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

Catalog No：E-BC-K318-M

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

Measuring instrument：Microplate reader（540-590 nm）

Detection range：0.0165-1 mg/mL

Elabscience® BCA Protein Colorimetric Assay Kit

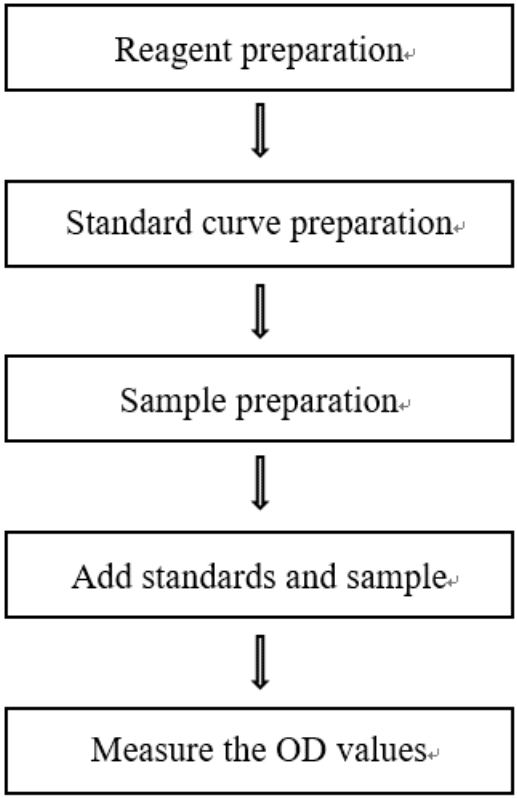
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary

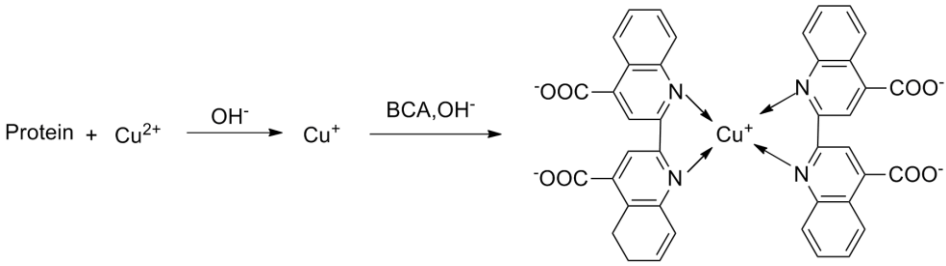


Intended use

This kit can be used to measure Total Protein (TP) content in serum, plasma, culture cells, tissue and cells samples.

Detection principle

Cu^{2+} can be reduced to Cu^{+} by protein in alkaline condition. Cu^{+} can combine with BCA reagent and form purple complex, which has a maximum absorption peak at 562 nm. The absorbance value is proportional to the protein concentration. Therefore, the protein concentration can be calculated according to the OD value.



Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	BCA Reagent	25 mL ×1 vial	50 mL ×2 vials	RT, 12 months
Reagent 2	Copper Salt Solution	0.5 mL ×1 vial	3 mL ×1 vial	RT, 12 months
Reagent 3	Standard	1 mg ×1 vial	1 mg ×5 vials	RT, 12 months
Reagent 4	Standard Diluent	15 mL ×1 vial	30 mL ×1 vial	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(540-590 nm), Vortex mixer, Micropipettor, Incubator

Reagents:

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

Reagent preparation

① The preparation of BCA working solution:

For each well, prepare 200 μL of BCA working solution (mix well 4 μL of copper salt solution and 196 μL of BCA reagent). Store at 4℃ for 24 h.

② The preparation of 1 mg/mL standard solution:

Dissolve a vial of standard with 1 mL standard diluent and mix fully. Aliquoted storage at -20℃ for 3 months

③ 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 standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.9, 1 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.9**	**1.0**
1 mg/mL standard solution (μL)	0	50	75	100	150	175	225	250
Standard Diluent (μL)	250	200	175	150	100	75	25	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℃ for a month.

