BCA Protein Assay Kit - Reducing Agent Compatible (#BN01034)

(Catalog #BN01034; 1000 assays, Store kit at Room Temperature)

I. Introduction:

Assay Genie's Reducing Agent Compatible BCA Protein Assay Kit is the only protein estimation kit on the market that is compatible with strong reducing agents such as TCEP up to 20 mM, DTT up to 10 mM & β-mercaptoethanol up to 35 mM. This kit is adapted from Assay Genie's BCA Assay Kit II and 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 alkaline conditions. Reducing agents in samples interfere with BCA assay due to their ability to reduce Cu^{+2}. Assay Genie's Reducing Agent Compatible BCA Protein Assay Kit is useful for determing protein concentrations of samples containing DTT, TCEP & β-mercaptoethanol. The assay is linear over the widest range of protein concentration between 25-2000 µg/ml. In general, protein concentrations are calculated 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 in the presence of reducing agents.

III. Kit Contents:

Components	BN01034	Cap Code	Part Number
BCA Reagent A	200 ml	NM	BN01034-1
BCA Reagent B	20 ml	NM	BN01034-2
BSA Standard (2 mg/ml)	10 x 1 ml	White	BN01034-3
Blocking Reagent (20 mg/tube)	20 x 1 vial	Amber	BN01034-4
Blocking Reagent Buffer	20 ml	WM	BN01034-5

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 of Standard, Sample & Reagents:

- **Preparation of BSA Standards:**
  Prepare BSA Standards as suggested in the table below by diluting BSA Standard using de-ionized water or same diluent (with or without the reducing agent)* as that of 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**)	100 of 2 mg/ml Stock	0	2000
2	100 of 2 mg/ml Stock	100	1000
3	100 of vial 2	100	500
4	100 of vial 3	100	250
5	100 of vial 4	100	125
6	100 of vial 5	400	25
7 (**Blank 1**)	0	100**	0
8 (**Blank 2**)	0	100***	0

Note:

*It is recommended to prepare the BSA Standards using water or protein sample diluent without the reducing agent and prepare both Blank 1 and Blank 2. However, the BSA Standards can also be made using water or protein sample diluent containing same amount of reducing agent as that of the protein samples. In that case, only Blank 1 is needed.

**Blank 1: Use water or protein sample diluent containing same concentration of reducing agent as that of the protein sample.

***Blank 2: Use water or protein sample diluent without reducing agent.

- **Preparation of Protein Samples**: Prepare different concentrations of protein samples by diluting with water or an appropriate diluent to a concentration within the assay range (25-2000 µg/ml). For unknown samples, it is recommended to use three different concentrations of samples & perform the assay in duplicates or triplicates.
- **Preparation of Blocking Reagent**: Dissolve one tube (20 mg) of the Blocking Reagent in 1 ml of Blocking Reagent Buffer. Vortex for 30 sec. This amount of solution is enough for the analysis of 50 samples (20 µl per sample).

  Note:

  Prepare the reagent freshly just before use & discard any unused solution. For less number of samples, one can weigh necessary amount (0.4 mg/well) and dissolve in appropriate volume of Blocking Reagent Buffer (20 µl/well).
- **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. 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 freshly. However, the prepared reagent is stable and can be stored at room temperature for several days in a closed container.

VII. Assay Protocol:

1. Add 20 µl of each BSA Standard, Blank(s) and protein samples into microtiter plate wells.
2. Add 20 µl of freshly prepared Blocking Reagent to all wells. Mix thoroughly for 30 sec. Cover the plate and incubate at 37°C for 30 min.
3. Add 200 µl of BCA Working Reagent to all wells. Mix thoroughly for 30 sec. Cover the plate and incubate at 37°C for 30 min. After incubation, cool the plate to room temperature.
4. Read the absorbance (OD_{562}) of all Standards and samples.
5. **Calculations:** Subtract OD_{562} of Blank 2 (0 Standard, # 8) from all BSA Standards. For samples, Subtract OD_{562} of Blank 1 (0 Standard, # **7**) from that of protein samples. Plot the Standard curve, OD_{562} (on Y-axis) vs Standard BSA concentration (on X-axis). Obtain the equation from the plot in the form of Y = aX + b. Use the obtained value of slope (a) to calculate protein concentration in samples.

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

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 as:

C = DX

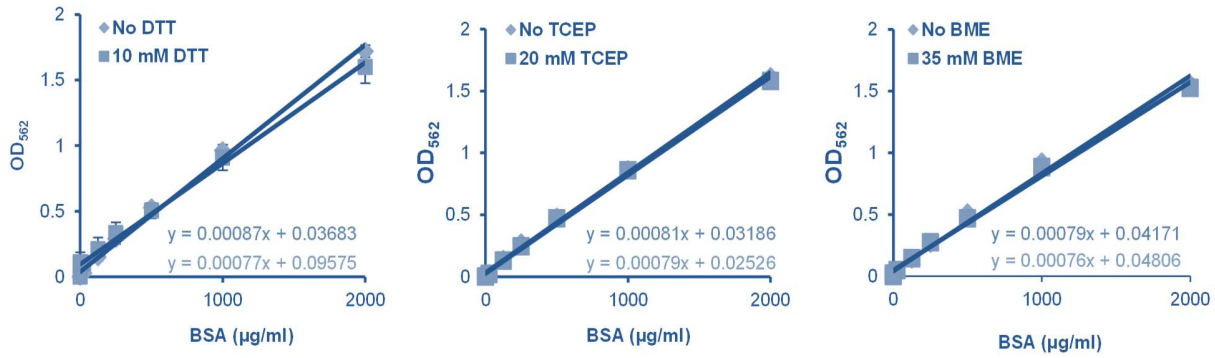

Figure: Standard Curves for BSA containing blocking reagent in the presence and absence of 10 mM DTT, 20 mM TCEP and 35 mM β-mercaptoethanol (BME). The experiments were performed in a microplate as outlined in the protocol given above.

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