Ready-to-use BCA Protein Assay Kit (Stable Version)

B1507333

Storage BCA Reagent A and Reagent B can be stored at room temperature or 2-8°C for one year. If precipitation occurs in BCA Reagent A or Reagent B during low-temperature or long-term storage, warm it at 37°C with stirring to ensure complete dissolution. If contamination is detected, discard it to avoid affecting the experimental results. Unopened BSA standard can be stored at 2-8°C for one year. After opening, it is recommended to aliquot and store at -20°C for long-term preservation.

Shipping Shipped under normal conditions. Please store under the specified storage conditions immediately upon receipt.

Introduction

The BCA (Bicinchoninic acid) method is currently one of the most widely used techniques for protein concentration determination. Based on the biuret reaction, proteins reduce Cu^{2+} to Cu^{+} in an alkaline environment, forming a purple-blue complex with a high absorbance value at 562 nm. The amount of the reaction product is directly proportional to the protein concentration. The Ready-to-use BCA Protein Assay Kit offers simplicity, sensitivity, rapidity, and stability in protein concentration determination. The protein standard provided in the kit facilitates the creation of a standard curve for users. This BCA protein assay kit can be used for both cuvette-based and microplate-based detection. While the former requires a larger volume (100 μL) of protein sample, it uses a 1:20 (v/v) ratio of protein sample to BCA working solution, reducing the impact of interfering substances. The latter is simple and convenient, requiring only a small volume (10-25 μL) of protein sample. However, due to its 1:10 (v/v) ratio of protein sample to BCA working solution, it has certain limitations on the tolerance of interfering substances and the minimum detectable level.

Product Features

1. High sensitivity, detection range: 20-2000 μg/mL.
2. Compatible with microplate readers and spectrophotometers.
3. High accuracy, the coefficient of variation is much lower than that of the Coomassie Brilliant Blue staining method.
4. Resistant to most chemical substances in samples. The greatest advantage of the BCA method for protein concentration determination is its tolerance to high concentrations of detergents, being compatible with up to 1% SDS, 1% Triton X-100, and 1% Tween 20, 60, 80 in samples. However, it is affected by chelating agents and slightly higher concentrations of reducing agents. Ensure that EDTA is below 10 mM, there is no EGTA, DTT is below 1 mM, and β-mercaptoethanol is below 0.01%. For more details, please refer to Table 1.
5. This kit contains a series of protein standard solutions (BSA solutions) at a range of

concentrations, which are ready to use without dilution, providing convenience and efficiency.

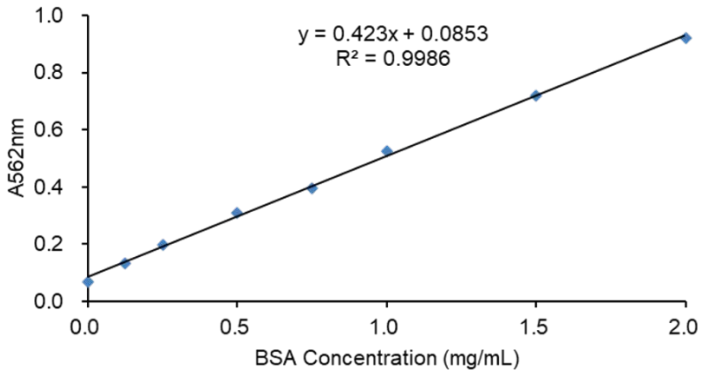

Figure 1. The actual measurement result of the protein standard curve of this kit. The data shown in the figure are for reference only; the actual detection results may vary slightly.

Kit Contents

B1507333	Components	500T	5×500T	Storage Temperature	Quantity Per Test
B1507333A	BCA Reagent A	60 mL	5×60 mL	Room Temperature	100 μL
B1507333B	BCA Reagent B	2 mL	5×2 mL	Room Temperature	2 μL
B1507333C	BSA Standard (2.0 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333D	BSA Standard (1.5 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333E	BSA Standard (1.0 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333F	BSA Standard (0.75 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333G	BSA Standard (0.5 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333H	BSA Standard (0.25 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333I	BSA Standard (0.125 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL
B1507333J	BSA Standard (0 mg/mL)	1 mL	5×1 mL	2-8°C	10 μL

Instruction for use

1. Preparation:

Prepare BCA working solution: Based on the number of samples, prepare an appropriate amount of BCA working solution by mixing reagent A and reagent B in a volume ratio of 50:1, and mix thoroughly. When the two are mixed, a precipitate will form, but it will disappear after thorough mixing. Note: Shake reagent A well before preparing the BCA working solution. The BCA working solution should be used as soon as it is prepared. The freshly prepared BCA

working solution can be stably stored at room temperature under sealed conditions for 24 hours.

