BCA Protein Assay Kit

Product Description:

BCA stands for Bicinchoninic Acid Assay, which is a protein quantification method based on the biuret principle. Under alkaline conditions, the protein peptide bond reduces Cu^{2+} in copper sulfate to Cu^{+}, and then BCA chelates Cu^{+} to form a purple complex that strongly absorbs light at a wavelength of 562 nm. The color of the chelate is related to the protein concentration, and the amount of Cu^{2+} or Cu^{+} obtained is proportional to the amount of protein present in the solution, so the protein concentration to be measured can be obtained by measuring the protein absorbance value.

This kit is characterized by ease of operation, high stability, high sensitivity, and high compatibility. The lower limit of detection concentration was 25 μg/mL, the minimum amount of detected protein was 0.5 μg, and the volume of samples to be measured was 1-20 μL. There is a good linear relationship in the concentration range of 50-2000 μg/mL.

Composition and storage conditions

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.			

Experimental manipulation

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 periods of time. *[Note] BCA Protein Assay Reagent components are overpacked and configured 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 L. The standard dilution is based on the contents of the solution in which the protein sample is

located, although the standard can also be diluted with 0.9% NaCl or PBS for ease of use. The diluted 0.5 mg/mL protein standard can be stored at -20°C for long-term storage.

2. **BCA working solution is prepared.**

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 AB (50:1), and mix thoroughly. For example, 5 ml Reagent A plus 100 μL Reagent B were 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. The protein standard solution (0.5 mg/mL) was added to the corresponding 96-well plate detection wells at 0, 1, 2, 4, 8, 12, 16, and 20 μL, and the standard dilution was added to 20 μL, which was equivalent to the concentration of the standard at 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL, respectively. Note: The standard diluent is the preservation system of the sample, such as Tris sodium chloride, PBS, etc.

b. Add the appropriate volume of sample to the sample wells of the new 96-well plate. If the sample is less than 20 μL, add the standard dilution to 20 μL and note the sample volume.

c. Add 200 μL of BCA solution to each of these wells and leave at 37°C for 20-30 minutes. *[Note] It can also be left at room temperature for 2 hours, or 60°C for 30 minutes. When the BCA method determines the protein concentration, the color darkens over time. And the color development reaction will be accelerated by the increase in temperature. If the concentration is low, it is appropriate to incubate at a higher temperature, or to extend the incubation time appropriately.*

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

e. The protein concentration of the sample species was calculated from the standard curve and the sample volume used.

Precautions

1. The kit has a good linear relationship in the concentration range of 50-2000 μg/ml.

2. The BCA method is independent of the chemicals present in most samples and is compatible with a wide range of detergents in the sample, such as up to 5% SDS, 5% Triton X-100, and 5% Tween 20, 60, and 80. However, due to the influence of chelating agents and slightly higher concentrations of reducing agents, it is necessary to ensure that EDTA is less than 10 mM, no EGTA, dithiothreitol (DTT) is less than 1 mM, and β-mercaptoethanol is less than 0.01%. When protein samples are not suitable for BCA testing, such as containing components such as reducing agents and chelating agents, it is recommended to try the Bradford Protein Assay Kit (K4103).

3. To speed up the determination of protein concentration by BCA, it can be heated in a microwave oven, but should not be overheated.

4. This product is for scientific research purposes only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com