipitated protein dissolved in SDS vs sarcosine



4. The comparison involves BSA standard solutions prepared using only sarcosine and 50 mM Tris pH 7 buffer, with incubation times of 30 minutes and 60 minutes. Notably, the removal of glycerol aims to mitigate the BCA assay's sensitivity to incubation duration.



5. For microplate loading:

- (1) Reverse pipetting: aspire 200 uL sample from the middle of the solution
- (2) Tip gently touches the side of the well, avoid bending. Dispense 200 uL into the microplate
- (3) Reverse pipet another 200 uL as replicate
- (4) Tip gently touches the side of the well, avoid bending. Dispense 200 uL into the microplate

PROTOCOL MATERIALS

☒ Tris Buffer 1M pH 7.0 **Fisher Scientific Catalog #BP1756-500** In 2 steps

☒ Sodium dodecyl sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L3771**

Step 4.2

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

Step 6

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

In 3 steps

☒ Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #23225** In 3 steps

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

Step 87

☒ Methanol (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #34860-4X2L-R**

Step 88

☒ Glycerol **Bioshop Catalog #GLY001.500** Step 4.1

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

Step 4.3

SAFETY WARNINGS

! Waste from BCA assay needs to be collected into waste container and gets further treated before disposal due to the negative impact on the activity of microorganism.

Solvent from protein precipitation (1) the mixture of water and methanol (2) the mixture of methanol and chloroform shall be disposed into separated waste container.

Sample collection

- 1 Microalgae samples

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

If using 500 uL extraction buffer, the minimum sampling volume (mL) = $750 / (\text{Chl-a_ug/L})$

If using 1000 uL extraction buffer, the minimum sampling volume (mL) = $2 \times 750 / (\text{Chl-a_ug/L})$

1.2 Filter microalgae in liquid media onto polycarbonate filters, using gentle vacuum pressure (130 mmHg).

1.3 Rinse filter tunnel with filtered artificial seawater (nutrient free) to avoid sample loss.

1.4 Fold the filter with two tweezers:

- (1) Fold in half along its diameter, creating a semicircular shape;
- (2) Fold once more in the same direction, resulting in a long strip;
- (3) Fold once more, halving its length, so that sample is secured.

1.5 Place folded filter in 2 mL cryogenic vial.

1.6 Filter blank media (without cells) through polycarbonate filter as blank.

1.7 Flash-freeze tubes with liquid nitrogen and store at  -80 °C

1.8 Freeze-dry samples before extraction.

2 Zooplankton samples

2.1 Grind freeze-dried samples in metal grinding tube (need dry ice)

5s

Equipment**Metal lysing matrix tube**

NAME

MPBio

BRAND

116992006

SKU

Equipment**CoolPrep™ adapter for 24 x 2 mL tube holder on FastPrep-24**

NAME

MPBio

BRAND

116002528

SKU

Equipment**FastPrep-24 5G**

NAME

Bead-beater

TYPE

MP Biomedicals

BRAND

116005500

SKU

<https://uk.mpbio.com/fastprep-24-5g-instrument>

LINK

- 2.2 Transfer ground sample into Lysing matrix tube (containing 1.4 mm ceramic spheres, 0.1 mm silica spheres and one 4 mm glass bead), weigh the biomass and log into sampling sheet.

Equipment

Lysing matrix E tube

NAME

2 mL

TYPE

MPbio

BRAND

116914050-CF

SKU

Equipment

Prefilled tubes

NAME

100 µm and 4 mm silica and 1.4 mm zirconia acid washed beads

TYPE

Cole-Parmer

BRAND

2303-MM3

SKU

- 2.3 Flash-freeze tubes with liquid nitrogen, store at  -80 °C .

Reagent

3

[M] 100 mM pH 7 Tris buffer

Dilute 1 part  Tris Buffer 1M pH 7.0 Fisher Scientific Catalog #BP1756-500 with 9 part MilliQ.