2. Prepare the instrument:

Turn on the microplate reader and set the reading wavelength (562nm). Preheat the incubation device: Set the water bath or oven to the required incubation temperature (usually 37°C for 30 minutes. High temperature can improve sensitivity and shorten time).

3. Preparation of samples to be tested:

Dilute the protein samples to be tested with the same buffer/diluent to an estimated concentration within the linear range of the standard curve (usually 20-2000 μg/mL). Record the dilution factor for subsequent calculation of the original concentration. It is recommended to perform 2-3 replicate wells for each sample to be tested.

4. Sample addition and reaction:

(1) Add the liquids to the 96-well plate according to the designed layout:

Standard curve wells: Add 10 μL of the corresponding concentration of standard protein solution (0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 mg/mL, etc.) to each well.

Blank control wells: Add 10 μL of buffer (0 mg/mL) to each well.

Sample wells: Add 10 μL of the diluted sample protein solution to each well.

(2) Add 100 μL of freshly prepared BCA working solution to each well (including standard wells, blank wells, and sample wells).

(3) Use a multi-channel pipette or single-channel pipette to add the BCA working solution quickly and gently to avoid generating bubbles. After addition, seal the plate with a sealing film or tap it gently on the table a few times, or use a plate shaker for a brief shake (low speed) to ensure thorough mixing of the liquid in each well.

5. Incubation:

Place the sealed 96-well plate in a preheated water bath or oven and incubate for the specified time (e.g., 30 minutes at 37°C). Strictly control the time and temperature!

6. Absorbance measurement:

After incubation, remove the plate from the water bath/oven immediately and let it cool to room temperature (about 10 minutes). Carefully dry any water vapor on the bottom of the plate. Place the plate in a microplate reader and read the absorbance value of each well at 562 nm.

7. Data processing and standard curve plotting:

(1) X-axis (horizontal axis): Standard protein concentration (μg/mL).

(2) Y-axis (vertical axis): Corrected absorbance value (Abs 562 nm).

(3) Curve fitting:

The BCA method usually shows a good linear relationship within the appropriate concentration range (typically 20-2000 μg/mL).

(4) Use software to perform linear regression and obtain the equation of the standard curve: $Y = aX + b$, where:

Y = corrected absorbance;

X = protein concentration (μg/mL);

a = slope (Slope);

b = Y-intercept (Intercept).

(5) Calculate the correlation coefficient (R^2). An ideal standard curve should have an R^2 value very close to 1 (typically > 0.99).

8. Calculation of sample concentration.

Matters needing attention

1. Accuracy: Pipetting operations must be precise. The standard is one of the main sources of error. Use calibrated pipettes and high-quality tips.
2. Replicates: Set up replicates (2-3) for both the standard curve and the sample to be tested, and take the average to reduce random errors.
3. Incubation conditions: Strictly follow the incubation temperature and time specified in the manual. An increase in temperature will accelerate the reaction and improve sensitivity, but it may also increase the background or instability.
4. Timely reading: The color reaction is stable for a period of time after incubation, but it is best to complete the reading as soon as possible after incubation to avoid prolonged storage.
5. Standard Curve: Ensure that the concentration of the sample to be tested (after dilution) falls within the linear range of the standard curve (usually between 20-2000 μg/mL in absorbance). If the absorbance is outside the linear range (too high or too low), the sample dilution should be adjusted and the measurement repeated. For each measurement, a corresponding standard curve needs to be made, because the color reaction is related to changes in temperature and time. Precise protein quantification should always involve making a standard curve.
6. Standard selection: BSA is a universal standard. Note: Different proteins may have slightly different reaction efficiencies with the BCA reagent (referred to as "color difference"). Therefore, the concentration of other proteins measured using the BSA standard curve is the "BSA equivalent concentration".
7. Cleanliness: Ensure that the cuvettes or microplate wells are clean and free of residues that may affect absorbance.
8. Data quality: Pay attention to the R^2 value of the standard curve. An $R^2 < 0.99$ usually indicates problems with the experimental operation or reagents, and the results are unreliable and need to be redone.

Table 1. Interferon substance table

Substance	Compatible concentration	Substance	Compatible concentration
Buffers		Detergent	
Glycine	1M	Brij35	1%
HEPES	0.2M	CHAPS	1%
MES	0.1M	NP-40	1%
MOPS	0.2M	SDS	1%
Sodium citrate	0.2M	Triton X-100	1%
Sodium phosphate	0.1M	Saccharides	
Sodium acetate	0.2M	Glucose	10mM
Tris	0.25M	Sucrose	1M
Salts		Chelating agents	
NaCl	1M	EDTA	10mM
Denaturing Agents		Reducing Agents	
GACl	4M	2-Mercaptoethanol	-
Urea	3M	DTT	-
Polar Compound		Other	
DMSO	10%	HCl	0.1M
Glycerol	10%	NaOH	0.1M