ab287853 – BCA Protein Assay Kit II

For quantifying the total protein concentration of pure proteins, extracts or lysates.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287853

Introduction

BCA Protein Assay kit provides a colorimetric detection and quantification of total protein content even in the presence of detergents, measuring total protein concentration of pure proteins, extracts or lysates. 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 µg/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.

Storage and Stability

Store all components of the kit at room temperature.

Materials Supplied

Item	Quantity (2500 test kit)	Quantity (5000 Test kit)	Storage Condition
BCA Reagent 1	500 ml	2 x 500 ml	RT
BCA Reagent B	20 ml	20 ml	RT
BSA Standard II	5 x 1 ml	10 x 1 ml	RT

PLEASE NOTE: BCA Reagent 1 was previously labelled as BCA Reagent A, and BCA Reagent B as BCA Reagent 2, and BSA Standard II as BSA Standard (2 mg/ml). The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Sterile Eppendorf tubes
- Test tubes
- Spectrophotometer
- Microplate
- Microplate reader.

Reagent Preparation

- Read the entire protocol before performing the experiment.

BCA working reagent: mix BCA Reagent 1 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 enough of BCA working reagent solution needed for all BSA Standards & Samples.

Δ 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.*

Standard Preparation

Prepare BSA Standards as suggested in the table below by diluting BSA Standard II 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 µg/ml. One tube of BSA Standard II 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 (µg/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

Sample Preparation

Prepare different concentrations of samples by diluting with water or an appropriate diluent to a concentration within the assay range (25-2000 µg/ml). It is recommended to use three different concentrations of samples & perform the assay in duplicates or triplicates.

Assay Protocol

- BCA Assay can be performed in a microtiter plate format or test tube format.

Microplate Procedure:

1. Add 25 µl of each BSA Standard II 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 (OD562).

Test Tube Procedure:

1. Add 100 µl of each BSA Standard II 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)
2. After incubation, cool the tubes to room temperature.
3. Set the absorbance wavelength of a spectrophotometer to 562 nm. Blank the instrument by using water or the diluent only.
4. Read absorbance (OD562) of all Standards and samples.

Calculation:

Subtract OD_{562} of Blank (0 Standard, #8) from all readings. Plot the Standard curve, OD_{562} (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.

Protein concentration in sample: **C = DX = Dilution Factor x (Y – B)/a = µg/ml**
Where:

- **Y** = OD_{562} 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

Technical Support

For all technical or commercial enquiries please go to:
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