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

Catalog No：E-BC-K168-M

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

Measuring instrument：Microplate reader（550-630 nm）

Detection range：0.046-0.6 mg/mL

Elabscience® Bradford 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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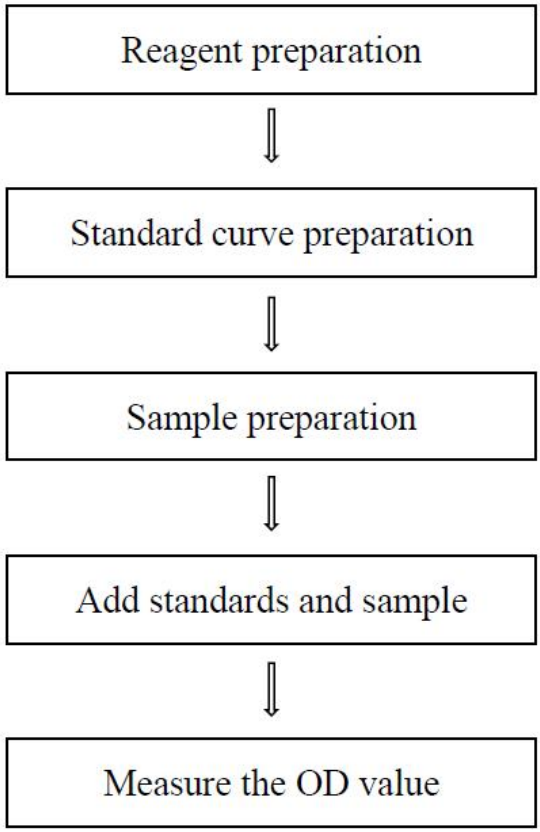
Assay summary



Intended use

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

Detection principle

Coomassie brilliant blue G-250 is red under the free state, and it has the maximum absorbance at 465 nm. When the Coomassie brilliant blue G-250 combined to protein, the compound will have the maximum at 595 nm. The absorbance value is directly proportional to the protein content, so the concentration of total protein can be calculated directly by measuring the OD value at 595 nm.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Chromogenic Agent Stock Solution	6 mL × 1 vial	30 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	1 mg Standard	1 mg × 2 vials	1 mg × 5 vials	RT, 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 (550 nm-630 nm, optimum wavelength: 595 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 Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic agent：

For each well, prepare 250 μL of chromogenic agent (mix well 50 μL of chromogenic agent stock solution and 200 μL of double distilled water). Store at 2-8°C for 7 days protected from light.

③ The preparation of 1 mg/mL standard solution：

Dissolve one vial of 1 mg standard with 1 mL of normal saline, mix well.

④ The preparation of standard curve：

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

Dilute 1 mg/mL standard solution with normal saline diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
1 mg/mL standard (μL)	0	5	10	20	30	40	50	60
Normal saline (μL)	100	95	90	80	70	60	50	40

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.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation = 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor 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	90-110
Human plasma	90-110
Rabbit serum	90-110
Rat plasma	90-110
Chicken serum	90-110
10% Mouse kidney tissue homogenate	15-20
10% Mouse lung tissue homogenate	15-20
10% Rat spleen tissue homogenate	15-20
10% Rat heart tissue homogenate	15-20
10% Rat liver tissue homogenate	20-25
293T	1
HL-60	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

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

Operating steps

① Standard tube: Add 10 μL of standard solution with different concentration to the well.

Sample tube: Add 10 μL of sample to the well.

② Add 250 μL of chromogenic agent to each well.

③ Mix fully with microplate reader for 10 s and stand at room temperature for 10 min.

④ Measure the OD value at 595 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:

$$\frac{\text{TP content}}{\text{(mg/mL)}} = (\Delta A_{595} - b) \div a \times f$$

[Note]

f: Dilution factor of sample before test.

ΔA_{595}: $OD_{Sample} - OD_{Blank}$.

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 (mg/mL)	0.10	0.24	0.52
%CV	3.5	3.1	3.0

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 (mg/mL)	0.10	0.24	0.52
%CV	8.1	7.4	9.1

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 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.08	0.24	0.57
Observed Conc. (mg/mL)	0.1	0.3	0.6
Recovery rate (%)	101	106	105

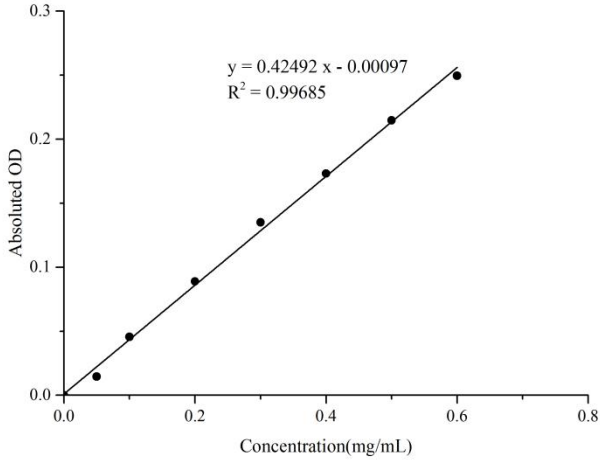
Sensitivity

The analytical sensitivity of the assay is 0.046 mg/mL. 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 (mg/mL)	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
OD value	0.320	0.337	0.358	0.405	0.452	0.502	0.528	0.568
	0.321	0.332	0.373	0.413	0.458	0.484	0.542	0.571
Average OD	0.320	0.335	0.366	0.409	0.455	0.493	0.535	0.569
Absoluted OD	0.000	0.015	0.046	0.089	0.135	0.173	0.215	0.249



Appendix II Example Analysis

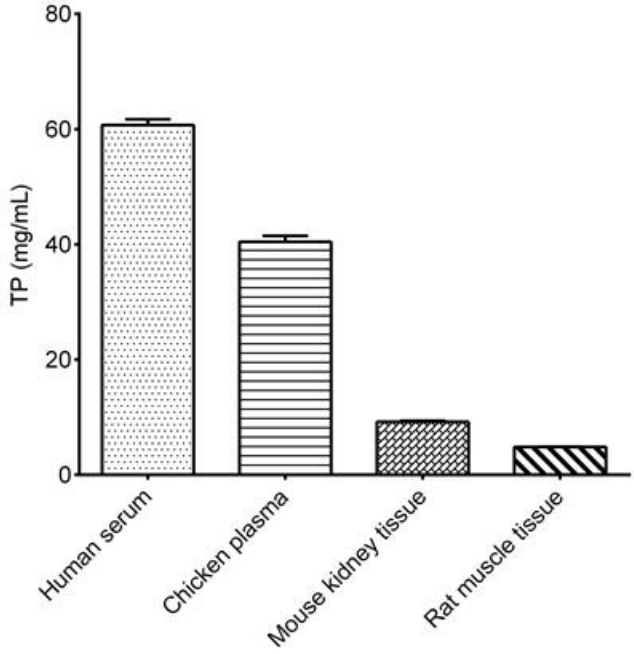
Example analysis：

Dilute human serum with normal saline (0.9% NaCl) for 100 times, take 10 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.3727 x + 0.0043, the average OD value of the sample is 0.550, the average OD value of the blank is 0.319, and the calculation result is:

$$\text{TP content (mg/mL)} = (0.550 - 0.319 - 0.0043) \div 0.3727 \times 100 = 60.83 \text{ mg/mL}$$

Detect human serum (dilute for 100 times), chicken plasma (dilute for 100 times), 10% mouse kidney tissue homogenate (dilute for 20 times) and 10% rat muscle tissue homogenate (dilute for 20 times) 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.