BCA Protein Assay Kit

for 20-2000 μg/ml protein

Catalog number: AR0146

A ready-to-use detergent-compatible western blot related total protein analysis reagent used for the quick determination of total protein concentration.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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BCA Protein Assay Kit

Catalog Number: AR0146

List of Components

Description	Quantity	Volume	Contents	Catalog Number
BCA Reagent A	1	100mL	containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1M sodium hydroxide	AR0146-A
BCA Reagent B	1	5mL	containing 4% cupric sulfate	AR0146-B
Albumin (BSA) Standards	1	20mL (2mg/mL)	containing bovine serum albumin (BSA) at 2mg/mL in 0.9% saline and 0.05% sodium azide	AR0146-C

Overview

Form Supplied	BCA Reagent A and B: ready-to-use 1X solutions BSA Standard: stock solution for preparing a series of standard protein dilutions
Assays per kit	Tube procedure: 50 assays Microplate procedure: 500 assays
Storage	Upon receipt store at room temperature. It is stable at room temperature for one year.
Note	If either Reagent A or Reagent B precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or evidence of microbial contamination.
Assay Range	20 - 2000μg/mL
Equivalent	Thermofisher (Product No. 23227), Assay Range: 20 - 2000μg/mL Millipore Sigma (Product No. BCA1), Assay Range: 200 - 1000μg/mL
Compatibility with reagents	**Compatible with** typical concentrations of most ionic and nonionic detergents **Incompatible with** chelating agents, strong acids or bases, and reducing agents: interfere with the reduction and chelating reactions of the assay mechanism

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Applications	Western blotting, protein expression assays, protein profiling and characterization, protein quantitation assays *Our Boster Guarantee covers the use of this product in the above tested applications.
Physical State of A+B Reagent mixture	Light blue liquid
Reagent Color/Absorbance	Blue / A562 nm
Activity	Active in alkaline medium
Description	BCA Protein Assay Kit is a ready-to-use detergent-compatible Western blot related total protein analysis reagent used for the quick determination of total protein concentration by measuring absorbance at 562 nm and comparing to a protein standard absorption vs. concentration curve, according to Smith. The protein quantification process can be finished in 45 minutes.
Cite This Product	BCA Protein Assay Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # AR0146)

Assay Information

Assay Type	Protein Quantitative Measurement
Sample Type	Serum, Plasma, Cell culture extracts, Tissue Extracts
Technique	Solution-based detection of spectral absorbance at 562 nm; Smith Protein assay
Detection Method	Colorimetric/Spectroscopic
Equipment needed	Test tubes or miscroplates; Spectrophotometer; Microplate Reader
Assay Purpose	Measure protein concentration in solution
Incubation Requirements	30 min at 37°C
To be used with	Lysates or homogenates, prepared with non-reducing lysis and extraction buffers: Mammal Tissue Protein Extraction Reagent, Mammal Cell Protein Extraction Reagent, RIPA Lysis Buffer, Mitochondria Extraction Reagent, Membrane Protein Extraction Kit, Cytoplasmic and Nuclear Extraction Kit, Bacterial Cell Protein Extraction Reagent

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Assay Principle

The BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantization 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 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000 μg/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 before the concentration of each unknown is determined based on the standard curve. If precise quantization 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. Two assay procedures are presented. Of these, the Test Tube Procedure requires a larger volume (0.1 ml) 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.

Additional Materials Required

- 37°C water bath or 37°C incubator
- Microplate reader and spectrophotometer capable of accurately measuring absorbance in the region 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 the influence of water evaporation.
6. Several ways for eliminating or minimizing the effects of interfering substances:
   - Remove interfering substances by dialysis or gel filtration.
   - Dilute the sample until substances no longer interfere.
   - Since detergents of high concentrations also affect the results, precipitate the proteins in the sample with trichloroacetic acid (TCA).
7. Avoid using substances including reducing substances, chelating agents, strong acid and alkali since they interfere with protein estimation even at very low concentration.

