Super BCA Protein Quantification Kit

Cat #: D-AKE3010

Size: 500T

Storage: Store according to the recommended storage conditions of each component, stable for 12 months.

Assay Principle

The BCA method protein quantification kit is one of the commonly used detection methods for protein concentration. Super BCA Protein Quantification Kit is similar to the traditional BCA protein quantitation method, but employs a completely new and specific chelator different from BCA, resulting in a rapid, stable, and sensitive determination of protein concentration. The principle of the method is that the protein reduces copper ion (Cu^{2+}) to cuprous ion (Cu^{+}) in basic conditions, and the generated Cu+ forms an orange yellow water-soluble complex with chelates and has a strong absorption peak at 480 nm, the absorbance value is proportional to the content of protein in the sample, and the protein concentration can be determined according to the absorbance value. The chelators in this kit can sensitively and specifically bind to Cu+ and complete the color reaction with only 5 min incubation at room temperature.

In response to the pain points of the traditional BCA assay method such as long time consuming (37°C up to 30-90 min), unstable results, and low sensitivity, Super BCA Protein Quantification Kit to effectively solve the problems in protein determination, which has the following worry-free advantages: (1) Fast and time-saving: 5 min room temperature color development reaction; (2) Wide measurement range: wide linear working range, 1-2000 μg/mL protein concentration; (3) Accurate absorbance: the colorimetric method is used to have the best absorbance at 480 nm; (4) High sensitivity: detection of protein concentration as low as 1 μg/mL; (5) Sample volume to be measured: 1-20 μL.

Materials Supplied and Storage Conditions

Kit components	Size（500T）	Storage conditions
Reagent A	100 mL	4°C, protected from light
Reagent B	2 mL	4°C
BSA Standard (10 mg/mL)	1 mL	-20°C

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 480 nm

·96-well plate

·Precision pipettes, disposable pipette tips

·PBS, 0.9% NaCl or deionized water

·EP tube

Reagent Preparation

BSA Standard (2 mg/mL): Before use, dilute 100 µL BSA Standard (10 mg/mL) with 400 µL PBS to 2 mg/mL. The remaining reagent can also be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

Working Solution: Prepare before use. Mix Reagent A and Reagent B at a ratio of 50:1. According to the experimental dosage, it is prepared and used now.

Note: 1) when reagent B is added to reagent A, a gray blue precipitate appears, but just by mixing for a few seconds, the precipitate disappears, forming a bright green solution. If there is a slight difference in color depth, it is a normal phenomenon, does not affect product performance. 2) Working Solution is prepared and used now. Working Solution will gradually deepen at room temperature, but will not affect the accuracy of quantification as long as it is used within 1.5 h. 3) Due to possible errors in sample addition, it is recommended that working Solution be prepared with 1-2 more wells.

Sample Preparation

Sample Solution: Dilute samples to fall within 1-2,000 μg/mL range.

Assay Procedure

1.Preheat the microplate reader for more than 30 min, and adjust the wavelength to 480 nm.

2.Sample measurement. (The following operations are operated in the EP tube)

Num.	2 mg/mL Standard Volume (μL)	PBS or 0.9% NaCl Volume (μL)	Standard Concentration (μg/mL)
Std.1	0	200	0
Std.2	2	198	20
Std.3	5	195	50
Std.4	10	190	100
Std.5	20	180	200
Std.6	50	150	500
Std.7	100	100	1,000
Std.8	200	0	2,000

3.Take 20 μL Standard or Sample into a 96-well plate, add 200 μL Working Solution, mix well, incubate at room temperature for 5 min, and measure the OD value at a wavelength of 480 nm. The Blank Well (0 μg/mL) is marked as A_{Blank}, the Standard Well is marked as $A_{Standard}$, and the Sample Well is marked as A_{Sample}. Finally, calculate $\Delta A_{Standard}=A_{Standard}-A_{Blank}$, $\Delta A_{Sample}=A_{Sample}-A_{Blank}$.

