

## BCA Protein Assay Kit (Enhanced)

### Introduction

BCA (Bicinchoninic Acid) Assay is a protein quantification method based on the biuret reaction principle. Under alkaline conditions, peptide bonds in proteins reduce  $\text{Cu}^{2+}$  in copper sulfate to  $\text{Cu}^+$ . BCA then chelates  $\text{Cu}^+$  to form a purple complex with a strong absorption peak at 562 nm. The color intensity of this chelate is correlated with the protein concentration. Since the decrease in  $\text{Cu}^{2+}$  or the amount of  $\text{Cu}^+$  generated is proportional to the amount of protein present in the solution, the protein concentration of a sample can be determined by measuring its absorbance. This method is widely used to determine the concentration of various biological samples, including cell lysates, tissue homogenates, purified proteins, subcellular fractions, and secreted proteins. It is an important sample preparation step for downstream applications such as enzyme activity assays, quantitative sample loading for electrophoresis, immunoblotting, and protein–protein interaction studies.

Compared with conventional BCA protein assay kits, this enhanced BCA kit offers three major advantages: (1) Higher sensitivity – the detection limit is as low as 10  $\mu\text{g}/\text{mL}$ , with a minimal detectable protein amount of only 0.2  $\mu\text{g}$  and a required sample volume of just 1–20  $\mu\text{L}$ . This performance is significantly better than that of standard BCA kits, making it particularly suitable for accurate quantification of low-concentration or limited protein samples; (2) Faster color development – at the same protein concentration, the required incubation time is markedly reduced, enabling absorbance measurements to be completed in a shorter time and greatly improving experimental throughput and efficiency; (3) Improved linear range – a good linear relationship is maintained over a concentration range of 20–2000  $\mu\text{g}/\text{mL}$ , ensuring the accuracy and reproducibility of quantitative results.

In terms of compatibility, this kit tolerates up to 5% SDS, 5% Triton X-100, and 5% Tween 20/60/80 in samples, and is therefore suitable for lysis buffers containing relatively high concentrations of detergents. However, chelating agents and reducing agents can interfere with the colorimetric reaction. It is recommended that EDTA be kept below 10 mM and that EGTA be avoided; DTT should be below 1 mM and  $\beta$ -mercaptoethanol below 0.01%. If such substances are present at relatively high levels in the samples, the Bradford Protein Assay Kit is recommended instead (e.g., the standard kit K4103 or the detergent-compatible kit K4104).

This kit contains all the components required to perform a complete BCA protein assay, including BCA Solution A (alkaline buffer), BCA Solution B (copper-containing color reagent), a protein standard, and the corresponding dilution buffer. To meet the needs of different experimental scales, the kit is available in three package sizes suitable for approximately 200, 500, or 5000 assays (the number of assays is estimated based on a 96-well plate format with a reaction volume of 200  $\mu\text{L}$  per well).

## Components and Storage

Components	200 Tests	500 Tests	5000 Tests	Storage
Reagent A	40 mL	100 mL	2× 500 mL	4°C
Reagent B	1.2 mL	3 mL	30 mL	4°C
Bovine serum albumin (BSA)	20 mg	30 mg	2× 30 mg	4°C
BCA Protein Assay Reagent	1 mL	1.5 mL	5 mL	4°C
Shipping: Blue ice	Shelf life: 12 months			

## Protocol

### 1. Preparation of Protein Standard Solution (for 500 tests as an example)

- a. Add 1.2 mL of BCA Protein Assay Reagent to one vial of Bovine Serum Albumin (30 mg) and dissolve thoroughly to prepare a 25 mg/mL stock solution. The stock solution can be used immediately or stored at  $-20^{\circ}\text{C}$  for long-term preservation.