Assay Procedure

A. Test Tube Procedure

1. Mix BCA Reagent A and BCA Reagent B at 50:1. i.e., mix 50 ml of BCA Reagent A with 1 ml BCA Reagent B.
   Note: Use the following formula to determine the total volume of working reagent required.
   (# standards+ # unknowns) x (# replicates) x (volume of working reagent per sample)= total volume of working reagent required.
2. Follow Table 1 to prepare a fresh set of standards. (Dilute Albumin (BSA) Standards with 0.9% NaCl or PBS)

Table 1: Preparation of Albumin (BSA) Standards

Tube Number	Volume of Diluent (µl)	Volume of BSA (µl)	Final BSA Concentration (µg/ml)
A	0 µl	900 µl of 2 mg/ml Stock	2000 µg/ml
B	100 µl	300 µl of tube A	1500 µg/ml
C	300 µl	300 µl of tube A	1000 µg/ml
D	200 µl	200 µl of tube B	750 µg/ml
E	300 µl	300 µl of tube C	500 µg/ml
F	300 µl	300 µl of tube E	250 µg/ml
G	300 µl	300 µl of tube F	125 µg/ml
H	400 µl	100 µl of tube G	25 µg/ml
I	300 µl	0	0 (blank)

3. Add 0.1 ml of each standard and protein samples into separate labeled test tubes.
4. Add 2 ml of BCA working reagent to each tube and mix well.
5. Incubate at 37°C for 30 minutes.

Note: Increasing the incubation time and temperature can increase the net 562 nm absorbance for each test and decreases both minimum detection level and test range of the kit.

6. Cool all tubes to room temperature (RT).
7. Set the wavelength of spectrophotometer at OD 562 nm. Calibrate the instrument to zero by using water. Subsequently, measure the absorbance of all samples within 10 minutes.
   Note: Color development continues even after cooling to RT. However, the subsequent development at RT is too weak to produce significant error if all absorbance measurements are made within 10 minute.
8. Subtract OD562 of Blank from all readings.
9. Plot the BSA standard curve: OD562 (on Y axis) vs BSA Standard concentration (on X axis). Use the standard curve to determine the protein concentration of each unknown sample.

B. Microplate Procedure

1. Mix BCA Reagent A and BCA Reagent B at 50:1. i.e., mix 50 ml of BCA Reagent A with 1 ml BCA Reagent B.
   Note: Use the following formula to determine the total volume of working reagent required.
   (# standards+ # unknowns) x (# replicates) x (volume of working reagent per sample)= total volume of working reagent required.
2. Follow Table 2 to prepare a fresh set of standards. (Dilute Albumin (BSA) Standards with 0.9% NaCl or PBS)

Table 2: Preparation of Albumin (BSA) Standards

Tube Number	Volume of Diluent (µl)	Volume of BSA (µl)	Final BSA Concentration (µg/ml)
A	0 µl	200 µl of 2 mg/ml Stock	2000 µg/ml
B	30 µl	90 µl of tube A	1500 µg/ml
C	60 µl	60 µl of tube A	1000 µg/ml
D	60 µl	60 µl of tube B	750 µg/ml
E	60 µl	60 µl of tube C	500 µg/ml
F	60 µl	60 µl of tube E	250 µg/ml
G	60 µl	60 µl of tube F	125 µg/ml
H	100 µl	25 µl of tube G	25 µg/ml
I	60 µl	0	0 (blank)

3. Add 25 µl of each standard and protein samples into separate microplate wells.
4. Add 200 µl of BCA working reagent to each well and mix well.
5. Seal plates and incubate at 37°C for 30 minutes.
6. Cool plate to room temperature (RT).

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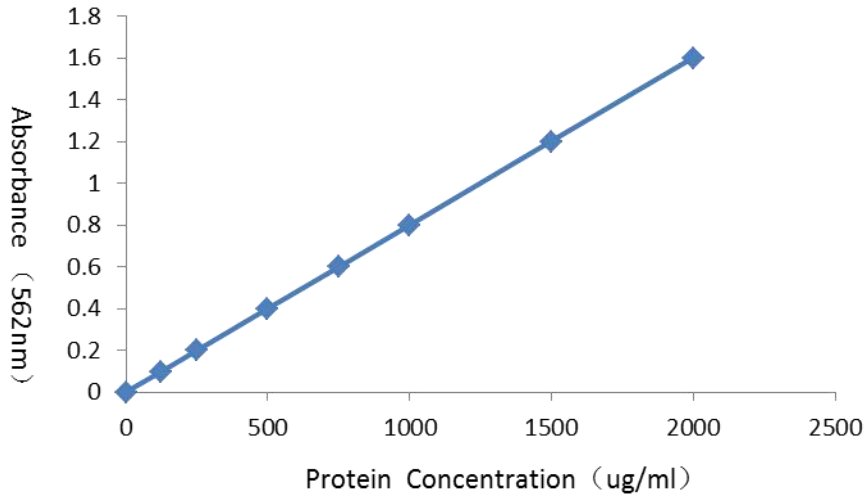
7. Measure the absorbance at 562 nm on a plate reader within 10 minutes.
8. Subtract OD_{562} of Blank from all readings.
9. Plot the BSA standard curve: OD562 (on Y axis) vs BSA Standard concentration (on X axis). Use the standard curve to determine the protein concentration of each unknown sample.

