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

Total protein (TP) Assay Kit (Coomassie Brilliant Blue Method)

Catalog No.: BC00007

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

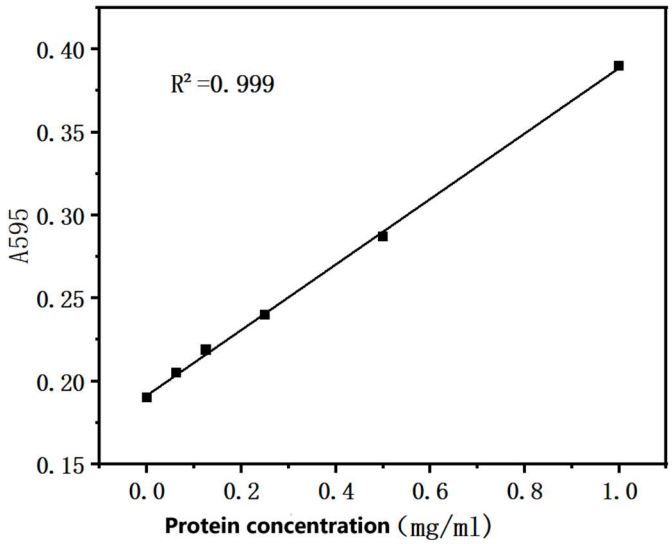
Product Name	Total protein (TP) Assay Kit (Coomassie Brilliant Blue Method)
Detection Method	Colorimetric
Sample Type	Serum,Plasma,Urine,Animal and Plant Tissues, Culture Medium,Cells
Assay Type	Quantitative
Detection Instrument	Microplate reader (560-610 nm, optimal detection wavelength 595 nm)

Product Introduction

This kit is a kit commonly used to determine the protein content in biological samples. Coomassie blue staining, also known as Bradford method, is a method for determining protein concentration.

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 complex has the maximum light absorption at a wavelength of 595 nm. The depth of its color is proportional to the protein content.



Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Chromogen Stock Solution	15mL	-20℃, avoid light, store at 2-8℃ after opening
Reagent 1	1mg/mL Standard	1.5mL	-20℃, avoid light, store at room temperature after opening
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

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

After opening the kit,please store it according to the requirements of "Components-Storage", valid for 6 months.

Experimental Preparation

- Sample processing

1. Liquid samples such as serum and plasma: can be measured directly.
2. Tissue samples: The homogenate medium was PBS (0.01 M, pH 7.4) or saline (0.9% NaCl). The homogenate was centrifuged and the supernatant was taken for measurement.
3. Cell samples:Take 1×10^6cells and add 300-500μL PBS (0.01 M,pH 7.4) or saline (0.9% NaCl) for homogenization.After homogenization, centrifuge at 10000×g for 10 min at 4℃ , and take the supernatant and place it on ice for testing.
4. If the sample needs to be diluted, the diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

- Preparation of the assay kit

1. Before testing, the reagents in the kit were equilibrated to room temperature.
2. Preparation of Chromogen working solution: Mix Chromogen Stock Solution and double distilled water in a volume ratio of 1:1. It can be stored at 4°C away from light for 7 days.
3. Dilution of standards of different concentrations : 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, 0 (blank well) mg /mL .

Operation process

1. Standard wells: Take 10μL of standard solution of different concentrations and add it to the corresponding standard wells. Assay wells: Take 10μL of sample and add it to the corresponding assay wells.

2. Add 250 μL of chromogen working solution to the standard wells and assay wells in step "1" .

3. Vibrate for 10s on a microplate reader,then let it stand at room temperature for 10 minutes, measure the OD values of each well at 595 nm.

The operation table is as follows:

	Standard well	Assay well
Standard solutions of different concentrations (μL)	10	--
Sample to be tested (μL)	--	10
Chromogen working solution (μL)	250	250
Vibrate for 10s on a microplate reader,then let it stand at room temperature for 10 minutes, measure the OD values of each well at 595 nm.		

