(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Total protein (TP) Assay Kit (Biuret Method)

Catalog No.: BC00047

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	Total protein (TP) Assay Kit (Biuret Method)
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma and other samples
Assay Type	Quantitative
Detection Instrument	Microplate reader (520-580 nm, optimal detection wavelength 540 nm)

Product Introduction

Protein content determination is one of the most commonly used and basic analytical methods in biochemical research.

Product Features

• This kit can rapidly measure protein in trace amounts. It is easy to operate, fast and time-saving, can save a lot of reagents, and can measure a large number of samples at one time.

Principle

Any compound containing two carbamoyl groups (-$CONH_2$) in the molecule can react with alkaline copper solution to form a purple complex. This reaction is called biuret reaction. There are many peptide bonds (-CONH-) in protein molecules that can react in this way. The color development degree of various proteins is basically the same. The absorbance can be detected at 520-580nm (the best detection wavelength is 540nm). The darker the purple color of the product, the higher the protein content, and vice versa. Based on this, the protein content can be calculated through colorimetric analysis.

Components

No.	Components	Size (100T)	Storage

Reagent 1	Copper Sulfate	powder ×1 vial	-20℃, after opening store at 2-8°C for 6 months.
Reagent 2	Alkaline Reagent	powder ×1 vial	-20℃, after opening store at 2-8°C in the dark for 6 months.
Reagent 3	100g/L Protein Standard	575μL ×1 vial	-20℃, after opening store at -20°C for 6 months.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months.

Preparation

- **Sample handling**

1. Liquid samples such as serum and plasma: 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. Dilution of samples: Before the formal test, it is necessary to select 2-3 samples with large expected differences and dilute them into different concentrations for preliminary experiments. According to the results of the preliminary experiments and the linear range of this kit: 10-100 g/L, the dilution ratios of different samples are as follows (for reference only):

Sample	Dilution factor	Sample	Dilution factor
Human serum	No dilution	Human plasma	No dilution
Rat serum	2-4	Mouse plasma	No dilution
Rabbit serum	No dilution	Chicken plasma	No dilution
Horse serum	1-3	Porcine plasma	1-3
Dog serum	2-4	10% rat spleen tissue	No dilution
10% mouse liver tissue	No dilution	10% mouse kidney	No dilution

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

- **Preparation of the kit**

1. Reagent 3 should be taken out of -20℃ and placed on ice to thaw slowly (avoid repeated freezing and thawing), and other reagents should be equilibrated to room temperature.
2. Preparation of reagent 1 working solution: Dissolve one bottle of reagent 1 in 10 mL of double distilled water and store at 2-8℃ for 3 months.
3. Preparation of Reagent 2 working solution: Dissolve one bottle of Reagent 2 in 20 mL of double distilled water and store at 2-8℃ for 3 months.
4. Preparation of Biuret working solution: Mix reagent 1 working solution and reagent 2 working solution in a volume ratio of 1:2. The prepared working solution can be stored at 2-8℃ for 1 day.

Operation process

1. Blank wells: Take 5μL of water and add it to the corresponding blank wells of the plate; Standard wells: Take 5μL of different concentrations of protein standards and add them to the corresponding standard wells of the plate; Measurement wells: Take 5μL of the sample to be tested and add them to the corresponding measurement wells of the plate.
2. Add 250 μL of biuret working solution to each well in step (1).
3. Vibrate the plate on the microplate reader for 5 seconds and incubate at 37 °C for 10 minutes.
4. Measure the OD values of each well at 540 nm.

The operation table is as follows:

	Blank well	**Standard well**	**Measurement well**
Double distilled water (μL)	5	--	--
Protein Standard(μL)	--	5	--
Sample to be tested (μL)	--	--	5
Biuret reagent (μL)	250	250	250

Vibrate the plate on the microplate reader for 5 seconds, incubate at 37°C for exactly 10 minutes, and measure the OD values at 540 nm.

Calculation

1. Standard Curve Method

Standard fitting curve: y = ax + b

The calculation formula for total protein content in liquid samples such as serum, plasma, and tissue homogenate is:

Total protein concentration = (ΔA540 - b) ÷ a × f (g/L)

y: Standard OD value – Blank OD value (OD value when the standard concentration is 0)

x: concentration corresponding to the absorbance

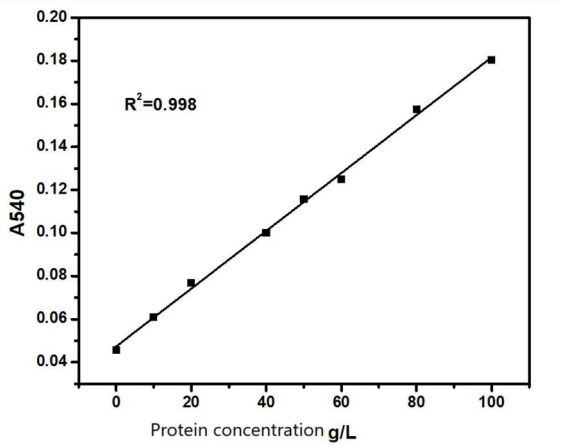
a: Slope of the standard curve

b: Intercept of the standard curve

ΔA540: Sample OD value - Blank OD value

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

The following standard curve is for reference only:



2. Single point calculation method

$$\text{Protein concentration of the sample to be tested (g/L)} = \frac{\text{Determine OD value - Blank OD value}}{\text{Standard OD value - Blank OD value}} \times \text{Protein standard concentration (g/L)}$$

Notes

1. Remember not to mix the powders of Reagent 1 and Reagent 2 and then add water to dissolve them.
2. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
3. Please wear lab coats and latex gloves for protection during the experiment.
4. This kit is suitable for detecting samples with protein content between 10 and 100 g/L. If the protein content is higher than 100 g/L, it must be diluted with physiological saline to within this range.
5. 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.