Pierce BCA Protein Assay Protocol Version 2

James P. O'Callaghan, Ph.D

Abstract

This is the protocol to be used in the determination of total protein in brain tissue by the Pierce BCA method. BSA (Bovine Serum Albumin) is used as the standard and samples are prepared by sonification in 1% (w/v) SDS.

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Guidelines

INTRODUCTION

The Thermo Scientific™ Pierce™ BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations over a broad working range (20-2000µg/mL). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together.

The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual color-producing functional groups. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve. If precise quantitation of an unknown protein is required, it is advisable to select a protein

standard that is similar in quality to the unknown; for example, a bovine gamma globulin (BGG) standard may be used when assaying immunoglobulin samples.

TRAINING REQUIREMENTS

Able to use a plate reader and to accurately dilute tissue samples.

REFERENCES

BCA Protein Assay Reagent (instruction manual), Pierce. Rockford, IL.

Softmax User's Manual, Molecular Devices Corporation. Sunnyvale, CA.

Materials

- Plate Reader by Contributed by users
- Pipettes by Contributed by users
- Incubator by Contributed by users
- Vortex Mixer by Contributed by users
- Microtubes 1.2 ml by Contributed by users
- 96 well microtiter plates by Contributed by users
- Pipette tips by Contributed by users

Pierce BCA Protein Assay Kit 23225 by Thermo Fisher Scientific

Pierce BCA Protein Assay Reagent A 23221 by Thermo Fisher Scientific

Pierce BCA Protein Assay Reagent B 23224 by Thermo Fisher Scientific

Bovine Serum Albumin (BSA) A7906 by Sigma Aldrich

Sodium Dodecyl Sulfate 1610416 by Bio-rad Laboratories

Protocol

REAGENT PREPARATION

Step 1.

1. **1% SDS**

A stock solution of 10%SDS is prepared w/v, 100 gms of SDS in 1 litre of deionized water. (Or 10% SDS purchased from vendor) The 1%SDS is a dilution of the stock- 10ml of 10X stock in 90 ml deionized water.

2. **BCA Reagent**

This reagent is made according to the directions provided by Pierce, 50ml of solution A and 1ml of solution B. Thoroughly mix while adding solution B.

STANDARD PREPARATION

Step 2.

Protein Standards: Bovine Serum Albumin (BSA) in 1% (w/v) SDS (sodium dodecyl sulfate).

1. Prepare 1mg/ml BSA in 1% SDS. Weigh out 0.100 gms of dry BSA on an analytical balance. Add the BSA to a 100 ml volumetric flask then gradually add 1% SDS to the flask. Mix gently after each addition of SDS to dissolve the BSA without a lot of bubbling. Continue adding the SDS until the total 100 ml has been added.

2. Aliquot a volume into microfuge tubes (600 µl is enough volume to do one standard curve). Store the tubes at -70°C . Thaw as needed, but do not re-freeze.

3. **Blank:** Remove 200µl of 1%SDS, place it in a dilution tube.

4. **1.0µg/10µl:** Remove 20µl of the prepared BSA standard (1mg/ml), place it in a dilution tube and add 180µl of 1%SDS.

5. **2.5µg/10µl:** Remove 50µl of the prepared BSA standard, place it in a dilution tube and add 150µl of 1% SDS.

6. **5.0µg/10µl:** Remove 100µl of the prepared BSA standard, place it in a dilution tube and add 100µl of 1% SDS.

7. **7.5µg/10µl:** Remove 150µl of the prepared BSA standard, place it in a dilution tube and add 50µl of 1% SDS.

8. **10.0µg/10µl:** Remove 200µl of the prepared BSA standard and place it in a dilution tube.

BSA Standard Curve

Tube #	µg Protein/ 10µl/well	µl of 1mg/ml BSA Standard	µl of 1%SDS
1	Blank	0	200µl
2	1.0µg	20µl	180µl
3	2.5µg	50µl	150µl
4	5.0µg	100µl	100µl
5	7.5µg	150µl	50µl
6	10.0µg	200µl	0

9. Repeat steps 3 to 8. This generates a second standard curve, independent of the first.

10. Vortex each tube of the diluted BSA standard, remove 10µl of the diluted standard and place it in the designated well on a microtiter plate.