4 Protein extraction buffer (PEB, 4X)

4.1 Use a transfer pipet to add  40 g  Glycerol **VWR International Catalog #GLY001.500** into a 100 mL reagent bottle

4.2 Weigh 8 g

 Sodium dodecyl sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L3771** and transfer to the same reagent bottle

4.3 Add  4 mL  EDTA buffer solution (0.5 M) **VWR International Catalog #4055-100ml**

4.4 Top to 100 g with  100 mM pH 7 Tris buffer.

4.5 Shake at  200 rpm, 37°C for complete dissolution.

Note

PEB (4X) might precipitate at room temperature due to the high concentration of glycerol and SDS. Redissolve by shaking at 37 degree.

5 50 mM pH 7 Tris buffer

Dilute five part  Tris Buffer 1M pH 7.0 **Fisher Scientific Catalog #BP1756-500** with 95 part MilliQ.

- 6** 20% Sarcosine
Dilute two part
 N-lauroylsacosine sodium salt solution (30%) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #61747**
 with one part 50 mM pH 7 Tris buffer
- 7** Protein extraction buffer (PEB, 1X): 2% SDS, 10% glycerol, 5 mM EDTA in 100 mM pH 7 Tris buffer:
 one part Protein extraction buffer (PEB, 4X)
 three part 100 mM pH 7 tris buffer

Protein extraction

- 8** Remove samples out of the ULT and keep them  On ice .
- 9** Prepare samples for homogenizing
 Zooplankton samples are already in the bead tubes.
 Phytoplankton samples need to be transferred from cryogenic tubes to bead tubes by following the steps below:

9.1 Rinse forceps with 95% ethanol and air dry

Equipment

Filter forceps	NAME
blunt end, stainless steel	TYPE
Millipore	BRAND
XX6200006P	SKU

9.2 Label Lysing matrix tubes (containing 1.4 mm ceramic spheres, 0.1 mm silica spheres and one 4 mm glass bead) and use clean forceps to transfer samples and blank filters into its corresponding tube.

9.3 Prior to transferring:

- (1) For filters folded into half-strip, unfold once to return to a strip.
- (2) For filters folded into quarter-circles, unfold once to return to a half-circle shape, then fold once along the dimension to form a strip.
- (2) For filters haphazardly into a compact mass, carefully unfold with two tweezers (avoiding losing biomass), fold once into a half-circle shape, then fold once more along the dimension to form a strip

9.4 Insert the strip into beads mixture.**9.5** Place the bead tubes  On ice .**10** Reverse pipet  1 mL PEB (1X) into each bead tube.**11** Turn on FastPrep**Equipment**

FastPrep-24 5G	NAME
Bead beater	TYPE
MP Biomedicals	BRAND
116005500	SKU
https://uk.mpbio.com/fastprep-24-5g-instrument	LINK

- 12 Turn on the refrigerate centrifuge, set temperature at 4 °C

Equipment

CENTRIFUGE 5430 R

NAME

Eppendorf

BRAND

MP2231000510

SKU

- 13 Check the cap of each tube to ensure cap is tightly closed. Organize the tubes in order, log the position of each tube, in case the labels get rubbed out during extraction.

- 14 Run 00:01:00 at 6.5 m/s

1m

- 15 Keep tubes On ice for 00:01:00

- 16 Check labels. Put tubes back into FastPrep.

- 17 Run 00:01:00 at 6.5 m/s

1m

- 18 Keep tubes On ice for 00:01:00

19 Check labels. Put tubes back into FastPrep.

20 Run  00:01:00 at 6.5 m/s

1m

21 Keep tubes  On ice for  00:01:00

22 Check labels. Put tubes back into FastPrep.

23 Run  00:01:00 at 6.5 m/s

1m

24 Keep tubes  On ice for  00:01:00

25 Check labels. Put tubes back into FastPrep.

26 Run  00:01:00 at 6.5 m/s

27 Keep tubes  On ice for  00:01:00

28 Check labels. Put tubes back into FastPrep.

29 Run  00:01:00 at 6.5 m/s

30 Keep tubes  On ice for  00:01:00

31 Check labels. Put tubes back into FastPrep.

32 Run  00:01:00 at 6.5 m/s

33 Keep tubes  On ice for  00:01:00

34 Check labels. Put tubes back into FastPrep.

35 Run  00:01:00 at 6.5 m/s

36 De-foam by centrifuging the extract.

 20000 rcf, 4°C, 00:05:00

5m

37 Transfer extract to 2 mL microtubes.

38 Centrifuging the extract.

 20000 rcf, 4°C, 00:05:00

5m

39

1. Transfer 100 uL supernatant to 2 mL microtube for crude protein measurement.
2. Transfere 100 uL supernatant to 2 mL microtube in duplicate for protein precipitation.
3. Save the remaining extract.

