IMMUNOLOGICAL SCIENCES Product Data Sheet

BCA Protein Quantification Assay Kit WB-RR-521 - WB-RR-522

CAT.#	Components	2500 Assays	5000 Assays	Storage
BCA-A	BCA Reagent A	500 mL	2X500 mL	+4°C – for 1 year
BCA-B	BCA Reagent B(blue)	15 mL	2X 15 mL	+4°C – for 1 year
BCA-C	BSA Standard Powder	10x1 mL (conc.5mg/mL)	20 x 1 mL (conc.5mg/ml)	-20°C- for 1 year
PBS	PBS powder	50 mL	2X 50 mL	+4°C – for 1 year

Introduction

BCA Protein Assay Kit 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 562 nm that is nearly linear with increasing protein concentrations over a broad **working range (20–2000 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 unknowns 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.

2 Assay Procedures are presented.

Test Tube Procedure requires a larger volume (0.1 mL) of protein sample;

The Microplate Procedure requires a smaller volume (10–20 µL) of protein sample

Preparation of standards and working reagent (required for both assay procedures)

The Working Reagent BCA-A and BCA-B ratio of 50:1 (v/v) – Mix 50 parts Reagent A to 1 Reagent B

For example: 50mL BCA-A and 1mL BCA-B are mixed very well to get the working reagent.

Note: Precipitation may be formed when they are mixed, the precipitation will disappear after shaking and fully dissolving.

The amount of working reagent required for each sample is:

- 200 µl for Micro-assy Plate Procedure
- 2.0ml for Test Tube procedure

A. The Microplate Assay Procedure（Working range 20-2000 µg/ml）:

1.Preparation of diluted albumin (BSA) standards： 20µl (5mg/ml BSA) standards was diluted by 100uL PBS to get the concentration 1 mg/ml.
2.Use the following table as a guide to prepare a set of protein standards.

Cat. #	0	1	2	3	4	5	6	7	8
	1 mg/ml BSA standards µL							5 mg/ml BSA standards µl	
BSA standards µl	0	0.5	2.5	5.0	10	15	20	6	8
PBS µl	20	19.5	17.5	15	10	5	0	14	12
BSA final concentration µg/ml	0	25	125	250	500	750	1000	1500	2000
Total volume µl	20 µl								

3.The test sample was added into a microplate well and the final volume is adjusted to 20uL using PBS
4.Add 200 µL of the Working Reagent to each well and mix plate thoroughly on a plate shaker for 30 seconds. Cover plate and incubate at 37°C for 30 minutes.
5.Cool plate to RT. Measure the absorbance at or near 562 nm on a plate reader.
6.Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
7.Prepare a standard curve by plotting the average Blank–corrected 562 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.
Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best–fit curve provides more accurate results than a purely linear fit. If plotting results by hand, a point–to–point curve is preferable to a linear fit to the standard points.

B.Test-tube procedure (Working range 20-2000 µg/ml)

1. Pipette 0.1 mL of each standard and unknown sample replicate into an appropriately labeled test tube.
2. Add 2.0 mL of the WR to each tube and mix well.
3. Cover and incubate tubes at selected temperature and time:
• Standard Protocol: 37°C for 30 minutes (working range = 20–2000 µg/mL)
• RT Protocol: RT for 2 hours (working range = 20–2000 µg/mL)
• Enhanced Protocol: 60°C for 30 minutes (working range = 5–250 µg/mL)

1.Preparation of diluted albumin (BSA) standards： 150µl - 5mg/ml BSA standards was diluted by 600uL PBS to get the concentration 1.0 mg/ml。
2.Use the following table as a guide to prepare a set of protein standards.

Cat N#	0	1	2	3	4	5	6	7	8
	1 mg/ml BSA standards µl							5 mg/ml BSA standards µl	
BSA standards µl	0	2.5	12.5	25	50	75	100	30	40
PBS µl	100	97.5	87.5	75	50	25	0	70	60
BSA final concentration µg/ml	0	25	125	250	500	750	1000	1500	2000
Total volume µl	100 µl								

3.The test sample was added into a test tube and the final volume is adjusted to 100uL using PBS.
4.Add 2mL of the Working Reagent to each test tube and mix thoroughly on a plate shaker for 30 seconds. Cover plate and incubate at 37°C for 30 minutes.
5.Cool all tubes to RT.
6.With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water, then measure the absorbance of all the samples within 10 minutes.
Note: Because the BCA assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.
7.Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562nm absorbance measurement of all other individual standard and unknown sample replicates.
8.Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.