**\*Note:** BCA Protein Assay Reagent is provided in excess.

- b. Take an appropriate amount of the above stock solution and dilute to a final concentration of 0.5 mg/mL. The diluent can be the same solution used for the protein samples, or 0.9% NaCl, or PBS. The diluted 0.5 mg/mL protein standard can be stored at  $-20^{\circ}\text{C}$  for long-term preservation. For example, add 20  $\mu\text{L}$  of the 25 mg/mL protein standard to 980  $\mu\text{L}$  of PBS to prepare 0.5 mg/mL protein standard solution.

### 2. Preparation of BCA Working Solution

Prepare an appropriate volume of BCA Working Solution by mixing 50 volumes of Reagent A with 1 volume of Reagent B (50:1, v/v) according to the number of samples, and mix thoroughly. For example, mix 5 mL of Reagent A with 100  $\mu\text{L}$  of Reagent B to prepare 5.1 mL of BCA Working Solution. The BCA Working Solution is stable at room temperature for 24 hours.

### 3. Protein Concentration Determination

- a. Add 0, 1, 2, 4, 8, 12, 16, and 20  $\mu\text{L}$  of the protein standard solution (0.5 mg/mL) to the corresponding wells of a 96-well plate, and bring the volume to 20  $\mu\text{L}$  with the diluent (the same solution used in step 1.b). The corresponding standard concentrations are 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively.
- b. Add an appropriate volume of samples to the wells of a new 96-well plate. If the sample volume is less than 20  $\mu\text{L}$ , bring the volume to 20  $\mu\text{L}$  with the diluent (the same solution used in step 1.b) and record the sample volume used.
- c. Add 200  $\mu\text{L}$  of BCA Working Solution to each of the above wells and incubate at  $37^{\circ}\text{C}$  for 20–30 minutes.

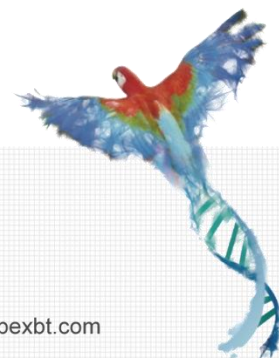
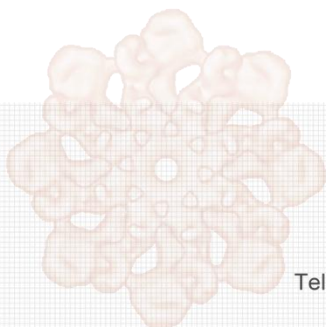
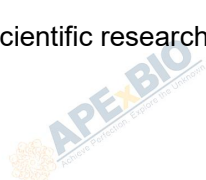
**\*Note:** Alternatively, incubate at room temperature for 2 hours, or at  $60^{\circ}\text{C}$  for 30 minutes. In the BCA assay, the color intensity

increases over time and the color development reaction is accelerated at elevated temperatures. For low-concentration samples, incubation at higher temperatures or extended incubation time is recommended.

- d. Measure the absorbance at 562 nm, or at other wavelengths between 540–595 nm, using a microplate reader.
- e. Calculate the protein concentration in the sample based on the standard curve and the sample volume used.

## Note

1. This kit exhibits a good linear relationship within the concentration range of 20–2000 µg/mL.
2. After preparation, the protein standard solution should be stored at –20°C.
3. The BCA assay is unaffected by most chemical substances commonly present in samples and is compatible with various detergents, including up to 5% SDS, 5% Triton X-100, and 5% Tween 20, 60, and 80. However, it is affected by chelating agents and slightly higher concentrations of reducing agents. Ensure that EDTA is below 10 mM, EGTA is absent, DTT is below 1 mM, and β-mercaptoethanol is below 0.01%. For protein samples that are not suitable for BCA detection due to the presence of reducing agents, chelating agents, or other interfering components, it is recommended to use the Bradford Protein Assay Kit (K4103 Bradford Protein Assay Kit).
4. To accelerate the BCA assay, a microwave oven may be used for heating, but care must be taken to avoid overheating.
5. This product is for scientific research use only.



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