PROMETHEUS™
Protein Biology Products

Instruction Manual

BCA Protein Assay with BSA Protein Standard — Catalog No.: 18-440 and 18-441

INTRODUCTION

Our BCA (Bicinchoninic Acid) Protein Assay is an improved, ready to use quick colorimetric method for total protein quantitation. This is a detergent-compatible formulation, based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total proteins. 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 (20-2,000µg/ml). The macromolecular structure of proteins, 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 tetra-peptides 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 is prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

The two assay procedures are presented; Cuvette and Microplate. Of these, the Cuvette procedure requires a larger volume (50 µl) of protein sample; however, because it uses a sample to working reagent ratio of 1:20 (v/v), the effect of interfering substances is minimized. The Microplate Procedure affords the sample handling ease of a microplate and requires a smaller volume (10-25 µl) of protein sample; however, because the sample to working reagent ratio is 1:8 (v/v), it offers less flexibility in overcoming interfering substance concentrations and obtaining low levels of detection. The BCA assay is more sensitive and applicable than either biuret or Lowry procedures and has less variability than the Bradford assay. The BCA protein assay demonstrates higher tolerances towards common interfering substances, such as nonionic detergents and buffer salts than the Lowry technique[1]. The BCA Protein Assay Kit can be used to measure the protein concentration of lysates or homogenates prepared with compatible lysis buffer, in microplate format. We recommend to use BCA compatible reagent and concentration to decrease interference from reducing agents, chelators, detergents, and other common ingredients found in most of the lysis buffers. The BCA assay has many advantages over other protein determination techniques:

Key Features

- Colorimetric method and read at 562nm
- Compatible with most ionic and non-ionic detergents
- Faster and easier than the Lowry method
- Linear working range for BSA: 20-2,000µg/ml
- Adaptable to microplates
- Less protein-to-protein variation than dye-binding methods

Items Supplied:

Item Name	Cat. No. 18-440	Cat. No. 18-441	Storage Condition*
BCA Reagent A	2 x 250 ml	2 x 500 ml	Room Temp
BCA Reagent B	12 ml	25 ml	Room Temp
BSA Standard (2 mg/ml)	2 x 5.0 ml	2 x 5.0 ml	4°C

**The kit is shipped at ambient temperature and upon receipt, store it at 4°C and the kit components are stable for 12 months, if stored and used as recommended.*

Additional Items Needed:

1. 37°C water bath or incubator
2. Spectrophotometer or Microplate reader, capable of accurately measuring absorbance in the range of 540-595nm (562nm is the optimal wavelength)

Important product information

1) If this kit is received or stored cold, a precipitate may form in Reagent A or Reagent B. To dissolve the precipitate, warm the solution slowly at 37°C while mixing or microwave for a few seconds. Discard the kit if it is contaminated by bacteria
2) If interference caused by reducing substances or metal-chelating substances contained in the sample remains, Bradford Assay Kit is recommended.
3) It is recommended that the standard of different concentrations and samples be assayed in duplicate. Standard curve should be plotted for each assay.
4) Newly formed green turbidity will disappear after mixing Reagent A and Reagent B thoroughly. It will not affect the performance.
5) Assayed sample amount will be reduced while using spectrophotometer to detect protein concentration. When using 37°C incubator, prevent water evaporation.
7) Avoid using substances including reducing substances, chelating agents, strong acid and alkali since they interfere with protein estimation even at very low concentration.

Interfering substances

Certain substances are known to interfere with the BCA Protein Assay, including those with reducing potential, chelating agents, and strong acids or bases. Because they are known to interfere with protein estimation at even minute concentrations, avoid the following substances as components of the sample buffer: EGTA, Impure Glycerol, Iron, Lipids, Hydrogen Peroxide, Melibiose, Ascorbic Acid, Catecholamines, Creatinine, Cysteine Hydrazides, Phenol Red, Impure Sucrose, Tryptophan, Tyrosine Uric acid. Other substances interfere to a lesser extent with protein estimation using the BCA Protein Assay, and these have only minor (tolerable) effects below a certain concentration in the original sample. Substances were compatible at the indicated concentration in the Standard Test Tube Protocol if the error in protein concentration estimation caused by the presence of the substance in the sample was less than or equal to 10%. The substances were tested using WR prepared immediately before each experiment. Blank-corrected 562nm absorbance measurements (for a 1,000 µg/ml BSA standard + substance) were compared to the net 562nm measurements of the same standard prepared in 0.9% saline. In the Microplate Procedure, where the sample to WR ratio is 1:8 (v/v), maximum compatible concentrations will be lower.

Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the BCA Protein Assay may be eliminated or overcome by one of several methods as below:

- Remove the interfering substance by dialysis or gel filtration.
- Dilute the sample until the substance no longer interferes. This strategy is effective only if the starting protein concentration is sufficient to remain in the working range of the assay upon dilution.
- Precipitate the proteins in the sample with Acetone or 10% Trichloro acetic acid (TCA). The liquid containing the substance that interfered is discarded and the protein pellet is easily solubilized in ultrapure water or directly in a suitable compatible buffer.
- Increase the amount of copper in the Working Reagent (prepare as 50:2 or 50:3, BCA Reagent A:B), which may eliminate interference by copper chelating agents.

Assay Protocols:

A. <u>Test Tube Protocol</u>

1. Prepare BCA Working Reagent (WR) by mixing 50 parts of BCA Reagent A with **<u>one</u>** part of BCA Reagent B (50:1 of BCA Reagent A & B). Combine 50ml of BCA Reagent A with 1ml of BCA Reagent B in a clean tube and mix it gently. Use the formula to determine the total volume of working reagent required: (No. of standards+ No. of unknowns) x (No. of replicates) x (volume of working reagent per sample) = total volume of working reagent required.

   <u>Note</u>: *When BCA Reagent B is first added to BCA Reagent A, a turbidity is observed that quickly disappears upon mixing and yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).*

2. Dilute the supplied 2mg/ml BSA standard, preferably in the same buffer (diluent) as the test sample(s) or in 0.9% NaCl, using the table on next page, as a guide for preparing a set of protein standards. **Note:** For the greatest accuracy, the protein standards must be treated identically to the sample(s).

Working Range 25 µg/ml to 2,000 µg/ml – Standard Protocol:

Tube No.	Volume of Diluent/Buffer	Volume and Source of BSA	Final BSA Concentration
B (Blank)	400 µl	0 µl	0 µg/ml
1	0	400 µl of 2 mg/ml	2,000 µg/ml
2	133 µl	400 µl of 2 mg/ml	1,500 µg/ml
3	200 µl	200 µl of Tube 1	1,000 µg/ml
4	200 µl	200 µl of Tube 2	750 µg/ml
5	200 µl	200 µl of Tube 3	500 µg/ml
6	200 µl	200 µl of Tube 5	250 µg/ml
7	200 µl	200 µl of Tube 6	125 µg/ml
8	400 µl	100 µl of Tube 6	25 µg/ml

Working Range 5 µg/ml to 250 µg/ml – Enhanced Protocol:

Tube No.	Volume of Diluent/ Buffer	Volume and Source of BSA	Final BSA Concentration
Blank	400 µl	0 µl	0 µg/ml
1	350 µl	50 µl of 2 mg/ml	250 µg/ml
2	200 µl	200 µl of Tube 1	125 µg/ml
3	225 µl	150 µl of Tube 2	50 µg/ml
4	200 µl	200 µl of Tube 3	25 µg/ml
5	200 µl	100 µl of Tube 4	5 µg/ml

3. Pipette 50 µl of each standard and unknown sample replicate into an appropriately labeled tubes.
4. Add 1 ml of the Working Reagent (WR) to each tube and mix well.
5. A. Standard Protocol - Cover the tubes and incubate at 37 °C for 30 minutes.
   B. Enhanced Protocol - Cover the tubes and incubate at 60 °C for 30 minutes.
   NOTE: *I. Increasing the incubation time or temperature increases the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.*
   II*. Use a water bath to heat the tubes for either Standard 37°C incubation protocol or Enhanced 60°C incubation protocol.* ***DO NOT USE*** *a forced-air incubator, which would introduce a significant error in color development because of uneven heat transfer.*
6. Cool down all tubes to room temperature (RT).
7. Set the spectrophotometer to 562nm and zero the instrument with a cuvette filled only with water, measure the absorbance of all the tubes within 10 minutes.
   NOTE: *Since, the BCA Assay does not reach a true end point, the color development will continue even after cooling the tubes to RT. However, since the rate of color development is low at RT, no significant error will be introduced, if the 562nm absorbance measurements of all the tubes are done within 10 minutes of each other.*
8. Subtract the average 562nm absorbance measurement of the Blank standard replicates from the 562nm absorbance measurement of all other individual standard and unknown sample replicates.
9. Prepare the BSA standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard on Y-axis Vs its concentration in µg/ml on X-axis. Use the standard curve to determine the protein concentration of unknown sample (s).

