BCA Protein Assay Kit

Catalog No. orb90441

Kit Contents

BCA Reagent A 100ml

BCA Reagent B 5ml

Albumin Standard Ampules, 10mg×5

Sufficient reagents for 50 test tube or 500 micro plate assays

Storage

BCA Reagent A & B should be stored at 4°C;

Albumin Standard Ampules should be stored at -20°C.

Expiration Date

One year.

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Introduction

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.2

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.

Protocol

Preparation of Standards and Working Reagent (required for both assay procedures)

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards.

1. BCA Regent A to BCA Regent B ratio = 50:1 (i.e. Add 1ml BCA Reagent B into 50ml BCA Reagent A).

2. Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 25–2,000 µg/ml) (Dilute the lyophilized Albumin Standard Ampules with 0.9% NaCl or PBS to 2,000 µg/ml working solution).

Tube	Volume of Diluent	Volume and source of BSA	Final BSA concentration
A	0 µl	600 µl	2000 µg/ml
B	100 µl	300 µl	1500 µg/ml
C	300 µl	100 µl of A dilution	1000 µg/ml
D	200 µl	200 µl of B dilution	750 µg/ml
E	300 µl	300 µl of C dilution	500 µg/ml
F	300 µl	300 µl of E dilution	250 µg/ml
G	300 µl	300 µl of F dilution	125 µg/ml
H	400 µl	100 µl of G dilution	25 µg/ml
I	300 µl	0 µl	0 mg/ml

Table 1. Preparation of Diluted Albumin (BSA) Standards.

- ## Test Tube Procedure (Sample to WR ratio = 1:20)

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:

a. Standard Protocol: 37°C for 30 minutes (working range = 25-2,000 µg/ml)

b. RT Protocol: RT for 2 hours (working range = 25-2,000 µg/ml)

c. Enhanced Protocol: 60°C for 30 minutes (working range = 5-250 µg/ml)

Notes:

Increasing the incubation time and 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.

Use a water bath to heat tubes for either Standard (37°C incubation) or Enhanced (60°C incubation) Protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.

4. Cool all tubes to RT.

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.

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.

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.

- ## Microplate Procedure (Sample to WR ratio = 1:8)

1. Pipette 25 µl of each standard or unknown sample replicate into a microplate well (working range = 25-2,000 µg/ml).

Note: If sample size is limited, 10 µl of each unknown sample and standard can be used (sample to WR ratio = 1:20).

However, the working range of the assay in this case will be limited to 125-2,000 µg/ml.

2. Add 200 µl of the WR 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 RT.

5. Measure the absorbance at or near 562 nm on a plate reader.

Notes:

a. Wavelengths from 540-595 nm have been used successfully with this method.

b. Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher562 nm measurements are desired, increase the incubation time to 2 hours.

- ## Troubleshooting

1. **When the temperature is law or storage tine is long, sediments may occur.** Please mix round or incubate at 37℃, or microwave for 30 seconds to melt it. If any bacteria contamination is found, please abandon it.
2. **If sample contains EDTA, EGTA, DTT, ammonia sulfate or lipid**, it will interrupt the result, please try Bradford protein concentration test kit; the detergent at high concentration will interrupt the result, try to remove them by TCA sediments.
3. **To get more exact protein concentration result**, make sure each SAAG and sample has a second well, try to deal with standard Ampules and sample in a same method(such as use a same dilution and regent). And erytime should make a standard curve.
4. **When Regent A mixes with Regent B, few sediments may occur.** It will fade away after be mixed thoroughly.
5. **Equipment required:** 37℃ water bath, incubator, ELISA reader, spectrophotometer (540-595 nm).