40 Freeze at  -80 °C .

Crude protein quantitation

41 Thaw protein extract ( [go to step #39](#))

42 Organize eight 2 mL microtubes in the tube rack, label the tubes from CS1 to CS8 (C denotes crude).

43 Mix well, and then transfer about 800 uL

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

from the original package into a 2 mL microtube.

44 PEB and BSA: transferred by reverse pipetting

Standard	PEB (4X) uL	100 mM Tris pH 7 (uL)	BSA (2 mg/mL) (uL)	Final Conc. (mg/mL)
CS1	125	375	0	0
CS2	125	370	5	0.02
CS3	125	363	12	0.048
CS4	125	350	25	0.1
CS5	125	325	50	0.2
CS6	125	275	100	0.4
CS7	125	175	200	0.8
CS8	125	125	250	1

45 Reverse pipetting: load  4 μ L of each standard solution onto microdrop plate.

Equipment

μDrop™ Plates

NAME

Thermo Scientific

BRAND

N12391

SKU

46 Read absorbance of eight standard solutions at 205 nm

Equipment

Varioskan LUX Multimode Microplate Reader

NAME

Thermo Fisher

BRAND

VL0L00D0

SKU

- 47** Subtract absorbance at 205 nm of blank standard from the 205 nm measurements of all other standard solutions
- 48** Plot the blank-corrected 205 nm measurement for each standard solution versus its concentration in mg/ml.
-
- | BSA (mg/mL) | Absorbance@ 205 nm |
|-------------|--------------------|
| 0.05 | 0.05 |
| 0.10 | 0.10 |
| 0.20 | 0.20 |
| 0.40 | 0.70 |
| 0.80 | 1.55 |
| 1.00 | 1.95 |
- Example of BSA standard curve: Absorbance read at 205 nm versus concentration (mg/mL)
- 49** 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 meets the requirement.
- 50** Organize eight 2 mL microtubes in the tube rack, label the tubes from CS1 to CS8. Reverse pipet $\text{100 } \mu\text{L}$ standard solution into its corresponding tube.

- 51 Use the following formula to determine the total volume of WR required. Consider leaving several mL of extra volume:

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

- 52 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 **VWR International Catalog #23225**

Equipment

Falcon® Centrifuge Tubes

NAME

Polypropylene, Sterile, 50 mL

TYPE

Corning®

BRAND

352070

SKU

- 53 Turn on incubator and preheat to  37 °C

Equipment

SHAKING INCUBATOR

NAME

71L

TYPE

Corning® LSE™

BRAND

6753

SKU

- 54 Using a single pipette tip and employing the reverse pipetting technique, add  800 µL of WR into each tube. Ensure that the tip does not come into contact with the solution to prevent cross-contamination of samples.

Note

Given the sensitivity of the BCA assay to reaction duration, it is advisable to employ reverse pipetting when dispensing the reagent into all tubes. While the BCA reagent is aqueous, this technique allows for quicker and more consistent dispensing, thereby minimizing any discrepancies in reaction duration between standards and samples, especially for crude protein in PEB with glycerol.

