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

Catalog No：E-BC-K165-M

Specification：96T(80 samples)/ 500Assays(484 samples)

Measuring instrument：Microplate reader（520-580 nm）

Detection range：0.58-100 g/L

Elabscience® Biuret Protein Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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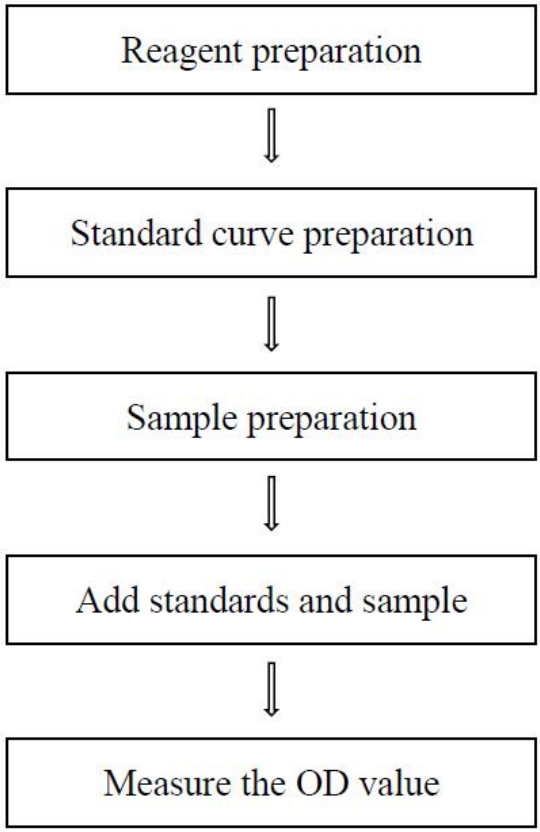
Assay summary



Intended use

This kit can be used to measure total protein (TP) content in serum, plasma and tissue samples.

Detection principle

Any compound that contains two -CONH2 in the molecule can react with alkaline copper solution to form a purple complex, which is known as the biuret reaction. Many peptide bonds (-CONH-) in protein molecules can perform this reaction, and the color degree of all kinds of proteins are essentially the same.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Copper Reagent	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months
Reagent 2	Alkali	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months, shading light
Reagent 3	100 g/L Protein Standard	1 mL × 1 vial	1 mL × 5 vials	-20°C, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (520-580 nm, optimum wavelength: 540 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge.

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Keep 100 g/L protein standard on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of copper working solution：
Dissolve one vial of copper reagent with 10 mL of double distilled water, mix well. Store at 2-8°C for 3 months.

③ The preparation of alkali working solution：
Dissolve one vial of alkali with 20 mL of double distilled water, mix well. Store at 2-8°C for 3 months.

④ The preparation of biuret working solution：
Before testing, please prepare sufficient biuret working solution according to the test wells. For example, prepare 300 μL of mersuring working solution (mix well 100 μL of copper working solution and 200 μL of alkali working solution). Store at 2-8°C for 1 day.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 g/L protein standard with normal saline diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 g/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (g/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
100 g/L standard (μL)	0	10	20	40	50	60	80	100
Normal saline (μL)	100	90	80	60	50	40	20	0

Sample preparation

① Sample preparation:

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human plasma	1
Rat serum	2-4
Mouse plasma	1
Rabbit serum	1
Chicken plasma	1
Horse serum	1-3
Porcine serum	1-3
Dog serum	2-4
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The time of incubation (37°C) should be accurately (10 min).

② Prevent the formulation of bubbles when adding the liquid to the microplate.

Operating steps

① Standard tube: Add 7 μL of standard solution with different concentration to the well.

Sample tube: Add 7 μL of sample to the well.

② Add 250 μL of biuret working solution to each well.

③ Mix fully with microplate reader for 5 s and incubate the microplate at 37°C for 10 min accurately.