SAMPLE PREPARATION

Step 3.

The unknown experimental samples are diluted two times, independently.

1. Remove samples from the freezer and allow to thaw at room temperature.

2. Vortex and then remove a 10µl aliquot from the sample and place it into a dilution tube. Add 190µl of 1% SDS to each sample tube.

3. Repeat step 2. This becomes the second dilution for this sample. (or take twice from 1 dilution)

4. Vortex the dilution tubes and remove a 10µl aliquot and place it in the appropriate well on the microtiter plate.

5. Repeat steps 1 through 4 for each experimental sample.

6. The following chart is an example of a typical microtiter plate layout, standard curve and 42 unknown samples.

Microtiter Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12

A	Blk	Blk	? 3	? 3	? 11	? 11	? 19	? 19	? 27	? 27	? 35	? 35
B	Std 1	Std 1	? 4	? 4	? 12	? 12	? 20	? 20	? 28	? 28	? 36	? 36
C	Std 2	Std 2	? 5	? 5	? 13	? 13	? 21	? 21	? 29	? 29	? 37	? 37
D	Std 3	Std 3	? 6	? 6	? 14	? 14	? 22	? 22	? 30	? 30	? 38	? 38
E	Std 4	Std 4	? 7	? 7	? 15	? 15	? 23	? 23	? 31	? 31	? 39	? 39
F	Std 5	Std 5	? 8	? 8	? 16	? 16	? 24	? 24	? 32	? 32	? 40	? 40
G	? 1	? 1	? 9	? 9	? 17	? 17	? 25	? 25	? 33	? 33	? 41	? 41
H	? 2	? 2	? 10	? 10	? 18	? 18	? 26	? 26	? 34	? 34	? 42	? 42

PROTEIN ASSAY

Step 4.

1. Add 200µl of reagent to each of the wells containing either the standard curve or sample.

2. Place the plate in the plate reader and mix the plate using the automatic mix button on the plate reader or vortex gently prior to placing the plate into the reader.

3. Incubate the plate at 37°C for 30 minutes.

4. At the completion of the incubation period, the plate is checked for bubbles and then read in the plate reader at a wavelength of 562nm. “Pop” any bubbles with a needle or a pipette tip prior to reading the plate.

CALCULATIONS

Step 5.

1. Software programs coupled to specific plate readers are used to calculate protein values based on comparing OD readings of the unknowns with those obtained from the standard curve. OD values and standard protein values are plotted in a linear fashion on both axes. The following is a typical standard curve for the BCA Protein Assay when bovine serum albumin is used for the standard. The table gives the actual values for each point on the standard curve and their corresponding OD readings.

