15. BCA protein concentration determination

·Material

BCA Protein Assay Kit

enzyme calibration

·Steps

① Preparation of protein standard:

a. Added 0.8 m protein standard solution to a tube protein standard (20 mg BSA), then prepare 25 mg/ml protein standard solution after full dissolution.

b. Take the appropriate amount of 25 mg / ml protein standard, diluted to 0.5 mg / ml. For example, take 20u1 25mg / ml protein standard, add 980u diluent can be self-made 0.5mg / ml protein standard.

② Preparation of BCA working solution:

According to the number of samples, add BCA reagent A and BCA reagent B (50:1) to prepare an appropriate amount of BCA working fluid (for example, 5ml BCA reagent A plus 100ul BCA reagent B), then fully mix them.

③ Determination of protein concentration:

a. Add different volume of the standards (0,2,4,8,12,16,20 μL/well) to the 96-well plates, then add the standard diluent to 20μL (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/ml).

b. Add sample into sample wells in 96-well plates.

c. Add 200ul BCA working solution to each well and place the well at 37 ° C for 20-30 minutes.

d. Determination of absorbance at wavelengths of A562(? nm by microplate reader.

e. Calculate the protein concentration of the sample according to the standard curve and the sample volume used.

·Note

① The protein standard can be used immediately after preparation, or it can be stored for a long time at -20°C.

② The standard should be diluted with the solution containing the protein sample. However, for simplicity, the standard can also be diluted with 0.9% NaCl or PBS. The diluted 0.5mg/ml protein standard can be stored for a long time at -20°C.

③ BCA working fluid is stable within 24 hours at room temperature.

④ As for *Step③c,* if the sample is less than 20ul, add standard diluent to make up to 20μL. Note down the sample volume. What's more, for this step, the well can also be placed at room temperature for 2 hours, or at 60°C for 30 minutes. When the BCA method is used to measure the protein fraction, the color will continue to deepen with the extension of time. And the color reaction will be accelerated due to the increase in temperature. If the concentration is lower, it is suitable to incubate at a higher temperature, or extend the incubation time appropriately.

⑤ Unless the time and temperature of the color reaction are precisely controlled, it is advisable to make a standard curve every time.