Urine Protein Assay Kit

(Cat/No.:BC141 Size:100T/96S)

1. Assay principle (CBB method)

In an acidic medium, CBB binds to the NH_3^+ groups of proteins, inducing a color change from brown to blue, with a maximum absorption peak at 595 nm.

2. Composition & Preparation (The validity period of the kit is 6 months)

Reagent 1: CBB Reagent: 60 mL × 1 bottle. Store at 4℃ for 6 months.For use, prepare the CBB Working Solution by mixing **CBB Reagent** and distilled water at a ratio of 1:4 (i.e., 5-fold dilution). Prepare fresh before use.

Reagent 2: Protein Standard Solution (524 mg/L): 0.5 mL × 1 vial. Storable at 4℃ for 1 month. (If long-term storage is required, aliquot the standard solution and store it frozen at -20℃, with a storage period of 6 months.)

3. Required instruments and reagents

Visible light spectrophotometer and 1 cm cuvette (or microplate reader (595nm) and 96-well plate), vortex mixer, distilled water.

4. Operation method

	Blank	Standard	Sample
Double-distilled water (mL)	0.05		
524mg/L protein standard solution (mL)		0.05	
Urine (mL)			0.05
CBB application solution (mL)	3.0	3.0	3.0
Mix well, let it stand at room temperature for 5 minutes. Set the wavelength to 595 nm, the optical path to 1cm. Zero the instrument with double-distilled water. Use a spectrophotometer to measure the absorbance values A of each tube (or take 200 μL of the reaction solution from each tube and add it to a 96-well plate, and read the value at 595nm with an enzyme reader)			

5. Calculation Formula

Urine protein concentration(mg/L)$=\frac{A_{sample}-A_{blank}}{A_{standard}-A_{blank}} \times C_{standard}(524mg/L) \times$

$Dilution\ Factor\ of\ Samples\ Before\ Testing$

6. Notes

1. If the sample protein concentration is too low, appropriately increase the sampling volume (e.g., take 0.1 or 0.2 mL). Meanwhile, dilute the standard solution with distilled water by 2-fold or 4-fold to match the sample volume. For calculation, divide the standard solution concentration by the corresponding dilution factor before substituting it into the formula. The volume of CBB Working Solution remains unchanged.
2. If the OD value of the assay tube is too high (i.e., the difference between the assay OD and blank OD is more than twice as large as the difference between the standard OD and blank OD), dilute the sample with physiological saline to a certain extent (select the dilution factor that results in an assay OD value close to the standard OD value after dilution) before retesting.
3. This kit can also be read using a microplate reader (e.g., after allowing the reaction solution to stand for 5 minutes, transfer 0.2 mL from each tube to a 96-well plate (note: avoid introducing air bubbles) and read the absorbance at 595 nm). The calculation formula remains unchanged.