Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

Protein Quantification Kit (BCA Assay)

Cat #: KTD3001 Size: 500 T/2500 T

	Protein Quantification Kit (BCA Assay)		
REF	**Cat #:** KTD3001	LOT	**Lot #:** Refer to product label
	Protein concentration range: 20-2,000 μg/mL		
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum and other samples		
	Storage: Store at room temperature, stable for 12 months		

Assay Principle

Abbkine Protein Quantification Kit (BCA Assay) is improved on the basis of BCA method, one of the commonly used protein concentration detection methods in the world. The principle of this method is that the protein reduces copper ions (Cu^{2+}) to cuprous ions (Cu^{+}) in alkaline conditions, and the generated Cu^{+} forms a purple complex with BCA, which has a strong absorption peak at 562 nm, and the absorbance value It is proportional to the protein content in the sample, and the protein concentration can be calculated according to the absorbance value. The BCA method is suitable for the detection of protein concentrations in the range of 20-2,000 μg/mL, the minimum detected protein amount is 0.4 μg, and the sample volume to be tested is 1-20 μL.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	500 T	2500 T	
BCA Reagent A	100 mL	500 mL	RT
BCA Reagent B	2 mL	10 mL	RT
BSA Standard	1 (20 mg)	1 (20 mg)×5	RT
BSA Standard Buffer	1.5 mL	6 mL	RT

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 562 nm
- 96-well plate
- 37℃ incubator
- Precision pipettes, disposable pipette tips
- PBS, 0.9% NaCl or deionized water
- EP tube

Reagent Preparation

Note: If crystals appear in BCA Reagent A, heat slowly until completely dissolved.

BSA Standard (2 mg/mL): Before use, Add 1 mL BSA Standard Buffer to 20 mg BSA Standard and mix well, the concentration is 20 mg/mL. Dilute 100 μL BSA Standard (20 mg/mL) with 900 μL PBS to 2 mg/mL. The remaining reagent can also be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

BCA Working Solution: Prepare before use. Mix BCA Reagent A and BCA Reagent B at a ratio of 50:1. According to the experimental dosage, it is prepared and used now. The prepared working solution can be stored at 4℃ for 24 h.

Sample Preparation

Sample Solution: Dilute samples to fall within 20-2,000 μg/mL range.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 562 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 BCA Working Solution, mix well, incubate at 37℃ for 20-30 min, and measure the OD value at a wavelength of 562 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: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. When the protein concentration was determined by BCA method, the color would deepen with time. And the color reaction will be accelerated by the increase of temperature. If the concentration is lower, it is suitable to incubate at a higher temperature, or appropriately prolong the incubation time.

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.

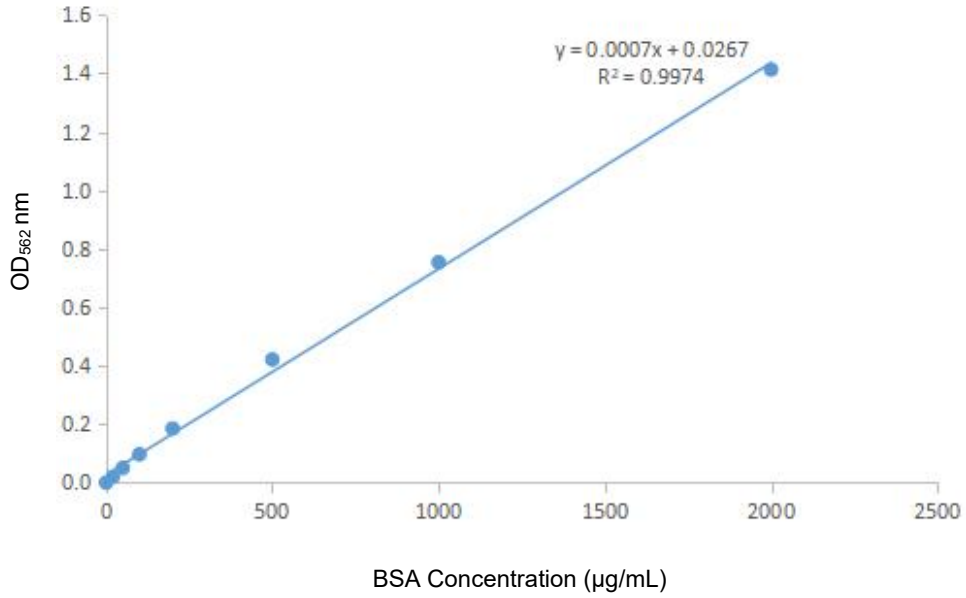
2. Calculation of the concentration

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. Use a microplate reader to measure the absorbance at 562 nm, or the absorbance at other wavelengths between 540-595 nm.
2. The protein concentration determined by BCA 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 BCA 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 EGTA, dithiothreitol (DTT) is less than 1 mM, and β-mercaptoethanol (β-Mercaptoethanol) is less than 0.01%.
3. It is recommended to make a standard curve for each measurement. Because the color of the BCA method will continue to deepen with the extension of time, and the color reaction will be accelerated due to the increase of temperature.
4. If the sample diluent or lysate itself has a high background, please try the Protein Quantification Kit (Bradford Assay) (Cat #: KTD3002).

FAQ

1. 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.

2. Can a spectrophotometer be used instead of an enzyme marker?

A: As long as the spectrophotometer can set the corresponding wavelength, it can be used. It should be noted that it is recommended to cooperate with the use of trace colorimetric dish, large volume colorimetric dish liquid surface is too low to be detected by the instrument, and it is necessary to increase the single determination reaction system, which will reduce the overall use of the kit.

3. There is no 562 nm option set for the enzyme label instrument, can 570 nm be used?

A: Yes, a wavelength range of 10 nm doesn't make a difference.

Recommended Products

Catalog No.	Product Name
KTD3002	Protein Quantification Kit (Bradford Assay)
KTD3010-EN	Super-Rapid Protein Quantification Kit (BCA Assay)
BMM3001	Colorcode Prestained Protein Marker (10-180 kDa)
BMM3002	Colorcode Prestained Protein Marker (15-130 kDa)

Disclaimer

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