BCA Protein Assay Kit

Cat #: orb1566791(manual)

Size：200T / 500T / 5000T

Product composition：

No.	200T	500T	5000T
BCA reagent A	40 ml	100 ml	500 ml×2
BCA reagent B	1.2 ml	3 ml	30 ml
Protein standard (BSA)	20 mg	50 mg	100mg ×5
Protein standard solution	1.0 ml	1.0 ml	5.0 ml

Preservation Method: BCA reagent A, reagent B and protein standard solution were stored at room temperature. Protein standard (BSA) should be stored at 2-8 ° C and at -20 ° C after preparation.

Product introduction:

Bicinchoninic acid (BCA) method is a widely used protein quantitative method recently. The principle of protein quantitation is similar to that of Lowery method, that is, protein complexation with Cu2 + and reduction of Cu2 + to Cu1 + in alkaline environment. BCA combines with CU1 + to form a stable violet-blue complex, which has a high light absorption value at 562 nm and is proportional to the protein concentration, according to which the protein concentration can be determined. Compared with Lowery method, the BCA protein determination method is more sensitive, simple, stable and less affected by interfering substances. Compared with Bradford method, the significant advantage of BCA method is that it is not affected by detergents.

BCA method has a good linear relationship in the range of 50 ~ 1000μg/ml, and the minimum detection amount is 25μg/ml.

Instructions:

1. Preparation of protein standard solution

a) Take out the 1.5 ml vertical centrifuge tube labeled with protein standard solution and protein standard (BSA) in testing kit, pipette 1 ml of protein standard solution, and add it into the 1.5 ml vertical centrifuge tube labeled with protein standard (BSA), and fully dissolve it. **Note: The mass of BSAs in test tubes of protein standard (BSAs) in different size products are different. The mass of BSAs in 200T, 500T and 5000T are respectively 20 mg, 50 mg and 100 mg. Similarly, after the solution is prepared, the concentration of BSAs in each strength size is**

respectively 20 mg/ml, 50 mg/ml and 100 mg/ml. The dissolved protein standard solution should be stored in -20℃.

b) Dilute the protein standard BSA solution to the mother solution of protein standard (BSA) with a concentration of 2 mg/ml. Refer to the following table for dilution. Care shall be taken to make sure that the diluent of the protein standard solution is the same as the solvent of the protein sample to be tested.

Size	Volume of diluted solution（μl）	Volume of protein standard BSA（μl）	Final concentration（mg/ml）
200T	900	100	2
500T	960	40	2
5000T	980	20	2

C). The protein standard BSA mother solution of 2 mg/ml was diluted to 0.025 mg/ml -2 mg/ml according to the following table, and then make a protein standard curve, Note that the diluent is the same as the solvent of the protein sample. The volume in the table is suitable for the 96-well plate assay. If the photometer plate is used, the volume of the solution should be scaled up according to the actual need.

No.	Volume of diluted solution（μl）	Volume of protein standard BSA（μl）	Final concentration（mg/ml）
A	0	Take 150 from mother solution	2
B	50	Take 150 from mother solution	1.5
C	150	Take 150 from mother solution	1
D	50	Take 50 from B tube	0.75
E	150	Take 150 from C tube	0.5
F	150	Take 150 from E tube	0.25
G	150	Take 150 from F tube	0.125
H	200	Take 50 from G tube	0.025
I	200	0	0

2. BCA Working Solution Preparation

a) Calculate the concentration of the working solution required for the experiment. When the microplate reader is used for testing, the volume of the working solution required for each test well is 200 µl. Count the number of protein standard samples and protein samples to be tested (pay attention to the number of wells required for parallel testing), and then multiply the total number by 200, which is the total volume of the working solution required (unit: Ml).

b) The BCA working solution was prepared in proportion to the volume ratio 50:1 of reagent (A) and reagent (B), i.e., fifty parts of reagent (A) and one part of reagent (B). The two reagent's were mixed well and the BCA working solution was stable within 24 h at room temperature. For example, if you plan to prepare 5.1 ml BCA working solution, you need to take 5 ml BCA reagent (A and 0.1 ml BCA reagent (B).

3. Protein standard and protein sample concentration determination

a) Take 20 µl of the diluted protein standard solution of different concentrations and the protein sample to be tested in the previous step and add them to the 96-well plate. Note If the protein sample concentration is too high, dilute the protein sample with the same solution as the protein standard diluent.

b) Add another 200 µl BCA of working solution into each well, shake for 30 s with the plate function of the microplate reader, or gently shake the plate by hand to mix the solution. Place at 37℃ for 30 min.

c) The absorbance of 562 nm was measured using a microplate reader. Take the concentration of protein standard as the abscissa and the absorption value as the ordinate. Note that the absorption value of blank control should be subtracted from the absorption value. Draw the standard curve to obtain the linear formula and R2 value.

d) According to the formula obtained in the previous step, calculate the concentration of the corresponding protein sample according to the measured absorption value of the protein sample to be tested.

e) If the spectrophotometer is used for determination, the solution volume can be enlarged as required. Because the BCA test results are dynamic, the absorbance value has been increasing, and the spectrophotometer measurement takes a long time, be careful to keep the test time within 10 minutes.

Precautions:

1. BCA protein quantitative testing kit is not affected by other components in most samples and is very compatible with SDSs, Triton X -100, Tween 20, Tween 80 at concentrations less than or equal to 5%.. However, the determination of protein concentration by BCA method is easily influenced by metal chelating agent and high concentration reducing

agent. Please ensure the concentration of EDTA ≦10mM; DTT concentration ≦1mM, 2-ME≦0.01% before the protein concentration is determined by BCA.

2. After the protein standard powder is dissolved in the protein standard preparation solution, the protein standard stock solution is obtained. The stock solution contains preservative and does not affect the subsequent test. The protein standard stock solution -20℃ is stored for a long time.

3. The standard curve is recommended for each measurement. Because the color will deepen with the time, and the rate of color reaction is related to the temperature, unless the time and temperature of color reaction are precisely controlled, the standard curve should be made every time for accurate determination.

4. The reagent can be dissolved by agitation or incubation at 37 ° C when precipitation occurs under low temperature or long-term storage, and should be discarded if bacterial contamination is found.

5. When solution A and solution B are mixed, there may be turbidity, but the mixture will disappear after mixing. BCA working solution is recommended to be prepared for use.

6. The A562 value of the tested sample should be within the range of the standard curve. If it is beyond this range, the sample should be diluted and determined again.

7. If there is no microplate reader, ordinary spectrophotometer can be used, but the minimum detection volume should be considered according to the cuvette. Increase the amount of BCA working solution proportionally so that the total volume is not less than the minimum test volume, and increase the amount of sample and standard proportionally. When using a spectrophotometer to determine protein concentration, the number of samples that can be determined per testing kit may be significantly reduced.

8.For your safety and health, wear a lab coat and disposable gloves.

9. In consideration of loss, we will give more protein standard preparation solution. Please follow the instructions during preparation.