Super-Rapid Protein Quantification Kit (BCA Assay)

Cat #: KTD3010-EN Size: 500 T

	Super-Rapid Protein Quantification Kit (BCA Assay)		
REF	**Cat #:** KTD3010-EN	LOT	**Lot #:** Refer to product label
	Protein concentration range: 1-2,000 μg/mL		
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum and other samples		
	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-Rapid Protein Quantification Kit (BCA Assay) 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 ℃ up to 30-90 min), unstable results, and low sensitivity, Abbkine has developed Super-Rapid Protein Quantification Kit (BCA Assay) 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, 1-2,000 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	Storage conditions
	500 T	
Reagent A	100 mL	-20℃, protected from light
Reagent B	2 mL	4℃
BSA Standard (10 mg/mL)	1 mL	-20℃
PBS	12 mL	4℃

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 480 nm
- 96-well plate
- Precision pipettes, disposable pipette tips
- EP tube

Reagent Preparation

Reagent A: Equilibrate to room temperature before use. Store and aliquot the remaining reagent at -20℃ for 12 months, protected from light to avoid repeated freezing and thawing. Store at 4℃ for 1 month in short term. If precipitation occurs after thawing is normal, redissolve at room temperature until completely dissolved.

Reagent B: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

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℃ 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, after mixing , 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: Samples were prepared according to experimental requirements and diluted in 1×PBS to the range of 20-2,000 µg/mL. If the sample is less than 20 µL, 1×PBS should be added to 20 µL, and the amount of samples to be measured can be estimated according to the empirical value.

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 96-well plate)

Num.	2 mg/mL Standard Volume (µL)	PBS Volume (µL)	Standard Concentration (µg/mL)
Std.1	0	20	0
Std.2	1	19	100
Std.3	2	18	200
Std.4	4	16	400
Std.5	8	12	800
Std.6	10	10	1,000
Std.7	15	5	1,500
Std.8	20	0	2,000

3. After preparation of 20 µL standard, add 20 µL sample to 96-well plate, then add 200 µL BCA Working Solution for each well , 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.

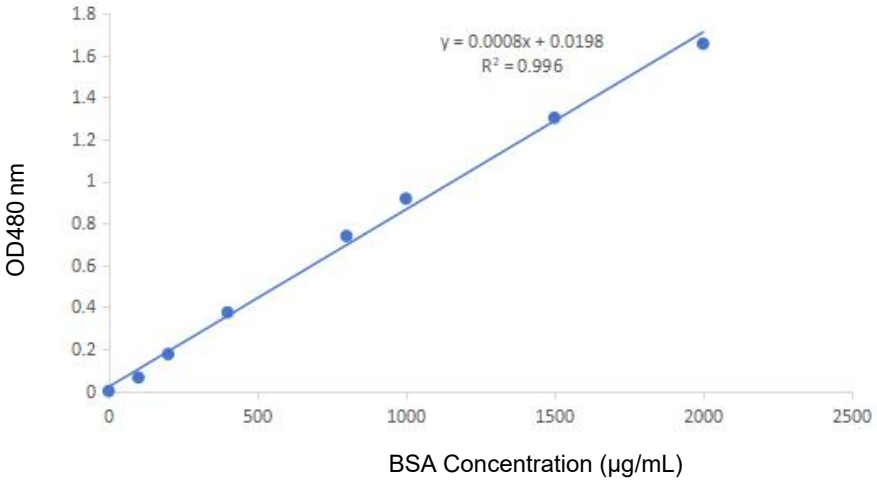
2. Calculation of the concentration

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 Assay) (Cat #: KTD3002).
4. The protein concentration range for this kit is 1-2,000 μg/mL.

Highlight moment: Super-Rapid Protein Quantification Kit (BCA Assay) is a kit developed by Abbkine for rapid protein quantification, which is very time-saving and convenient (RT, 5 min color development), which can quickly perform protein detection while ensuring stable, sensitive and reliable results, which is the best choice for your protein quantification experiments.

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 vary from 1-20 μL. If the sample is less than 20 μL, it should be supplemented with PBS or sample buffer to 20 μL, keeping consistent with the dosage of standard product. Do not arbitrarily reduce the volume of standard product and samples, otherwise it may lead to low of standard curve, resulting in 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 results, it is suggested to plot the standard curve for each experiment. The standard curve in the instruction manual is only for reference.

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

A: This kit can be used to cell culture supernatant unless the samples contain a large number of surfactants and reducing agents that may interfere with the quantitative reaction.

Recommended Products

Product Name	Catalog No.	Recommended Reasons
KTP3007	ExKine™ Pro Total Protein Extraction Kit for Animal Cultured Cells/Tissues	Complete protein extraction in 7 min
KTD3001	Protein Quantification Kit (BCA Assay)	Good linearity: linear working range from 20 to 2000 μg/mL
KTD3002	Protein Quantification Kit (Bradford Assay)	Time saving: almost immediate color development
BMP2010	WB Lysis Buffer	Denaturing lysis for a wide range of applications
BMM3001	Colorcode Prestained Protein Marker (10-180 kDa)	Optimized for SDS-PAGE and Western blotting
BMM3002	Colorcode Prestained Protein Marker (15-130 kDa)	Optimized for SDS-PAGE and Western blotting

Disclaimer

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