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Ready-to-use Protein Quantification Kit (BCA Assay)

Cat #: KTD3004 Size: 500 T/2500 T

	Ready-to-use Protein Quantification Kit (BCA Assay)		
REF	**Cat #:** KTD3004	LOT	**Lot #:** Refer to product label
	Protein concentration range: 50-2,000 μg/mL		
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum and other samples		
	Storage: Store the **BSA standard** at −20°C. Store all other components at room temperature. Shelf life: 12 months.		

01 Background and Principles

BCA (bicinchoninic acid) assay is a widely used method for protein quantitation. Its detection principle is based on the biuret reaction: under alkaline conditions, proteins reduce cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}). The generated Cu^{+} binds with BCA to form a purple complex, which has a strong absorption peak at 562 nm. The absorbance is proportional to the protein concentration in the sample, so the protein content can be calculated by measuring absorbance at 562 nm.

This kit is based on the BCA method. Compared with the traditional Lowry method, it features better reagent stability and higher tolerance to detergents, and is suitable for high-throughput 96-well plate assays. It contains pre-diluted BSA protein standards of a series of concentrations plus blank controls, which are ready-to-use without further dilution for convenient and efficient operation.

02 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
Blank (BSA, 0 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.1 (BSA,100 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.2 (BSA,200 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.3 (BSA,400 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.4 (BSA,800 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.5 (BSA,1000 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.6 (BSA,1500 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃
Std.7 (BSA,2000 μg/mL)	1 (1mL)×1	1 (1mL)×5	-20℃

***Please check the quantities of each component before the experiment.**

03 Required Instruments and Reagents

Resource Category	Item Name	Remarks
Instrument	Microplate Reader	Capable of measuring absorbance at 562 nm
Consumables	96-well microplate	Standard transparent plate
Other	Homogenizer (for tissue samples), incubator, ice maker, refrigerated centrifuge, adjustable pipettes and pipette tips	Multi-channel pipettes are recommended for high-throughput assays to improve efficiency

04 Notes

- **Before formal detection, it is recommended to select 2–3 samples with largely different expected concentrations for a pre-experiment.**
- The BCA assay for protein concentration measurement is tolerant to most chemicals in samples. It is compatible with samples containing up to 5% SDS, 5% Triton X-100, and 5% Tween 20, 60, 80. However, the BCA assay is interfered by chelating agents and high concentrations of reducing agents. Ensure EDTA < 10 mM, no EGTA, DTT < 1 mM, and β-Mercaptoethanol < 0.01%. If the sample diluent or lysis buffer has high intrinsic background, switching to the Bradford Protein Quantification Kit (Cat. No. KTD3002) is recommended.
- Prepare a standard curve for each assay. The color developed in the BCA reaction deepens over time, and the reaction rate increases with temperature.
- Absorbance measurement is preferably performed at 562 nm. If this wavelength is unavailable, wavelengths within 552–572 nm are acceptable.

• The diluent for BSA standards in this kit is compatible with test samples prepared in PBS or 0.9% NaCl. If samples are prepared in other matrix solvents, the matrix may introduce background interference. It is recommended to prepare solvent blanks matching the sample matrix; use the blank absorbance for correction and include it in standard curve calculation to improve detection accuracy.

• The working solution after preparation normally appears light blue. Minor color variations between batches are normal and will not affect test results.

• For research use only. Not for clinical diagnostic applications.

05 Experimental Procedure

05-1 Reagent Preparation

Reagent Name	Reagent Preparation	Precautions
Blank、Stds	Thaw on ice and mix thoroughly before use.	Keep on ice during the experiment. Repeated freeze-thaw cycles will reduce kit accuracy. It is recommended to aliquot and store components at -20 °C for up to 12 months.

05-2 Sample Preparation

Prepare samples according to experimental requirements. Dilute samples with sample diluent to a concentration within the range of 50–2,000 μg/mL. If the sample volume is less than 20 μL, add the sample solvent to make up to 20 μL. The loading volume of test samples can be estimated based on preliminary tests.

