

Samples & all BSA Standards.

Note:

We recommend to prepare fresh BCA Working Reagent. 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 50 µl of each BSA Standard, Blank(s) and protein samples into a clean test tube.
2. Add 50 µl of freshly prepared Blocking Reagent to each tube. Mix thoroughly for 30 sec. Cover the tubes and incubate at 37°C for 30 min.
3. Add 1 ml of BCA Working Reagent to each tube. Mix thoroughly for 30 sec. Cover the tubes and incubate at 37°C for 30 min. After incubation, cool the tubes to room temperature.
4. Read the absorbance (OD₅₆₂) of all Standards and samples.
5. **Calculations:** Subtract OD₅₆₂ of Blank 2 (0 Standard, # 8) from all BSA Standards. For samples, Subtract OD₅₆₂ of Blank 1 (0 Standard, # 7) from that of protein samples. Plot the Standard curve, OD₅₆₂ (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 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 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:

$$C = DX$$

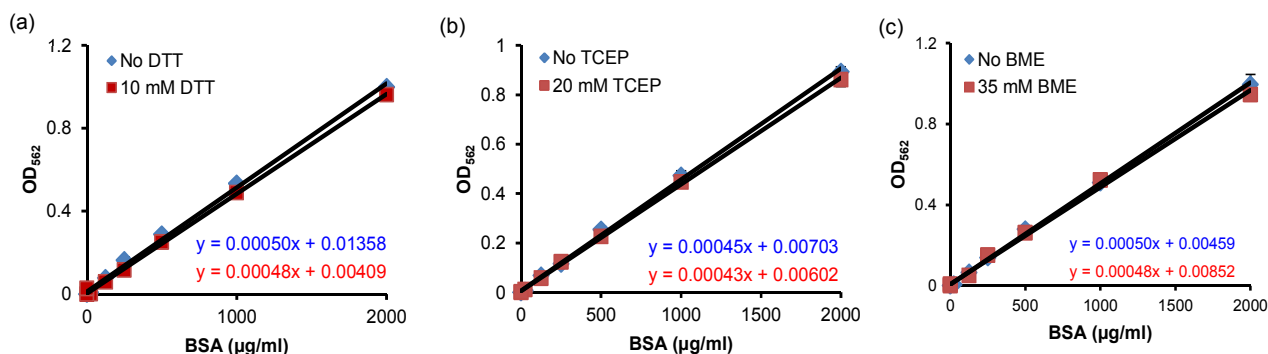


Figure: Standard Curves for BSA containing blocking reagent in the presence and absence of 10 mM DTT (a), 20 mM TCEP (b) and 35 mM β-mercaptoethanol (BME) (c). The experiments were performed in test tubes as outlined in the protocol given above.

VIII. RELATED PRODUCTS:

- EZLys™ Bacterial Protein Extraction Reagent (8001)
- EZLys™ Tissue Protein Extraction Reagent (8002)
- EZLys™ lysozyme, human (8005)
- Western Blot Substrate Kit (K820)
- Extra Sense™ BCA Protein Assay Kit (K814)
- 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, K813)
- BCA Protein Assay Kit – Reducing Agent Compatible (K818)
- Protease & Phosphatase inhibitor cocktails (K283, K284)

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