Result Image

Figure 1: Standard Curve of Net Absorbance versus protein sample concentration (Incubate 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 working reagent (e.g., use 50:2, Reagent A:B)
Color of samples appears darker than expected	Protein concentration is too high	Dilute sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids3
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

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Interfering substances

Certain substances are known to interfere with the BCA 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:

Ascorbic acid	**EGTA**	**Iron**	**Impure sucrose**
Catecholamines	**Impure glycerol**	**Lipids**	**Tryptophan**
Creatinine	**Hydrogen peroxide**	**Melibiose**	**Tyrosine**
Cysteine	**Hydrazides**	**Phenol Red**	**Uric acid**

Other substances interfere to a lesser extent with protein estimation using the BCA assay, and these have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the Standard Test Tube Protocol are listed in Table 3

Table 3: Compatible substance concentrations in the BCA Protein Assay:

Substance	Compatible Concentration
Sodium bicarbonate	100 mM
Sodium phosphate	25 mM
2-Mercaptoethanol	0.01%
Glycercol (pure)	10%
Glycine-HCl, pH 2.8	100 mM
HEPES	100 mM
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
Nickel chloride (in TBS, pH8.0)	10 mM
Nonidet P-40 (NP-40)	5% (w/v)
Octyl β -glucoside	5% (w/v)
Potassium thiocyanate	3.0 M
SDS	5%
Sodium acetate, pH 4.8	200 mM
Sodium azide	0.20%
Sodium hydroxide	100 mM
Sucrose	40%
Triton X-100	5%
Triton X-114, X-305,X-405	1%

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Tween-20, Tween-60, Tween-80	5%
Zwittergent	1%
ACES, pH 7.8	25 mM
Acetone	10%
Acetonitrile	10%
Ammonium sulfate	1.5 mM
Aprotinin	10 mg/L
Bicine, pH 8.4	20 Mm
Bis-Tris, pH 6.5	33 mM
Borate, pH 8.5	50 mM
Brij-35	5%
Brij-52	1%
Brij-56, Brij-58	1%
BugBuster protein Extraction Reagent	no interference (undiluted)
Calcium chloride (in TBS, pH 8.0)	10 mM
CelLytic B Reagent	no interference (undiluted)
Cesium bicarbonate	100 mM
CHAPS	5%
Cobalt chloride (in TBS, pH 8.0)	0.8 mM
CytoBuster Protein Extraction Reagent	no interference (undiluted)
Deoxycholic acid	5%
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
DMF	10%
DMSO	10%
EDTA	10 mM
EPPS, pH 8.0	100 mM
Ethanol	10%
Ferric chloride (in TBS, pH 8.0)	10 mM
Glucose	10 mM
Glycerol	10%
Guanidine-HCl	4 M
Imidazole, pH 7.0	50 mM

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MES, PH6.1	100mM
Methanol	10%
MOPS, pH7.2	100mM
N-Acetyglucosamine(10mM)in PBS, pH7.2	10mM
Octyl β-thioglucpyranoside	5%
PIPES, pH6.8	100mM
PMSF	1 mM
PopCulture Reagent	no interference (undiluted)
Reportasol Extraction Buffer	no interference (undiluted)
Sodium chloride	1 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium ortho-vanadate in PBS, pH7.2	1mM
Span 20	1%
TBS (150 mM NaCl, 100 mM Tris-HCl, pH 8.0)	no interference (undiluted)
Thimerosal	0.01%
TLCK	0.1mg/L
TPCK	0.1mg/L
Tricine, pH 8.0	25 mM
Triethanolamine, pH 7.8	25 mM
Tris	250 mM
Tris(hydroxypropyl)phosphine (THP)	1 mM
Urea	3M
Zinc chloride (in TBS, pH 8.0)	10 mM