Result calculation

Standard fitting curve: y = ax + b

The calculation formula for serum (plasma) and animal tissue total protein (TP) concentration is:

Total protein (TP) content (mg/mL) = (ΔA595 - b) ÷ a × 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

x: concentration corresponding to the absorbance

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. A microplate reader capable of detecting wavelengths between 560-610 nm is required, with the optimal detection wavelength being 595 nm. A 96-well plate is also required.
2. This product is for Research Use Only, and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences. .

1. Preparation of protein standards
Take 0.8 ml of protein standard solution and add it to a tube of protein standard (20 mg BSA). After fully dissolving, prepare a 25 mg/ml protein standard solution. Take an appropriate amount of 25 mg/ml protein standard and dilute it to a final concentration of 1 mg/ml. The dilution solution of the standard is generally the same as the solution of the sample to be tested. The standard can also be diluted with 0.9% NaCl or PBS. The protein standard solution and the diluted protein standard solution can be stored for a long time at -20ºC.
2. Preparation of BCA working solution
According to the number of samples, prepare an appropriate amount of BCA working solution by adding 50 volumes of BCA reagent A to 1 volume of BCA reagent B (50:1) and mix thoroughly. For example, add 5ml of BCA reagent A to 100μl of BCA reagent B, mix well, and prepare 5.1ml of BCA working solution. BCA working solution is stable at room temperature for 24 hours.

Operation process

1. 1 mg /ml standard solution was diluted in half to 0.5mg/ml , 0.25mg/ml , 0.125mg/ml , 0.0625mg/ml , and 0mg/ml (blank well), and 20 μl of each was added to the standard wells of a 96-well plate.
2. Add an appropriate volume of sample to the sample wells of the 96-well plate. If the sample volume is less than 20μl, add standard diluent to make it up to 20μl.The protein concentration range determined by this kit is 0.02-1.5mg/ml.If the sample protein

concentration is too high, dilution is required.
3. Add 200μl BCA working solution to each well and incubate at 37ºC for 30 minutes.
4. The absorbance at A562 or other wavelengths between 540-595 nm was measured using a microplate reader.
The operation table is as follows:

	Standard well	Determination well
Standards of different concentrations (μl)	20	--
Sample to be tested (μl)	--	20
BCA working solution (μl)	200	200
Incubate for 30 minutes at 37ºC with a microplate reader, and measure the OD value of each well at 562nm.		

Result calculation

Calculate the protein concentration of the sample based on the standard curve and the sample volume used.

Notes

1. A microplate reader is required, and the measurement wavelength is between 540-595nm , with 562nm being the best. Requires 96- well plate. If there is no microplate reader, you can also use an ordinary spectrophotometer for measurement, but when measuring, you need to appropriately increase the amount of BCA working solution according to the minimum detection volume of the cuvette so that it is not less than the minimum detection volume. The dosage can be scaled up accordingly or remain unchanged. When using a spectrophotometer to measure protein concentration, the number of samples that can be measured per kit may be significantly reduced.
2. The BCA method for determining protein concentration is not affected by the chemicals in most samples and is compatible with up to 5% SDS, 5% Triton X-100, and 5% Tween20, 60, and 80 in the sample. However, this kit is affected by chelator and slightly higher concentrations of reducing agents. It is necessary to ensure that EDTA is less than 10mM, there is no EGTA, dithiothreitol (DTT) is less than 1mM, and β-mercaptoethanol (β-Mercaptoethanol) is less than 0.01%. If the sample diluent or lysate itself has a high background, it will affect the accuracy of the kit.
3. When the BCA method is used to determine protein concentration, the color will deepen over time. The color development reaction will accelerate as the temperature rises. If the concentration is low, it is appropriate to incubate at a higher temperature or extend the incubation time appropriately.

4. Unless the time and temperature of the color development reaction are precisely controlled, it is recommended to make a standard curve each time if you need to accurately determine the protein concentration.
5. This product is for Research Use Only, and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences. .