BCA Protein Assay Kit

Catalog# BWR1023

Size: 200 tests

Lot # Check on the product label

Introduction

BCA (bicinchoninic acid) is a perfect protein assay method, it serves the purpose of the Folin reagent in the Lowry assay, namely to react with complexes between copper ions and peptide bonds to produce a purple end product. This method is favored by professionals for its high sensitivity, stability, reliability, and small variation coefficient on different kinds of protein detections. Determination of the protein concentration with BCA is not influenced by chemical substances in majority of the samples. For example, in tissue and cell lysate experiments, the test results were not affected by common detergents (SDS, Triton X-100, Tween), but will be influenced by chelant (EDTA, EGTA), deoxidizer (DTT, mercaptoethanol) and lipid. So, in the assay, if the background of the sample diluent buffer or lysis buffer is high, end user can try to use Bradford Protein Assay Kit (Catalog #BWR1027). This kit can work well here.

Principle

In an alkaline medium, Cu^{2+} was reduced to Cu^{+} by protein, and the purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with Cu^{+}. This complex exhibits a strong absorbance at 562nm, and since the absorbancy strength is proportional to the protein concentration, determine the absorption value at 562nm, then compare with the standard curve, the protein concentration can be calculated.

Kit Components

Components	Size	Storage Instruction
BCA Reagent A	40 ml	Store at 4°C for one year.
BCA Reagent B	1.2 ml	
Lyophilized Albumin Standard Ampules (5mg BSA)	5 mg	

Protocol

- **Test Tube Method**

1. Preparation of BCA working solution: BCA Reagent A to BCA Reagent B ratio=50:1 (i.e. Combine 1 ml of BCA Reagent B with 50 ml of BCA Reagent A), and mix thoroughly.

2. **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 the liquid Albumin Standard Ampules: Dilute with 0.9% NaCl or PBS to adjust Albumin Standard Ampules final concentration to 25~2000 μg/ml.

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923

7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China

www.biospes.com

Here is the dilution instruction:

Tube No.	Volume of Diluent	BSA Standard	Final concentration
A	360 μl	240 μl	2000 μg/ml
B	280 μl	120 μl	1500 μg/ml
C	300 μl	300 μl (Pipette from Tube A)	1000 μg/ml
D	200 μl	200 μl (Pipette from Tube B)	750 μg/ml
E	300 μl	300 μl (Pipette from Tube C)	500 μg/ml
F	300 μl	300 μl (Pipette from Tube E)	250 μg/ml
G	300 μl	300 μl (Pipette from Tube F)	125 μg/ml
H	400 μl	100 μl (Pipette from Tube G)	25 μg/ml
I	300 μl	0 μl	0 μg/ml（blank）

3. Pipette 0.1 ml of each Albumin Standard Ampules and sample replicate into an appropriately labeled test tube.

4. Add 2 ml of BCA working solution into each tube, incubate at 37℃ for 30 min. (**Note:** Increasing the incubation time or temperature can increase 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.)

5. Cool the tubes to room temperature

6. Measure the absorbance at or near 562 nm on a Microplate Reader within 10 min, calculate the protein concentration according to the standard curve.

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.

- **Microplate Method**

1. Preparation of BCA working solution. (Ditto)
2. **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 the liquid Albumin Standard Ampules. (Ditto)
3. Pipette 25 μl of each Albumin Standard Ampules and properly diluted sample replicate into a microplate well.
4. Add 200 μl of BCA working solution into each well, and mix thoroughly.
5. Cover plate and incubate at 37℃ for 30 min.
6. Cool plate to room temperature, measure the absorbance at or near 562 nm on a Microplate Reader, calculate the protein concentration according to the standard curve.

Notes

1. Precipitate may appear under law temperature or long term storage. Mix or incubate at 37℃or microwave for dozens of seconds to dissolve. Abandon the reagent if bacterial contaminated.
2. If sample contains EDTA, EGTA, DTT, ammonium sulfate, lipid, it will affect the test result, try to use Bradford Protein Assay Kit. Highly concentrated detergent will affect the test result as well, try to remove them by TCA precipitate.
3. To get more accurate result, each protein gradient and sample should be done the duplicate well, and the Albumin Standard Ampules and sample should be treated in the same way (for example, use the same solution to dissolve), make a standard curve each test.
4. A turbidity is observed while reagent A and reagent B mixed, but it will disappear if mix thoroughly.
5. Required instruments: 37 ℃ water bath kettle or incubator, microplate reader or spectrophotometer (wavelength: 540-595 nm, 562 nm is optimal).

   Microplate reader should be used with 96-well plate. While determining the protein concentration with the spectrophotometer, the quantity of sample can be tested by this kit will reduce. And avoid the water evaporation while incubating with the incubator.

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com