Note: 1) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. When the protein concentration is determined, the color deepens continuously with time, and the color reaction is accelerated by increasing temperature. 2) Due to the faster color reaction, it was guaranteed that the reading value was completed within 10 min, which would otherwise affect the accuracy of protein quantification. If the value cannot be read in time, add 50 μL 1 M HCl to stop the reaction.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1.Drawing of standard curve

With $\Delta A_{Standard}$ as the y-axis and the concentration of the standard as the x-axis, draw the standard curve y=kx+b.

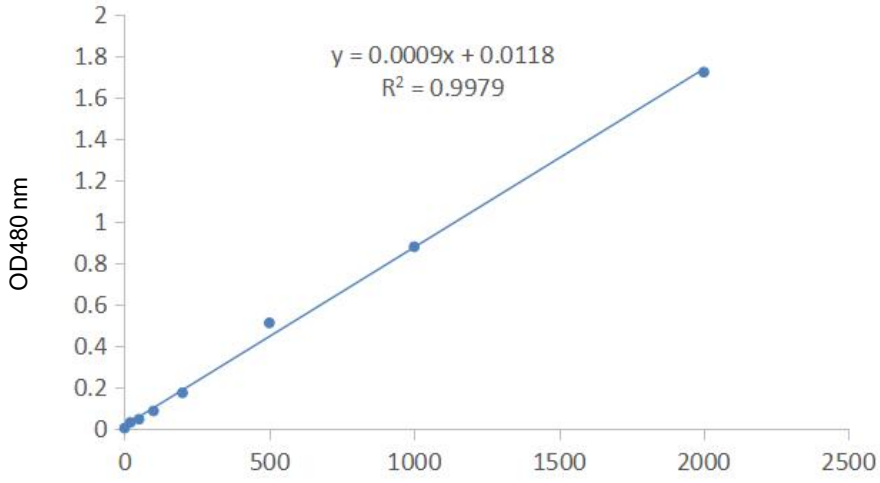
2.Calculation of the concentration

Bring the ΔA_{Sample} of the sample into the equation to get the x value (μg/mL).

Note: If the sample is further diluted, it needs to be multiplied by the further dilution factor n.

Typical Data

Typical standard curve-the following data and curves are for reference only, the experimenter needs to establish a standard curve according to the experiments.



Precautions

1.The protein concentration determined by method is not affected by chemical substances in most samples, and can be compatible with up to 5% SDS, 5% Triton X-100, 5% Tween20, 60, 80 in the sample. However, the method is affected by chelating agents and slightly higher concentrations of reducing agents. It is necessary to ensure that EDTA is less than

10 mM, no dithiothreitol (DTT) and β-mercaptoethanol.

2.It is recommended to make a standard curve for each measurement. Because the color of the method will continue to deepen with the extension of time, and the color reaction will be accelerated due to the increase of temperature.

3.If the sample diluent or lysate itself has a high background, please try the Protein Quantification Kit (Bradford Protein Assay Kit) (Cat #:D-AKE3002).

FAQ

1.What if the protein sample is not enough for the required 20 μL?

A: The volume of the sample to be measured can be anywhere from 1-20 μL. If the sample is less than 20 μL, it should be supplemented with PBS to 20 μL, keeping consistent with the dosage of standard product. Do not arbitrarily reduce the volume of standard and sample amount, otherwise it is easy to lead to low standard curve reading, sample reading beyond the range of standard curve.

2.Does the standard curve need to be remade for each quantitative experiment? Is it possible to directly use the standard curve made in the first experiment or directly apply the standard curve data in the manual?

A: Considering the influence of various factors such as environment and operation on the quantitative reaction process, in order to obtain more accurate data results, it is suggested to re-make the standard curve for each experiment. The standard curve in the instruction manual is for reference only.

3.Can the supernatant of cell culture be quantified by the kit?

A: As long as the sample does not contain a large number of surfactants and reducing agents that may interfere with the quantitative reaction, it can be used.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.