BCA Protein Assay Kit

Product Description

BCA, fully named Bicinchoninic Acid Assay, is a protein quantification method based on the biuret reaction principle. Under alkaline conditions, protein peptide bonds reduce Cu^{2+} (from copper sulfate) to Cu^{+}. Subsequently, BCA chelates Cu^{+} to form a purple-colored complex, which strongly absorbs light at a wavelength of 562 nm. The intensity of the colored complex is proportional to protein concentration, as the reduction of Cu^{2+} or the amount of Cu^{+} formed directly reflects the amount of protein present in the solution. Thus, protein concentration in an unknown sample can be determined by measuring absorbance.

This assay kit features easy operation, high stability, high sensitivity and excellent compatibility. It can detect protein concentrations as low as 25 μg/mL, with a minimum detection limit as low as 0.5 μg protein. It requires a sample volume of only 1–20 μL. The assay shows good linearity in the concentration range of 50–2000 μg/mL.

Components and Storage

Components	K4101-200 T	K4101-500 T	K4101-1000 T
Reagent A	40 mL	100 mL	2 x 100 mL
Reagent B	1.2 mL	3 mL	6 mL
Bovine Serum Albumin (BSA)	10 mg	15 mg	30 mg
BCA Protein Assay Reagent	6 mL	9 mL	18 mL
Store the components at 4°C for a year.			

Protocol

1. Preparation of protein standards (200 T as an example)

a. Add 5 mL of BCA Protein Assay Reagent to a tube of Bovine serum albumin (10 mg), dissolve thoroughly, and make a 2 mg/mL protein standard solution. It can be used immediately after formulation or can be stored at -20°C for long period of time. *[Note] Each component is supplied in slightly greater amounts than indicated in the table above. Please prepare them to a final 2 mg/mL concentration.*

b. Take an appropriate amount of 2 mg/mL protein standard and dilute to a final concentration of 0.5 mg/mL. The diluent can be the same buffer used to dilute the protein sample. Alternatively, 0.9% NaCl or PBS can also be used. The diluted 0.5 mg/mL protein standard can be stored at -20°C for long-term storage.

2. Preparation of BCA working solution

According to the number of samples, prepare an appropriate amount of BCA working solution in the ratio of 50 volumes of Reagent A to 1 volume of Reagent B (50:1), and mix thoroughly. For example, 5 mL Reagent A plus 100 µL Reagent B are mixed to make 5.1 mL BCA working solution. The BCA working solution is stable within 24 hours at room temperature.

3. Protein concentration determination

a. Add 0, 1, 2, 4, 8, 12, 16, and 20 µL of the protein standard solution (0.5 mg/mL) into the corresponding wells of a 96-well plate. Then add standard diluent to bring the total volume to 20 µL in each well. This results in final standard concentrations of 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively. *[Note] The standard diluent should be the same as the sample storage buffer, such as Tris-NaCl, PBS etc.*

b. Add an appropriate volume of the sample to the designated wells of a new 96-well plate. If the sample volume is less than 20 µL, supplement with standard diluent to reach a final volume of 20 µL, and record the volume of the sample added.

c. Add 200 µL of BCA working solution to each well described above, and incubate at 37°C for 20–30 minutes. *[Note] Alternatively, the reaction can be carried out at room temperature for 2 hours or at 60°C for 30 minutes. In the BCA assay, color development intensifies over time, and higher temperatures accelerate the reaction. For samples with low protein concentration, incubation at a higher temperature or extending the incubation time is recommended.*

d. Measure the absorbance value at A562, or other wavelengths between 540-595 nm, with a microplate reader.

e. Calculate the protein concentration in the samples based on the standard curve and the volume of sample used.

Note

1. This kit exhibits good linearity within the concentration range of 50–2000 µg/mL.

2. The BCA method for protein quantification is generally unaffected by most chemical substances present in samples and is compatible with various detergents, including up to 5% SDS, 5% Triton X-100, and 5% Tween 20, 60, or 80. However, it is sensitive to chelating agents and slightly high 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%. If the protein sample contains interfering substances such as reducing

or chelating agents that are incompatible with the BCA assay, we recommend using the Bradford Protein Assay Kit (K4103) as an alternative.

3. To accelerate the BCA protein assay, microwave heating may be used with caution. However, do not overheat the samples.

4. This product is for research use only.

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