④ Measure the OD value at 540 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

For sample:

$$\underset{(g/L)}{\text{TP content}} = (\Delta A_{540} - b) \div a \times f$$

[Note]

y: $OD_{Standard} - OD_{Blank}$.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

ΔA_{540}: $OD_{Sample} - OD_{Blank}$.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (g/L)	2.50	38.50	95.00
%CV	4.2	3.4	4.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (g/L)	2.50	38.50	95.00
%CV	6.2	7.5	5.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (g/L)	15	56	72
Observed Conc. (g/L)	15.2	52.6	71.3
Recovery rate (%)	101	94	99

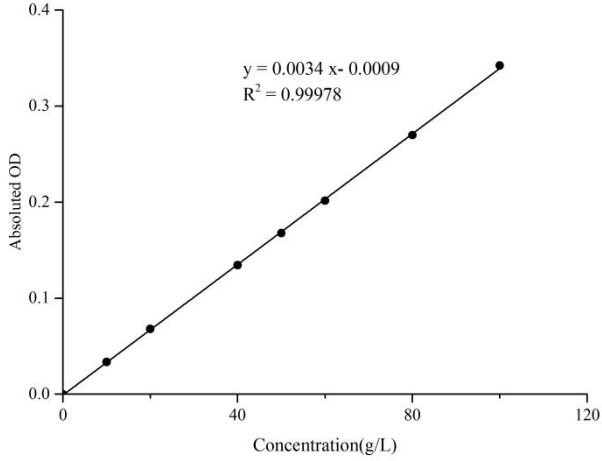
Sensitivity

The analytical sensitivity of the assay is 0.58 g/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (g/L)	0	10	20	40	50	60	80	100
OD value	0.122	0.155	0.189	0.255	0.289	0.323	0.391	0.463
	0.122	0.156	0.191	0.257	0.290	0.324	0.393	0.465
Average OD	0.122	0.156	0.190	0.256	0.290	0.324	0.392	0.464
Absoluted OD	0.000	0.034	0.068	0.134	0.168	0.202	0.270	0.342



Appendix II Example Analysis

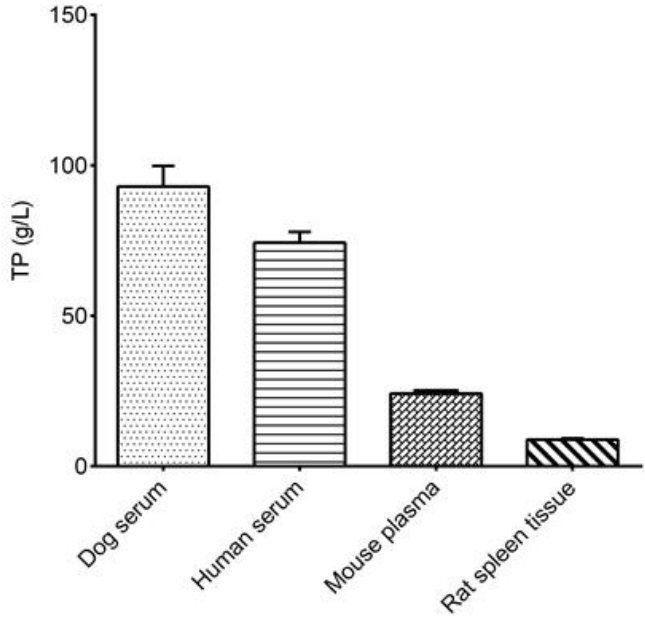
Example analysis：

Take 7 μL of human serum and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0052 x - 0.0057, the average OD value of the sample is 0.497, the average OD value of the blank is 0.121, and the calculation result is:

$$\text{TP content (g/L)} = (0.497 - 0.121 + 0.0057) \div 0.0052 = 73.40\text{g/L}$$

Detect dog serum (dilute for 3 times), human serum, mouse plasma, 10% rat spleen tissue homogenate according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.