Product Manual

BCA Protein Assay Kit

Catalog No.: abx090642

Size: 200 tests / 500 tests

Linear range: 0.5 µg/ml – 20 µg/ml

Minimum detection concentration: 0.5 µg/ml

Storage: Store all reagents at 4°C for up to one year.

Introduction

The BCA protein assay is a protein determination formulation based on bicinchoninic acid (BCA) for the colorimetric detection. This method combines the reduction of Cu^{2+} to Cu^{+} by proteins in an alkaline medium (the biuret reaction) and the soluble purple-colored reaction product from the complexing of Cu^{+} and BCA. This purple-colored complex exhibits a maximum absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (0-2 mg/ml). Since it is highly sensitive and simple to use, the BCA protein assay is adopted by many laboratories and companies, and is one of the main protein quantitative methods as well as the Bradford assay.

Kit components (200 tests)

1. Reagent A: 20 ml
2. Reagent B: 20 ml
3. Reagent C: 1 ml
4. Lyophilized BSA standard ampoules: 5 mg

Kit components (500 tests)

1. Reagent A: 50 ml
2. Reagent B: 50 ml
3. Reagent C: 3 ml
4. Lyophilized BSA standard ampoules: 5 mg

Materials required but not provided

1. 37°C incubator
2. Spectrophotometer or plate reader (wavelength 562 nm)
3. Test tubes or 96-well plate
4. Distilled water (or reagent used to dilute samples)

A. Preparation of stock standard and working standard solutions

1. Reconstitute the lyophilized Albumin Standard Ampoules with 1 ml distilled water to obtain a stock standard solution of 5 mg/ml.

2. Prepare working standard concentrations by diluting the 5 mg/ml BSA standard into concentrations of 200, 40, 20, 10, 5, 2.5, 1 and 0.5 µg/ml. A control/blank (0 mg/ml BSA) should also be prepared. The BSA dilutions can be frozen and stored at -20°C, and thawed and warmed to room temperature when used.

Tube	Diluent Volume	BSA Standard Volume	Final Concentration
1	7.68 ml	320 µl (from 5 mg/ml stock solution)	200 µg/ml
2	8.0 ml	2.0 ml (from Tube 1)	40 µg/ml
3	4.0 ml	4.0 ml (from Tube 2)	20 µg/ml
4	4.0 ml	4.0 ml (from Tube 3)	10 µg/ml
5	4.0 ml	4.0 ml (from Tube 4)	5 µg/ml
6	4.0 ml	4.0 ml (from Tube 5)	2.5 µg/ml
7	4.8 ml	3.2 ml (from Tube 6)	1 µg/ml
8	4.0 ml	4.0 ml (from Tube 7)	0.5 µg/ml
9	8.0 ml	0 ml	0 µg/ml (blank)

B. Preparation of BCA working solution

1. Calculate the volume of BCA working solution required using the following formula.

$$V_{\text{Total}} = (N_{\text{Standards}} + N_{\text{Samples}}) \times N_{\text{Replicates}} \times V_{\text{Test}}$$

Where:

- V_{Total} is the total volume of BCA working solution required (rounded up)
- $N_{\text{Standards}}$ is the number of standards used
- N_{Samples} is the number of samples used
- $N_{\text{Replicates}}$ is the number of replicates for each standard and sample
- V_{Test} is the volume of working solution per standard or sample (1 ml if using the Test Tube Procedure, or 150 µl if using the Microplate Procedure)

2. Prepare the BCA working solution by mixing Reagent A, Reagent B and Reagent C in the ratio 50:48:2 (e.g. add 5 ml Reagent A, 4.8 ml Reagent B and 200 µl Reagent C to a separate tube and mix thoroughly).

Note: When Reagent C is added to a mixture of Reagent A and Reagent B, turbidity may be

observed. This disappears upon mixing to yield a clear, green solution. The working solution is stable for 1 day after preparation when stored in a sealed container at room temperature. It is recommended to prepare slightly more BCA working solution than calculated (e.g. if the calculated V_{Total} is 22 ml, it is recommended that the end user prepares 25 ml of BCA working solution).

C. Procedure

96-well microplate procedure

1. Pipette 150 µl of controls, standards and samples into the appropriate wells.
2. Add 150 µl of BCA working solution to each well. Mix thoroughly using plate shaker for 30 seconds.
3. Cover the plate and incubate at 37°C for 2 h, or at 60°C for 1 h.
4. Cool the plate to room temperature, then measure the absorbance at 562 nm on a plate reader.
5. Subtract the average blank absorbance values from the absorbance values of the other wells. Draw a standard curve by plotting absorbance reading for each BSA standard versus its concentration. Use the standard curve to determine the protein concentration of each unknown sample.

Test tube procedure

1. Pipette 1.0 ml of controls, standards and samples into the appropriate tubes.
2. Add 1.0 ml of BCA working solution to each tube. Mix thoroughly.
3. Cover the tubes and incubate at 60°C for 1 h using a water bath.
4. Cool all tubes to room temperature. Using a spectrophotometer set at 562 nm, zero the instrument using a cuvette filled with distilled water only, and then measure the absorbance of all samples and standards within 10 minutes.
5. Subtract the average blank absorbance values from the absorbance values of the other wells. Draw a standard curve by plotting absorbance reading for each BSA standard versus its concentration. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: The rate of color development is reduced at low temperatures. No significant error is observed if all tubes are measured within 10 minutes of the first measurement.