BCA Protein Assay Kit

Cat No. BCA-M-001-500
Size: 500 Reactions
Albumin (BSA) Standard should be stored at -20°C
Store other reagents at 4°C

MOLEQULE-ON®

Kit Components	Volume
Albumin (BSA) (5mg/ml)	1 ml
BCA reagent A	100 ml
BCA reagent B	3ml
PBS	30ml

Description

The 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 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.

Protocol

Ⅰ. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards for Test Tube Procedure.

Table 1. Preparation of Diluted Albumin (BSA) Standards for Test Tube Procedure.

VIAL	0	1	2	3	4	5	6	7
Albumin (BSA) Standard (µl) (0.5µg/µl)	0	5	10	20	40	60	80	100
Distilled water or PBS (µl)	100	95	90	80	60	40	20	0

Use Table 2 as a guide to prepare a set of protein standards for Microplate Procedure.

Table 2. Preparation of Diluted Albumin (BSA) Standards for Microplate Procedure, make the final concentration of BSA to 0.5ug/ul for standard curve.

VIAL	0	1	2	3	4	5	6	7
Albumin (BSA) Standard (µl) (0.5ug/µl)	0	1	2	4	8	12	16	20
Distilled water or PBS (µl)	20	19	18	16	12	8	4	0

Ⅱ . Preparation of the BCA Working Reagent

1. Use the following formula to determine the total volume of BCA Working Reagent required:

Note: 1.0 ml of the BCA Working Reagent is required for each sample in the Test Tube Procedure, while only 200 µl of BCA Working Reagent is required for each sample in the Microplate Procedure.

2. Prepare BCA Working Reagent by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A: B).

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

Ⅲ. Test Tube Procedure

1. Pipette 0.1 ml of each standard and unknown sample replicate into an appropriately labeled test tube.
2. Add 1.0 ml of the BCA Working Reagent to each tube and mix well.
3. Cover and incubate tubes at 37°C for 30 minutes
4. Cool all tubes to room temperature.
5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.
6. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement 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.

Ⅳ. Microplate Procedure

1. Pipette 20 µl of each standard or unknown sample replicate into a microplate well.
2. Add 200 µl of the BCA Working Reagent to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37°C for 30 minutes.
4. Cool plate to room temperature.
5. 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.