BCA Protein Assay Kit (Enhanced)

Catalog# BWR1026

Size: 500 tests

Lot # Check on the product label

Introduction

1. Hypersensitive, minimum concentration of detection: 0.5 μg/mL.
2. Linear range: 0.5～20 μg/mL, especially suitable for the sample with low concentration.
3. It's not sensitive to most ionic and non-ionic detergents, but is sensitive to reductant and chelant of Cu^{+}.
4. It is more superior than Lowry Assay, can finish assay within 60 min.
5. The reagent can be stable for 2 years at room temperature, and the working solution can be effective within one day.
6. Its end product is stable, and the variation coefficient on different kinds of protein detections is smaller than Coomassie Brilliant Blue Assay.
7. Since this kit can detect dipeptide, it is suitable for detecting proteins with small molecular weight, however, Bradford Assay has the requirement for protein size.
8. It is suitable for Test Tube and Microplate Methods.

Kit Components

Components	Size	Storage Instruction
BCA Reagent A	50 ml	Store at 4°C for one year.
BCA Reagent B	50 ml	
BCA Reagent C	3 ml	
Lyophilized Albumin Standard Ampules (5mg BSA)	5 mg	

Preparation of standard and working solution

A. Dilute Albumin Standard Ampules

Reconstitute the lyophilized Albumin Standard Ampules with 1ml of distilled water to get the liquid Albumin Standard Ampules with concentration 5mg/ml first, then, dilute Albumin Standard Ampules according to the following table:

Tube No.	Volume of Diluent	BSA Standard	Final concentration
	7.68 ml	320 μl (From 5mg/ml tube)	200 μg/ml
A	8.0 ml	2.0 ml (From 200μg/ml tube)	40 μg/ml
B	4.0 ml	4.0 ml (Pipette from Tube A)	20 μg/ml
C	4.0 ml	4.0 ml (Pipette from Tube B)	10 μg/ml

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D	4.0 ml	4.0 ml (Pipette from Tube C)	5 μg/ml
E	4.0 ml	4.0 ml (Pipette from Tube D)	2.5 μg/ml
F	4.8 ml	3.2 ml (Pipette from Tube E)	1 μg/ml
G	4.0 ml	4.0 ml (Pipette from Tube F)	0.5 μg/ml
H	8.0 ml	0	0 μg/ml (blank)

B. Preparation of micro-BCA working solution

1. Use the following formula to determine the total volume of working solution required:

(# standards + # unknowns) × (# replicates) × (volume of working solution per sample) = total volume working solution required

Example: for the Standard Test Tube Method with 3 unknowns and 2 replicates of each sample:

(8 standards + 3 unknowns) × (2 replicates) × (1 ml) = 22 ml of BCA working solution (end user can prepare 25ml)

Note: 1 ml of working solution is required for each sample in the Test Tube Method, while only 150 μl of working solution required for each sample in the Microplate Method.

2. Preparation of BCA working solution

Prepare BCA working solution by mixing 50 parts of BCA Reagent A with 48 parts of BCA Reagent B and 2 parts of BCA Reagent C (BCA Reagent A: B: C=50:48:2).

i.e. Combine 5 ml of Reagent A with 4.8 ml of Reagent B and 200 μl of Reagent C.

Note: When Reagent C is first added to Reagent A and B, a turbidity is observed that quickly disappears upon mixing to yield a clear and green working solution. Prepare sufficient volume of working solution according to the number of samples to be assayed. The working solution is stable for 1 day when stored in a closed container at room temperature.

Protocol

- **Microplate Method**

1. Pipette 150 μl of each Albumin Standard Ampules and unknown sample replicate into a microplate well.
2. Add 150 μl of working solution to each well and mix thoroughly on the plate shaker for 30 seconds.
3. Cover plate and incubate at 37℃ for 2 hours or place at 60℃ for 1 hour.
4. Cool plate to room temperature.
5. Measure the absorbance at or near 562 nm on a microplate reader.
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 & its concentration (μg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

- **Test Tube Method**

1. Pipette 1.0 ml of each Albumin Standard Ampules and unknown sample replicate into an appropriately labeled test tube.
2. Add 1.0 ml of working solution to each tube and mix thoroughly.
3. Cover and incubate tubes in water bath at 60 ℃ for 1 hour.
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. Then, measure the absorbance of all the samples within 10 minutes.

   Note: BCA assay does not reach a true end point, color development will continue even after cooling to room temperature. However, since the rate of color development is low at room temperature, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made 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 & its concentration (μg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

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