B. Microplate Protocol

1. Prepare BCA Working Reagent (WR) by mixing 50 parts of BCA Reagent A with **one** part of BCA Reagent B (50:1 of Solution A & B). Combine 50ml of BCA Reagent A with 1ml of BCA Reagent B in a clean tube and mix it gently. Use the formula to determine the total volume of working reagent required: (No. of standards+ No. of unknowns) x (No. of replicates) x (volume of working reagent per sample) = total volume of working reagent required.
   Note: *When BCA Reagent B is first added to BCA Reagent A, a turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).*
2. Dilute the supplied 2mg/ml BSA standard, preferably in the same buffer (diluent) as the test sample(s) or in 0.9% NaCl, using the table below as a guide for preparing a set of protein standards.

Working Range: 25 µg/ml to 2,000 µg/ml)

Tube No.	Volume of Diluent/Buffer	Volume and Source of BSA	Final BSA Concentration
B (Blank)	60 µl	0 µl	0 µg/ml
1	0 µl	200 µl of 2 mg/ml	2000 µg/ml
2	30 µl	90 µl of tube 1	1500 µg/ml
3	60 µl	60 µl of tube 1	1000 µg/ml
4	60 µl	60 µl of tube 2	750 µg/ml
5	60 µl	60 µl of tube 3	500 µg/ml
6	60 µl	60 µl of tube 5	250 µg/ml
7	60 µl	60 µl of tube 6	125 µg/ml
8	100 µl	25 µl of tube 7	25 µg/ml

3. Add 25 µl of each standard and protein sample(s) into separate microplate wells.
4. Add 200 µl of BCA Working Reagent (WR) to each microwell and mix it well.
5. Seal the microplate(s) and incubate at 37°C for 30 minutes.
6. Cool plate to room temperature (RT).
7. Measure the absorbance at 562 nm on a plate reader within 10 minutes.
8. Subtract OD_{562} of Blank from all readings.
9. Plot the BSA standard curve: OD_{562} (on Y axis) vs BSA Standard concentration (on X axis). Use the standard curve to determine the protein concentration of each unknown sample.

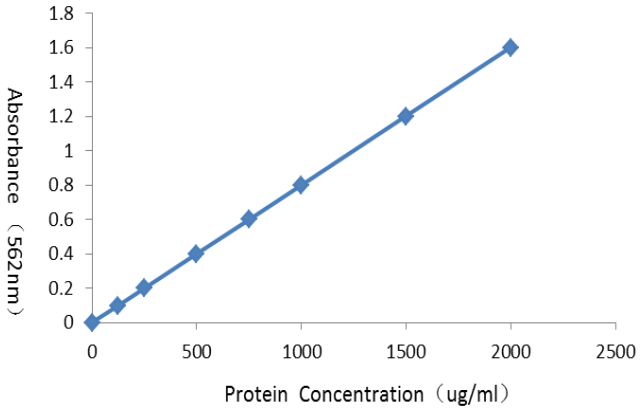

Figure-1: A Typical Standard Curve of Protein Concentration Vs Net Absorbance (Incubated at 37°C for 30 minutes).

Troubleshooting:

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt or dilute sample Increase copper concentration in the Working Reagent (WR), e.g., use 50:2, Reagent A:B. Remove interfering substances from sample, using acetone or 10% trichloroacetic acid (TCA) precipitation method.
Blank absorbance is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 562nm
Color of samples appears darker than expected	Protein concentration is too high	Dilute the sample(s)

	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids[2]
		Remove interfering substances from sample, using acetone or 10% trichloroacetic acid (TCA) precipitation method.
All tubes (including blank) are dark purple	Buffer contains a reducing agent	Dialyze or dilute sample Remove interfering substances from sample, using acetone or 10% trichloroacetic acid (TCA) precipitation method.
	Buffer contains a thiol	
	Buffer contains biogenic amines (catecholamines)	
Need to measure color at a different wavelength	Spectrophotometer or plate reader does not have 562nm filter	Color may be measure at any wavelength between 540nm and 590nm, although the slope of standard curve and overall assay sensitivity will be reduced

