Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

BCA Protein Assay Kit II

rev. 9/14

(Catalog # K813-2500; K813-5000, Store at Room Temperature)

I. Introduction:

Biovision'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 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.

II. Applications:

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

III. Kit Contents:

Components	K813-2500	K813-5000	Cap Code	Part Number
BCA Reagent A	500 ml	2 X 500 ml	NM	K813-xxxx-1
BCA Reagent B	25 ml	25 ml	NM	K813-xxxx-2
BSA Standard (2 mg/ml)	5 x 1 ml	10 x 1 ml	White	K813-xxxx-3

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 µg/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 (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

- **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 µg/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 (OD_{562}) of all Standards and samples.

C. Calculations: 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.

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

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

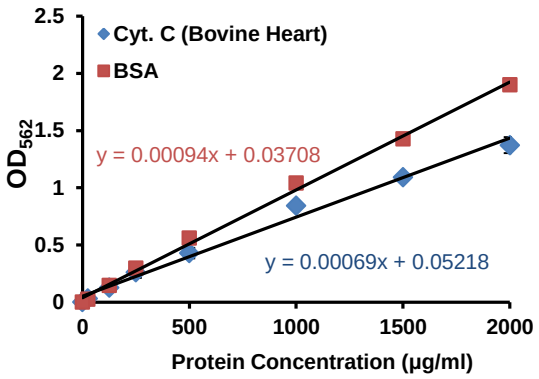

Figure: Typical absorbance plots obtained for BSA and Cytochrome C from Bovine Heart (Cat. # 2120) using a microplate procedure (37°C for 30 min).

VIII. RELATED PRODUCTS:

EZLys™ Bacterial Protein Extraction Reagent (8001)
EZLys™ Tissue Protein Extraction Reagent (8002)
EZLys™ lysozyme, human (8005)
Western Blot Substrate Kit (K820)
Protein Carbonyl Content Assay Kit (K830)
Protease inhibitor cocktails (K271, K272, K277, K278, K279)
EZLys™ Yeast Protein Extraction Reagent (8003)
EZLys™ Mammalian Protein Extraction Reagent (8004)
Protein Quantitation kit (K810)
BCA Protein Quantitation Kit (K812, K814)
Protease & Phosphatase inhibitor cocktails (K283, K284)

FOR RESEARCH USE ONLY! Not to be used on humans.