

BCA Protein Assay Kit II

Catalog#: BN01029 -2500, -5000. Store at Room Temperature

I. Introduction:

Assay Genie's BCA Protein Assay kit provides a colorimetric detection and quantification of total protein content even in the presence of detergents. The Kit is based on the chelation of bicinchoninic acid (BCA) with the cuprous cation (Cu^{+1}), which is generated by reduction of cupric cation (Cu^{+2}) with the protein in an alkaline condition. The Cu^{+1} -BCA chelate is a water-soluble complex and exhibits a strong absorbance at 562 nm that is linear over a wide range of protein concentrations between 25-2000 $\mu\text{g/ml}$. In general, protein concentrations are estimated with reference to a commonly used protein standard. The Kit also includes Bovine Serum Albumin (BSA) as a protein standard for estimation of total protein content of samples.

II. Applications:

Measuring total protein concentration of pure proteins, extracts or lysates.

III. Kit Contents:

Components	BN01029 2500 assays	BN01029 5000 assays	Cap Code
BCA Reagent A	500 ml	2 X 500 ml	NM
BCA Reagent B	25 ml	25 ml	NM
BSA Standard (2 mg/ml)	5 x 1 ml	10 x 1 ml	White

IV. User Supplied Reagents and Equipment:

- Sterile Eppendorf tubes, test tubes, spectrophotometer, microplate and microplate reader.

V. Storage and Handling:

Store all components of the kit at room temperature. Read the entire protocol before performing the experiment.

VI. Preparation:

• Preparation of BSA Standards:

Prepare BSA Standards as suggested in the table below by diluting BSA Standard using de-ionized water or same diluent as that of the protein samples. Other similar dilutions can also be used within the assay range of 25-2000 $\mu\text{g/ml}$. One tube of BSA Standard is sufficient to make diluted solutions in triplicates. The diluted standard solutions can be used for up to one week when stored at 4 °C.

Vial	Volume of BSA (μl)	Volume of diluent (μl)	Final BSA Concentration ($\mu\text{g/ml}$)
1 (Stock)	300 of 2 mg/ml Stock	0	2000
2	300 of 2 mg/ml Stock	100	1500
3	300 of 2 mg/ml Stock	300	1000
4	300 of vial 3	300	500
5	300 of vial 4	300	250
6	300 of vial 5	300	125
7	100 of vial 6	400	25
8 (Blank)	0	400	0

- **Preparation of Protein Samples:** Prepare different concentrations of samples by diluting with water or an appropriate diluent to a concentration within the assay range (25-2000 $\mu\text{g/ml}$). It is recommended to use three different concentrations of samples & perform the assay in duplicates or triplicates.

- **Preparation of BCA working reagent:** To prepare BCA working reagent, mix BCA Reagent A with BCA Reagent B in the ratio of **50:1**. Upon mixing, green colored turbidity will be observed that should disappear upon further mixing to give a green colored solution. Each sample replicate requires 200 μl of BCA working reagent for microplate assay or 2 ml for test tube procedure. Prepare sufficient amount of BCA working reagent solution needed for all BSA Standards & Samples.

Note: It is recommended that BCA working reagent should be prepared fresh. However, the prepared reagent is stable and can be stored at room temperature for several days in a closed container.

VII. Assay Protocol: BCA Assay can be performed in a microtiter plate format or test tube format.

A. Microplate Procedure:

1. Add 25 μl of each BSA Standard and protein samples into microtiter plate wells.
2. Add 200 μl of BCA working reagent to the Standard & sample wells, mix thoroughly for 30 s.
3. Cover the plate and incubate at 37 °C for 30 min or room temperature for 2 h. After incubation, cool the plate to room temperature.
4. Set the absorption wavelength of a microplate reader to 562 nm and read all Standards and samples (OD_{562}).

B. Test Tube Procedure:

1. Add 100 μl of each BSA Standard and protein samples into a 4 ml test tube.

2. Add 2 ml of the BCA working reagent and mix well.
 3. Cover the tubes and incubate under either one of following conditions:
 - 37 °C for 30 min or at room temperature for 2 h (Assay range is 25-2000 µg/ml)
 - 60 °C for 30 min (Assay range is 5-250 µg/ml)
 4. After incubation, cool the tubes to room temperature.
 5. Set the absorbance wavelength of a spectrophotometer to 562 nm. Blank the instrument by using water or the diluent only.
 6. Read absorbance (OD₅₆₂) of all Standards and samples.
- C. Calculations:** Subtract OD₅₆₂ of Blank (0 Standard, #8) from all readings. Plot the Standard curve, OD₅₆₂ (on Y-axis) vs Standard BSA concentration (on X-axis). Obtain the equation from the plot $Y = aX + b$. Use the obtained value of slope (a) to calculate protein concentration in samples.

$$\text{Protein concentration in sample} : C = DX = \text{Dilution Factor} \times \frac{(Y-b)}{a} = \mu\text{g/ml}$$

Where **Y** = OD₅₆₂ of protein sample

X = concentration of protein sample

a = Slope of the BSA Standard curve

b = Y-intercept of the Standard Curve

D = Dilution factor of protein sample

Alternatively, get the sample concentration from the Standard curve. Then calculate protein concentration in sample:

$$C = DX$$

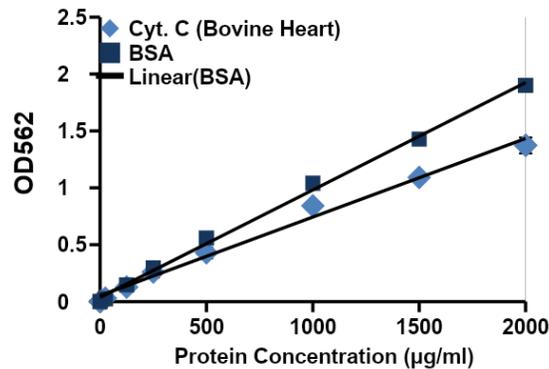


Figure: Typical absorbance plots obtained for BSA and Cytochrome C from Bovine Heart using a microplate procedure (37°C for 30 min).

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