05-3 Assay Protocol

1. **Microplate reader setup:** Preheat for at least 30 min and set the wavelength to 562 nm.

2. **Preparation of Working Reagent:** Prepare freshly before use. Each well requires 200 μL Working Reagent. To avoid loss, prepare a 204 μL per-well system: combine 200 μL Reagent A and 4 μL Reagent B, and mix well.

3. Assay setup in a 96-well plate as follows:

Reagent	Blank Well (μL)	Standard Well (μL)	Sample Well (μL)
Sample	0	0	20
Blank (BSA, 0 μg/mL)	20	0	0
Stds.	0	20	0
Working Reagent	200	200	200

4. **Absorbance measurement:** After mixing, incubate at 37 ° C in the dark for 20–30 min. Read absorbance at 562 nm, recorded as A_{Blank}, $A_{Standard}$ and A_{sample} respectively.

06 Calculation of Results

The derived calculation formula and simplified calculation formula are provided below.

1. Data Processing

Calculate $\Delta A_{Standard} = A_{Standard} - A_{Blank}$ and $\Delta A_{sample} = A_{sample} - A_{Blank}$.

2. Plotting the Standard Curve

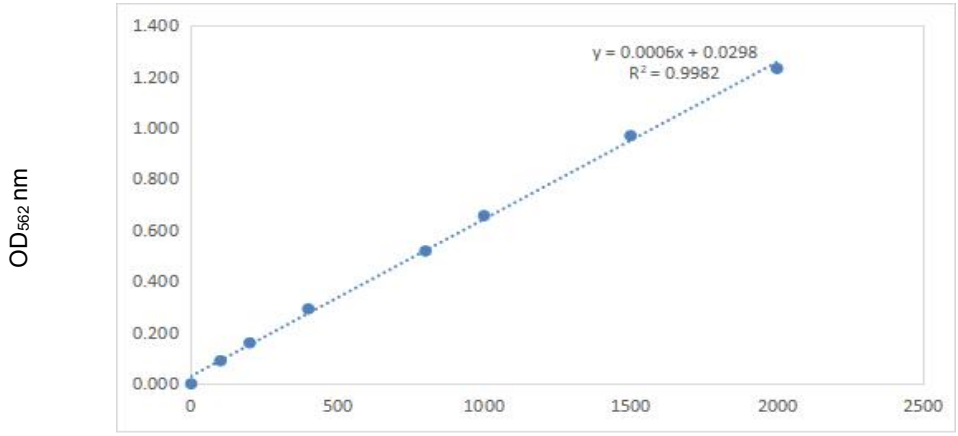
Plot the standard curve with $\Delta A_{Standard}$ on the y-axis and standard concentration on the x-axis.

3. Calculating Sample Protein Content

Substitute ΔA_{sample} into the regression equation to obtain the value of x (μg/mL). If the sample was diluted, multiply the result by the corresponding dilution factor to get the final concentration.

07 Results

Typical standard curve:y=0.0006x+0.0298，R2=0.9982



Example 1: Take 20 μL of cell supernatant, follow the assay procedure, and perform the assay using a 96-well plate. $\Delta A_{sample} = A_{sample} - A_{Blank} = 0.628 - 0.103 = 0.525$. Substitute this value into the standard curve to calculate x = 825.33. Thus, the sample concentration is 825.33 μg/mL.

08 FAQ

1. Can a spectrophotometer be used if a microplate reader is unavailable?

Detection can be performed using a spectrophotometer that supports the target detection wavelength. A microcuvette is preferred. If standard large-volume cuvettes are used, the single reaction volume should be scaled up accordingly.

Recommended Products

Catalog No.	Product Name
KTD3001	Protein Quantification Kit (BCA Assay)
KTD3002	Protein Quantification Kit (Bradford Assay)
KTD3005	Pre-Diluted Protein Standards
KTD3010	Super-Rapid Protein Quantification Kit (BCA Assay).
KTB2223	CheKine™ Micro Total Protein (TP) Content Assay Kit (Biuret Method)