

BCA Protein Assay Kit

Catalog No.: K001

Size: 100 T/500 T/2500T

Storage: Store at room temperature for one year except for BSA at -20°C.

Kit Contents:

Extraction Times	100T	500T	2500T	Storage
I Reagent A	20 ml	100 ml	500 ml	RT
II Reagent B	1 ml	5 ml	25 ml	RT
III BSA(5 mg/ml)	0.4 ml	2 ml	10 ml	-20°C

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 protein 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). According to highly sensitivity and simple to use, the BCA protein assay is adopted by laboratories and companies, and becomes one of the prior protein quantitative methods as well as the Bradford assay.

The Fine BCA Protein Assay Kit has the characteristics of high sensitivity and light background, and the measured range can be up to 3 mg/ml.

Procedure

1. 96-Microplate Procedure

- (1) Prepare the Working Reagent. Working reagent, clear liquid in the color of yellow green, is mixed with the Reagent A and Reagent B at ratio of 50:1.
- (2) Prepare the Standard Reagent. Dilute the 5 mg/ml BSA Standard into lateral concentrations of 0, 0.25, 0.5, 0.75, 1, 1.5, 2 mg/ml. The BSA dilutions can be frozen and stored at -20°C, and thawed and warmed to room temperature when used.
- (3) Mark the wells with Standard or Test. Add at least 20 μl of the diluted Standard Reagent to each Standard well and add equal volume of testing samples each Test well.
- (4) Add all the wells with 200 μl Working Reagent and incubate for 30 min at 37°C.

(5) Measure the absorbance at 562 nm on a plate reader. Subtract the average absorbance reading of at least 3 blank 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.

2. Test Tube Procedure

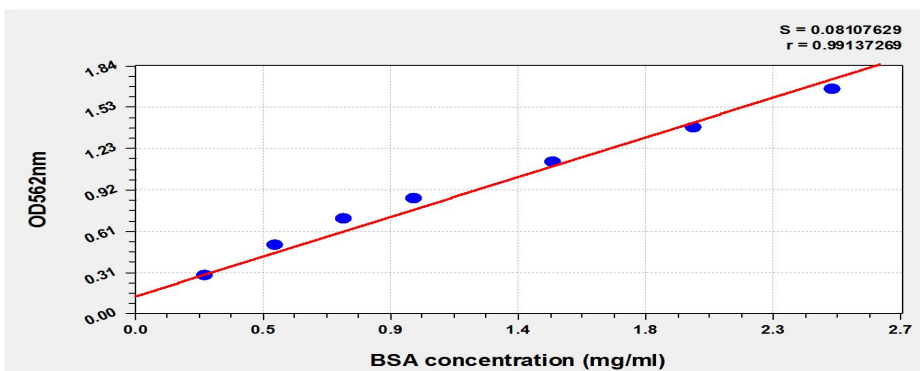
(1) Prepare the Working Reagent. Working reagent, clear liquid in the color of yellow green, is mixed with the Reagent A and Reagent B at ratio of 50:1.

(2) Prepare the Standard Reagent. Dilute the 5 mg/ml BSA Standard into lateral concentrations of 0, 0.25, 0.5, 0.75, 1, 1.5, 2 mg/ml. The BSA dilutions can be frozen and stored at -20°C , and thawed and warmed to room temperature when used.

(3) Mark the tubes with Standard or Test. Add at least 50 μl of the diluted Standard Reagent to each Standard tube and add equal volume of testing samples into each Test tube.

(4) Add all the tubes with 1 ml Working Reagent and incubate for 30 min at 37°C .

(5) Measure the absorbance at 562 nm with a spectrophotometer. Subtract the absorbance average reading of at least 3 blank tubes. 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.



Additional Testing Information

1. If lacking time for incubation, the mixed reagents can be heated by microwave on low with 20 seconds.

Prevented from violent boiling, the mixed reagents should be heated 10 seconds twice separately and waggled slightly in the heating gap.

2. Using the same protein with known concentration to prepare Standard Reagent can enhance the accuracy of concentration determining, e.g. determine the concentration of serum protein with Standard Reagent of BSA, or determine the concentration of antibodies with Standard Reagent of IgG.