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Extra Sense™ BCA Protein Assay Kit

rev. 6/13

(Catalog # K814-2500; K814-5000, Store kit at Room Temperature)

I. Introduction:

BioVision's Extra Sense™ BCA Protein Assay kit provides an ultra-sensitive method to detect and quantify total protein concentration even in the presence of detergents. The Kit has been optimized for determining total protein content in low protein concentration samples (0.5-40 µg/ml). The assay is based on the reaction of bicinchoninic acid (BCA) with the cuprous cation (Cu^{+1}), which is generated by reduction of cupric cation (Cu^{+2}) with the protein in alkaline conditions. The Cu^{+1}-BCA chelate is a water-soluble complex and exhibits a strong absorbance at 562 nm that is linear over a concentration range of 0.5-40 µg/ml. In general, protein concentrations are estimated with reference to a commonly used protein standard. The Kit includes Bovine Serum Albumin (BSA) as a protein standard for estimation of total protein content of samples.

II. Application:

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

III. Kit Contents:

Components	K814-2500	K814-5000	Cap Code	Part Number
BCA Reagent A	190 ml	380 ml	WM	K814-xxxx-1
BCA Reagent B	190 ml	380 ml	NM	K814-xxxx-2
BCA Reagent C	9 ml	18 ml	NM	K814-xxxx-3
BSA (2 mg/ml)	5 x 1 ml	10 x 1 ml	White	K814-xxxx-4

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:** It is recommended that same diluent should be used to make the BSA Standard solutions as that of the protein samples. Prepare BSA Standard working solution to 200 µg/ml by diluting 0.5 ml of BSA Standard in 4.5 ml of de-ionized water or the diluent. Using this working solution, prepare BSA Standard solutions as suggested in the table below using same diluent. Other similar concentrations can also be used within the assay range of 0.5-40 µg/ml. One tube of BSA Standard is sufficient to make diluted solutions in triplicates.

Vial	Volume of BSA (ml)	Volume of diluent (ml)	Final BSA Concentration (µg/ml)
1	1 ml of 200 µg/ml Stock	4	40
2	4 ml of tube 1	4	20
3	4 ml of tube 2	4	10
4	4 ml of tube 3	4	5
5	4 ml of tube 4	4	2.5
6	3.2 ml of tube 5	4.8	1
7	4 ml of tube 6	4	0.5
8 (**Blank**)	0	8	0

- **Preparation of Protein Samples**: Prepare different concentrations of protein samples by diluting with de-ionized water or an appropriate diluent to a concentration within the assay range (0.5-40 µg/ml). It is recommended to use three different dilutions of a sample & perform the assay in duplicates or triplicates.
- **Preparation of BCA Working Reagent**: To prepare BCA Working Reagent, mix BCA Reagent A, BCA Reagent B and BCA Reagent C in the ratio of 25:25:1. Upon mixing, green colored turbidity will be observed that should disappear upon further mixing to give a green colored solution. Each sample replicate requires 150 µl of BCA working reagent for microplate assay, or 1 ml for test tube procedure. Prepare sufficient amount of BCA reagent working solution for all BSA Standards as well as protein samples.

  Note: It is recommended that BCA reagent working should be prepared fresh.

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

A. Microplate Procedure:

1. Add 150 µl of each BSA Standard and protein samples into microtiter plate wells.
2. Add 150 µl of BCA reagent working solution to Standards and samples, mix thoroughly for 30 s.
3. Cover the plate and incubate at 37 °C for 2 h.
4. After incubation, cool the plate to room temperature.
5. Set the absorption wavelength of a microplate reader to 562 nm and record the absorbance (OD_{562}) of all Standards and samples.

B. Test Tube Procedure:

1. Add 1 ml of each BSA Standard and protein samples into a 4 ml test tube.
2. Add 1 ml of the BCA reagent working solution and mix well.
3. Cover the tubes and incubate at 60 °C for 1 h.
4. After incubation, cool the tubes to room temperature.
5. Set the wavelength of spectrophotometer at 562 nm. Blank the instrument by using water or the diluent only.
6. Measure the absorbance (OD_{562}) of all the BSA Standards (Sample 1-7) and protein samples.

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

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

Where **Y** = OD_{562} of protein sample
X = concentration of protein sample
a = Slope of 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

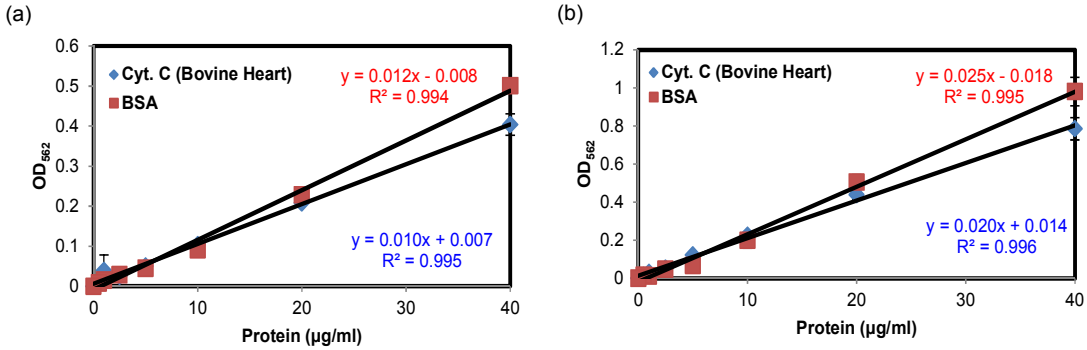

Figure: Typical absorbance plots obtained for BSA and Cytochrome C from Bovine Heart (Cat. # 2120) by using microplate procedure (37°C for 2 h) [a] and test tube procedure (60°C for 1 h) [b].

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.