BCA Total Protein Standard

Curve

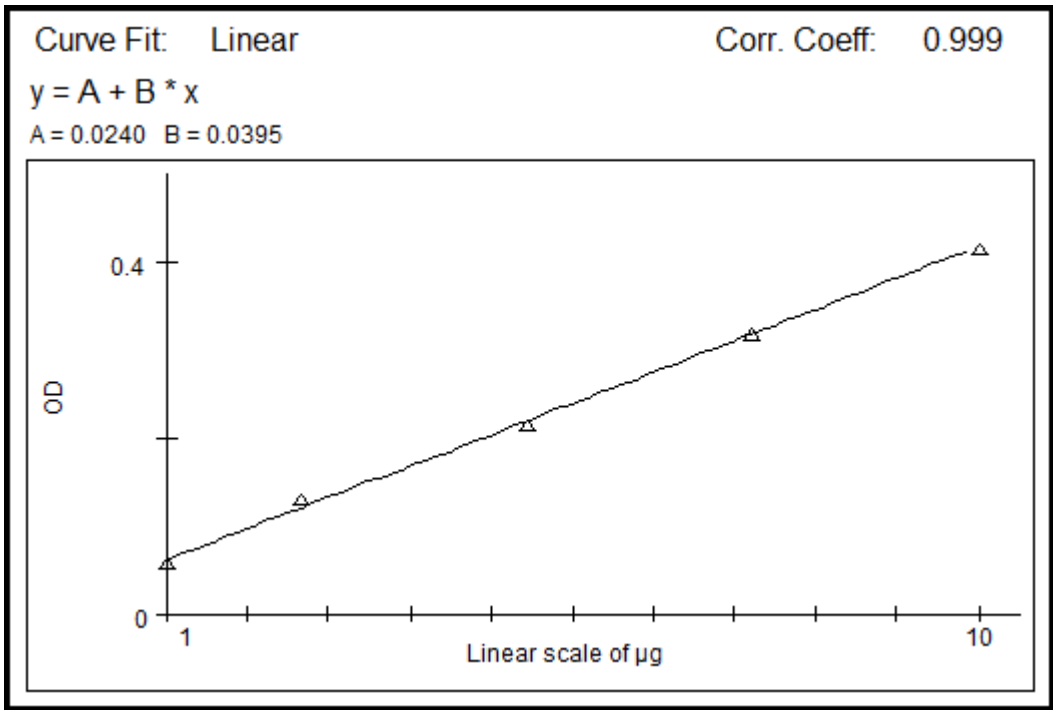

Plate Blank	Well	OD	Mean	Std Dev	CV
BL	A1	0.083	0.082	0.001	1.725
	A2	0.081			

STANDARD	Value	Well	OD	Mean	Std Dev	CV
STD01	1.000 µg	A3	0.06	0.058	0.002	3.626
		A4	0.057			
STD02	2.500 µg	A5	0.131	0.131	7.07E-04	0.542
		A6	0.13			
STD03	5.000 µg	A7	0.22	0.218	0.003	1.297
		A8	0.216			
STD04	7.500 µg	A9	0.311	0.321	0.014	4.406
		A10	0.331			
STD05	10.00 µg	A11	0.419	0.418	0.001	0.338

A12	0.417	

2. The blank can be automatically subtracted from all other OD readings or it must be done manually if this feature is not available.

3. The dilution factors can be entered into the programs, otherwise they must be considered in the final calculations.

4. The final printout will include a plot of the standard curve and the protein concentration of each unknown expressed as mg/ml or µg/µl. The table below gives the calculated protein values for unknown hippocampus samples in mg/ml or µg/µl. These values include the subtraction of the blank value and a dilution factor of 2.

UNKNOWN	Mean	Std Dev	CV	Well	OD	Value	Dil. Factor
UNK01	9.325	0.215	2.306	B1	0.211	9.477	2
				B2	0.205	9.173	
UNK02	10.44	*****	*****	B3	0.23	10.44	2
				B4	0.23	10.44	
UNK03	9.883	0.072	0.725	B5	0.218	9.832	2
				B6	0.22	9.934	
UNK04	11.18	1.254	11.23	B7	0.227	10.29	2
				B8	0.262	12.06	
UNK05	11.45	0.717	6.258	B9	0.24	10.95	2

	B10	0.26	11.96

5. Example of calculations, if samples are prepared as listed above:

a. Hippo#3 is prepared as a 1:20 dilution (10µl of standard or unkown sample are placed in a dilution tube and brought up to a final volume of 200µl)

b. OD reading for this sample is 0.219

c. OD of .219 corresponds to » 4.942µg/10µl or 0.4942µg/µl total protein

d. This sample has been diluted 1:20 so the value is actually 0.4942µg/µl x 20= 9.883µg/µl (since the protein sample was loaded in 10 µl volumes the value can be determined by using a dilution

factor of 2, That is, 4.942µg/10µl x2=9.883µg/µl).

Warnings

CAUTIONARY NOTES/SPECIAL CONSIDERATIONS

BCA reagent is stable for up to one week; standard practice dictates the use of 50ml quantities, enough to do two full microtiter plates.

SDS serves as a wetting agent and pipetting SDS-containing samples can lead to pipetting errors.

Therefore, use only a single pipette tip per sample and withdraw the sample only a single time per tip. This procedure holds for SDS and all SDS-containing samples.