Reagents & Buffers compatible with BCA Protein Assay if standard recommended procedure is used:

Salts & Buffers	Concentration
ACES, pH 7.8	25 mM
Ammonium sulfate	1.5 M
Asparagine	1 mM
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
Borate (50mM), pH 8.5	undiluted
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4	Undiluted
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0	1:8 dilution in ultrapure water
Na-Citrate (0.6M), MOPS (0.1M), pH 7.5	1:8 dilution in ultrapure water
Cobalt chloride in TBS, pH 7.2	0.8 mM
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
Glycine-HCl, pH 2.8	100 mM
Guanidine-HCl	4.0 M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	50 mM
MES, pH 6.1	100 mM
MES (0.1M), NaCl (0.9%), pH 4.7	undiluted
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4	undiluted
Nickel chloride in TBS, pH 7.2	10 mM
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer *[50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0]*	undiluted
Sodium acetate, pH 4.8	200 mM
Sodium azide	0.2 %
Sodium bicarbonate	100 mM

Detergents	Concentration
Brij-35	5.0 %
Brij-58	1.0 %
CHAPS	5.0 %
CHAPSO	5.0 %
Deoxycholic acid	5.0 %
Octyl β-glucoside	5.0 %
Nonidet P-40 (NP-40)	5.0 %
Octyl β-thioglucopyranoside	3.0%
SDS	5.0 %
Triton X-100	5.0 %
Triton X-114	1.0 %
Tween-20	5.0 %
Tween-80	5.0 %

Chelating agents	Concentration
EDTA	10 mM
EGTA	Not Compatible
Sodium citrate	200mM

Reducing & Thiol Containing Agent	Concentration
N-acetylglucosamine in PBS, pH 7.2	10 mM
Ascorbic acid	Not Compatible
Cysteine	Not Compatible
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
Glucose	10 mM
Melibiose	Not Compatible
β-Mercaptoethanol	0.01 %
Potassium thiocyanate	3 M
Thimerosal	0.01 %

Sodium chloride	1.0 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium phosphate	100 mM
Tricine, pH 8.0	25 mM
Triethanolamine, pH 7.8	25 mM
Tris	250 mM
TBS; Tris (25mM), NaCl (0.15M), pH 7.6	Undiluted
Tris (25mM), Glycine (192mM), pH 8.0	1:3 dilution in ultrapure water
Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3	undiluted
Zinc chloride in TBS, pH 7.2	10 mM

Other Reagents & Solvents	Concentration
Acetone	10 %
Acetonitrile	10 %
Aprotinin	10 mg/L
DMF	10 %
DMSO	10 %
Ethanol	10 %
Glycerol (Fresh)	10 %
Hydrazide (Na2BH4 or NaCNBH3	Not Compatible
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
Methanol	10 %
Phenol Red	Not Compatible
PMSF	1 mM
Sodium hydroxide	100 mM
Sucrose	40 %
TLCK	0.1 mg/L
TPCK	0.1mg/L
Urea	3.0 M
o-Vanadate (sodium salt), in PBS, pH 7.2	1 mM

REFERENCE(S):

1. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76-85.
2. Kessler, R. and Fanestil, D. (1986). Interference by lipids in the determination of protein using bicinchoninic acid. Anal. Biochem. 159, 138-142.

RELATED PRODUCTS:

1. **Protease Inhibitor Cocktails (Cat. No. 18-420, 18-425, 18-427, 18-430, 18-433 and 18-436)**
   For inhibiting protease activity in the protein extracts: General, Bacterial, Mammalian, Plant, Recombinant and Yeast/Fungi.
2. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409, 18-411**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples.
3. **RIPA Lysis Buffer (18-415, 18-416 and 18-417:**
   For extracting proteins from different species samples.
4. **Protein Loading Buffer [2X], Cat. No. 20-309:**
   Non-reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.
5. **Protein Loading Buffer [2X], Cat. No. 20-310:**
   Reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.

For Other Related Products, please visit our website or contact us.