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Urine Protein Assay Kit (Coomassie Brilliant Blue Method)

Catalog No.: BC00052

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

Email (Sale) order@enkilife.com

Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

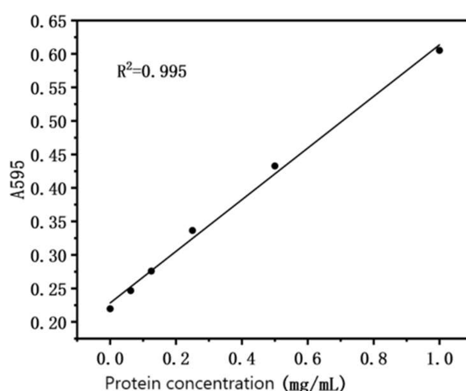
Product Name	Urine Protein Assay Kit (Coomassie Brilliant Blue Method)
Detection Method	Colorimetric
Sample Type	Tissue, cell, serum, plasma, urine and other samples
Assay Type	Quantitative
Detection Instrument	Microplate reader (550-630 nm, optimal detection wavelength 595 nm)

Product Introduction

Normally, the amount of protein in urine is very low and usually cannot be detected by routine urine testing methods. However, certain diseases or abnormal physical conditions may cause increased protein in the urine, which is called proteinuria.

Principle

Coomassie brilliant blue G-250 is red in the free state, with the maximum light absorption at 465nm; when it binds to protein, it turns cyan, and the protein-pigment conjugate has the maximum light absorption at a wavelength of 595 nm. The depth of its color is proportional to the protein content. The figure below shows the standard curve of this kit for detecting urine protein.



Components

No.	Components	Size (100T)	Storage
Reagent 1	Chromogen Stock Solution	15 mL	-20℃, protect from light.
Reagent 2	1 mg Standard	1 mg × 2 vials	-20℃, protect from light.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20°C for 6 months.

Preparation

- **Sample handling**

1. Liquid samples such as urine, serum, plasma, etc.: can be measured directly.
2. Tissue samples: Homogenize in PBS (0.01 M, pH 7.4) or physiological saline (0.9% NaCl), centrifuge after homogenization, and take the supernatant for testing.
3. Cell samples: Take 1×10^6 cells and add 300-500 μL PBS (0.01 M, pH 7.4) or physiological saline (0.9% NaCl) for homogenization. After homogenization, centrifuge at 10000×g for 10 min at 4 °C, take the supernatant and place it on ice for testing.

Note: The diluent is physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

- **Preparation of the kit**

1. Before testing, equilibrate the reagents in the kit to room temperature. Double distilled water, physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) are required.
2. Preparation of colorimetric reagent working solution: Mix reagent 1 and double distilled water in a volume ratio of 1:1. Store at 2-8 °C away from light for 7 days.
3. Preparation of 1 mg/mL standard solution: Add 1 mL of physiological saline to each vial of reagent 2 and dissolve and mix to obtain 1 mg/mL standard solution.
4. Dilution of different concentrations of standards: Dilute the standard solution with

physiological saline by half dilution method to different concentrations such as 1, 0.5, 0.25, 0.125, 0.0625, and 0 (blank well) mg /mL.

Operation process

1. Standard wells: Take 10 μL of different concentrations of standard solution and add to the corresponding standard wells. Sample wells: Take 10 μL of sample and add to the corresponding sample wells.
2. Add 250 μL of the colorimetric working solution to the standard wells and sample wells in step (1).
3. Oscillate on a microplate reader for 10 s, let stand at room temperature for 10 min, and measure the OD value of each well at 595 nm.

	Standard well	**Measurement well**
Different concentrations of standard solutions (μL)	10	--
Sample to be tested (μL)	--	10
Color developer working solution (μL)	250	250
Oscillate on a microplate reader for 10 s, let stand at room temperature for 10 min, and measure the OD value of each well at 595 nm.		

Calculation

Standard fitting curve: $y = ax + b$

Protein concentration calculation formula: Total protein (TP) content (mg/mL) = $(\Delta A595 - b) \div a \times f$

Annotation:

y: OD value of standard well - OD value of blank well (OD value when the concentration of standard is 0)

x: concentration corresponding to the absorbance

a: slope of the curve

b: intercept of the curve

ΔA595: Sample OD value - blank OD value (OD value when the standard concentration is

0)
f: dilution factor of the sample before adding it to the detection system

Notes

1. The optimal detection wavelength of the microplate reader is 595 nm, and detection can be performed in the range of 550 nm-630 nm.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.