- 55 Vortex each tube, shake and incubate at 37 °C for 00:30:00 at 200 rpm. 30m
- 56 Remove samples from the incubator and centrifuge 13300 rpm, Room temperature, 00:05:00 5m
- 57 Each microplate can hold eight standard solutions and forty samples+blanks, all in duplicate

Equipment**96-Well Microplates**

NAME

Polystyrene, Clear,

TYPE

Greiner Bio-One

BRAND

82050-760

SKU

- 58 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

Calculate protein content per filter

- 59 Subtract the average absorbance at 562 nm of the blank standard replicates from the 562 nm measurements of all other individual **standards**.
- 60 Subtract the average absorbance at 562 nm of the blank sample (filter) replicates from the 562 nm measurements of all other individual **samples**.
- 61 Create a response curve where the corrected absorbance at 562 nm for each BSA standard is plotted against its concentration in mg/mL.
- 62 Standard curve
 - 62.1 For crude protein, the standard curve is quadratic.
 $Abs = aConc^2 + bConc + c$
Where Abs is the blank corrected absorbance at 562 nm and $Conc$ is the concentration of protein.
 - 62.2 For precipitated protein, the standard curve is linear.
 $Abs = aConc + b$

Where Abs is the blank corrected absorbance at 562 nm and $Conc$ is the concentration of protein.

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

64 Actual detection limit of the assay ($L - LOD$)

64.1 Enter the standard deviation (SD) of the absorbance values from the blank as Abs in the standard curve to determine the $Conc$.

64.2
$$L - LOD = 3.3 * Conc$$

65 If the calculated sample concentration is lower than $L - LOD$ but higher than 5 ug, go to Section:

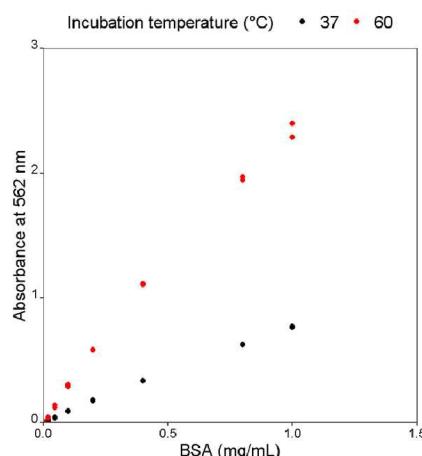
Enhanced Pierce BCA assay for protein 5 to 200 ug/sample

66 If the calculated sample concentration is lower than 5 ug, then use "<LOD" as the result, indicating the protein content is undetectable.

67 Protein_mg/filter = Protein_mg/mL X PEB_mL

Enhanced Pierce BCA assay for protein 5 to 200 ug/sample

68



Response of absorbance at 562 nm to BSA concentration after 30-min incubation at 37 and 60 °C

- 69 Thaw extract: remove extract ([go to step #39](#)) from ULT and centrifuge 5m
- 13300 rpm, Room temperature, 00:05:00

- 70 Reverse pipetting: Transfer 100 µL supernatant to a new 2 mL microtube.
Return the remaining extract to ULT as soon as possible.

- 71 Turn on dry bath and preheat to 60 °C

Equipment

Digital dry bath	NAME
LSE	TYPE
Corning	BRAND
6875SB	SKU

72

Prepare BSA standard: [M] 0.4 mg/mL

- 72.1 Well mix the package and then directly reverse pipet 300 μL BSA standard into a 2 mL microtube (do not return remaining solution back into the bottle).

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

- 72.2 Forward pipet 600 μL + 600 μL 100 mM pH 7 Tris buffer into the tube, vortex.

- 73 Organize eight 2 mL microtubes in the tube rack, label the tubes from CS1 to CS8.

- 74 PEB and BSA: transferred by reverse pipetting

	Standard	PEB (4X) (μL)	100 mM Tris pH 7 (μL)	BSA (0.4 mg/mL) (μL)	Final Conc. (mg/mL)
	CS1	25	75	0	0
	CS2	125	370	5	4
	CS3	125	365	10	8
	CS4	125	355	20	16
	CS5	125	345	30	24
	CS6	125	315	60	48
	CS7	125	250	125	100
	CS8	125	125	250	200

- 75 Prepare seven 2 mL microtubes for CS2 to CS8.

Vortex and then use reverse pipetting: transfer 100 μL standard solutions into the corresponding tubes, except for CS1 (it has already been 100 μL).

