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BCA Protein Quantification Detection Kit

Product Number: S011123

Shipping and Storage

The entire set of reagents can be transported at room temperature; Protein standard (BSA) is stored at 2-8℃ and has a shelf life of 12 months. After preparing the protein standard solution, it needs to be stored at -20℃ and used within 6 months. The remaining reagents are stored at room temperature and have a shelf life of 12 months.

Component

Component	C012155-200T	C012155-1000T
BCA reagent	40mL	2×100mL
Copper sulfate solution	1.2mL	5×1.2mL
Protein Standard (BSA)	25mg	5×25mg
Protein standard preparation solution	1.5mL	5×1.5mL

Description

There are currently two most commonly used methods for protein concentration quantification, namely Bradford method and BCA method. The basic principle of BCA method for quantitative detection of proteins is based on the biuret reaction, which means that under alkaline conditions, $Cu^{2+}$is reduced by proteins to Cu^{+}, and then Cu^{+}undergoes chelation reaction with BCA (Bicinchonic acid, a color developer). Every two molecules of BCA chelate one Cu^{+}, generating a purple water-soluble complex. This substance has the highest absorption value at 562nm, and the color depth is proportional to the protein concentration within a certain range. Therefore, it can be used for quantitative detection of proteins. The protein concentration has a good linear relationship in the concentration range of 50-2000μg/mL.

This method is not affected by the majority of chemical substances in the sample and is compatible with high concentrations of detergents in the sample, including SDS with concentrations up to 5%, Triton X-100 with 5%, Tween-20, 60, 80 with 5%, etc. However, chelating agents and high concentrations of reducing agents can affect the detection results. It is necessary to ensure that there is no EGTA in the sample, EDTA concentration is less than 10mM, DTT is less than 1mM, and β- mercaptoethanol is less than 1mM. If the sample contains chelating agents or reducing agents, other products of our company, S011125 Bradford protein quantification detection kit, can be considered.

Protocol

1. Preparation of protein standard storage solution: Take 1mL of protein standard preparation solution and add it to a protein standard tube (BSA). Dissolve 25mg of protein standard completely to obtain a protein standard storage solution with a concentration of 25mg/mL. The prepared protein standard solution can be stored for a long time at -20℃.
2. Preparation of protein standard working solution: Take an appropriate amount of 25mg/mL protein standard storage solution, dilute 50 times with PBS or physiological saline, and obtain a final concentration of 0.5mg/mL protein standard working solution. Pay attention to diluting according to a 10 fold gradient method to ensure accurate dilution.
3. Draw standard curves (enzyme-linked immunosorbent assay): Add protein standard working solution to a 96 well plate at concentrations of 0, 1, 2, 4, 8, 12, 16, and 20μL. Then, add 20, 19, 18, 16, 12, 8, 4, and 0μL of PBS or physiological saline in sequence to make up the gradient working solution to 20μL. Obtain gradient curves of protein concentrations of 0, 25, 50, 100, 200, 300, 400, and 500μg/mL.
4. Prepare the sample to be tested: Dilute the protein sample to be tested appropriately (pre experimental testing can be used to ensure that the protein concentration of the sample is within the standard curve range and the test results are reliable), and add 20μL of each sample to a 96 well plate. Dilute the test sample with the same solution as the protein standard.
5. Preparation of BCA color developing working solution: Mix BCA reagent and copper sulfate solution in a volume ratio of 50:1

thoroughly to obtain BCA color developing working solution. BCA color developing working solution can be stored at room temperature and used within 24 hours. Each sample to be tested requires 200μL. It is recommended to prepare it as needed to avoid waste.

6. Detection: Add 200μL of BCA colorimetric working solution to each well of the standard curve sample well and the test sample well, mix well (a 96 well plate can be shaken on an oscillator for 30 seconds), react at 37℃ for 30 minutes, and use standard curve No. 0 as a reference for colorimetric measurement at a wavelength of 562 nm. Record the absorbance values of each well. (Note: It can also be reacted at room temperature for 2 hours or at 60℃ for 30 minutes.). If the protein concentration is low, it is recommended to react at 60℃
7. Calculation: Plot the standard curve with gradient protein content (μg/mL) as the horizontal axis and absorbance value as the vertical axis. Based on the absorbance value of the measured sample, the protein concentration (μg/mL) of the sample in the corresponding well can be obtained on the standard curve, and then multiplied by the sample dilution factor to obtain the actual protein concentration of the sample.

Note

1. The BCA method for determining protein concentration is greatly affected by temperature and time, and the absorbance value will change with the extension of time or the increase of temperature. If the time and temperature of the color reaction cannot be accurately controlled, it is recommended to make a standard curve for each measurement.
2. When preparing protein standard storage solution, it is necessary to ensure sufficient dissolution. When diluting and preparing protein standard working solution, it is recommended to dilute it in a 10 fold gradient instead of diluting it 50 times at once to avoid significant errors.
3. To ensure accurate quantification of protein, it is best to use the same buffer solution for sample extraction and dilution preparation of protein standards, while ensuring that the detection conditions for both are consistent. If the buffer itself has a high background value, it is recommended to use other methods for measurement.
4. When BCA reagent crystallizes and precipitates under low temperature conditions, it can be dissolved in a 37 ℃ bath without affecting its use.
5. Please wear lab clothes and disposable gloves during operation.