- 76 Use the following formula to determine the total volume of WR required. Consider leaving several mL of extra volume:

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

- 77 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 **VWR International Catalog #23225**

Equipment

Falcon® Centrifuge Tubes

NAME

Polypropylene, Sterile, 50 mL

TYPE

Corning®

BRAND

352070

SKU

- 78 Reverse pipetting: Add 800 µL WR into each tube.

- 79 Vortex each tube, incubate at 60 °C for 00:30:00

- 80 Cool down samples to room temperature, and centrifuge 13300 rpm, Room temperature, 00:05:00 5m

- 81 Each microplate can hold eight standard solutions and forty samples+blanks, all in duplicate

Equipment**96-Well Microplates** NAME

Polystyrene, Clear, TYPE

Greiner Bio-One BRAND

82050-760 SKU

- 82** 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

- 83** Calculation refers to the section: **Calculate protein content per filter**

Protein precipitation

50m 30s

- 84** Protein extract from  [go to step #39](#)

- 85** Turn on refrigerate centrifuge, set temperature at  4 °C

Equipment

CENTRIFUGE 5430 R

NAME

Eppendorf

BRAND

MP2231000510

SKU

86 Add  300 µL MilliQ into each tube with extract.

87 In the fume hood, vortex after adding  100 µL

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

88 In the fume hood, add  400 µL

 Methanol (HPLC grade) **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #34860-4X2L-R

89 Vortex until the mixture turns milky.

90 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

91

Incubate in the fridge for  00:30:00

30m

92

 20000 rcf, 4°C, 00:10:00

10m

93

In the fume hood, remove upper phase (about 700 μ L)

Note

Do not disturb the interphase.

94

In the fume hood, add  300 μ L methanol. Invert the tube gently at a slight angle to avoid letting precipitated protein and liquid touch the inside of the cap.

95

 20000 rcf, 4°C, 00:10:00

10m

96 In the fume hood, remove all solvent by using 100 uL pipet tip.

Note

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

97 With microtube lid open and vacuum pump running, dry pellet in vacuum desiccator for 00:15:00 at 15m

Room temperature

Note

Methanol and chloroform interfere the BCA assay. However, do not dry protein pellet for too long, otherwise it might lead to difficulties in resolubilizing the pellet .

98 Store at -80 °C .

BCA assay to quantify precipitated protein

50m 30s

99 Re-solubilization buffer (RSB):

Add 500 µL 20% sarcosine (go to step #6) into 9.5 mL 50 mM Tris (go to step #5)

100 Reverse pipetting, add 100 µL RSB (go to step #99) to each tube with precipitated protein (go to step #98).

Vortex for complete re-solubilization.

101 Transfer about 800 uL

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

to a 2 mL microtube.

102 Organize eight 2 mL microtubes in the tube rack, label the tubes from PS1 to PS8, P denotes precipitated.

103 BSA standard solutions

	Standards	20% sarcosine (uL)	50 mM Tris pH 7 (uL)	2 mg/mL BSA (uL)	Final Conc. (mg/mL)
	PS2	25	470	5	0.02
	PS3	25	463	12	0.048
	PS4	25	450	25	0.1
	PS5	25	425	50	0.2
	PS6	25	375	100	0.4
	PS7	25	275	200	0.8
	PS8	25	225	250	1

104 Reverse pipet 100 µL RSB ([go to step #99](#)) into PS1.

105 Prepare seven 2 mL microtubes for PS2 to PC8.

106 Vortex and then use reverse pipetting: transfer 100 µL standard solutions (PS2 to PS8) into the new tubes.

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

$$(\# \text{ standards} + \# \text{ samples}) \times \text{ 800 } \mu\text{L} = \text{total volume WR required}$$

108 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 VWR International Catalog #23225](#)

109 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.

110 Vortex each tube, shake and incubate at 37 °C for 00:30:00 at 200 rpm.

30m

111 Remove samples from the incubator, and centrifuge 13300 rpm, Room temperature, 00:05:00

5m

112 Each microplate can hold eight standard solutions and forty samples+blanks, all in duplicate

Equipment

96-Well Microplates

NAME

Polystyrene, Clear,

TYPE

Greiner Bio-One

BRAND

82050-760

SKU

113 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

114 Calculation refers to the section: **Calculate protein content per filter**