Tissue sample:

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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

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

Cells:

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 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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
10% Mouse brain tissue homogenization	8-12
10% Mouse kidney tissue homogenization	8-12
Human serum	100-200
10% Rat liver tissue homogenization	15-20
10% Mouse heart tissue homogenization	8-12
Rat serum	100-200

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 should be accurately.

② The concentration of the sample protein must be diluted to 1 mg/mL or less with normal saline, and it will show a good linear range below this concentration.

③ Prevent the formulation of bubbles when adding the reagents to the microplate.

Operating steps

The measurement of samples

① Standard well: add 20 μL of standard solution with different concentration.
Sample well: add 20 μL of tested samples.

② Add 200 μL of BCA working solution into each tube.

③ Oscillate for 20 s to mix fully and incubate at 37 ℃ for 30 min.

④ Measure the OD values of each well at 562 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).

The sample:

$$\text{Protein content (mg/mL)} = (\Delta A_{562} - b) \div a \times f$$

[Note]

ΔA_{562}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

f: Dilution factor of sample before test.

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)	0.08	0.28	0.65
%CV	2.5	2.3	1.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.08	0.28	0.65
%CV	4.2	4.5	4.8

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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.25	0.45	0.85
Observed Conc. (mg/mL)	0.3	0.4	0.9
Recovery rate (%)	101	99	100

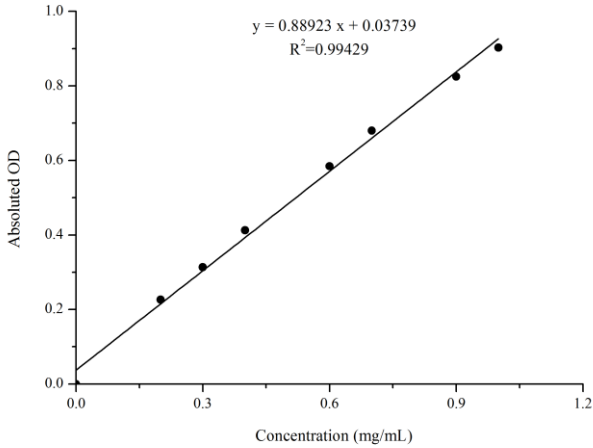
Sensitivity

The analytical sensitivity of the assay is 0.0165 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	0.2	0.3	0.4	0.6	0.7	0.9	1
Average OD	0.081	0.308	0.395	0.494	0.666	0.761	0.906	0.984
Absoluted OD	0	0.227	0.314	0.413	0.585	0.680	0.825	0.903



Appendix II Example Analysis

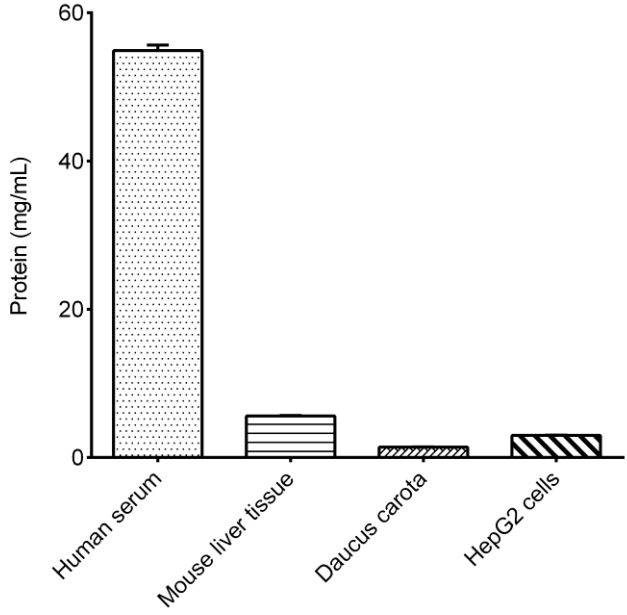
Example analysis:

Dilute human serum with PBS (0.01 M, pH 7.4) for 50 times, take 0.02 mL of diluted human serum and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.88923x +0.03739, the average OD value of the sample well is 1.100, the average OD value of the blank well is 0.087, and the calculation result is:

$$\text{Protein content (mg/mL)} = (1.100 - 0.087 - 0.03739) \div 0.88923 \times 50 = 54.88 \text{ mg/mL}$$

Detect human serum (dilute for 50 times), 5% mouse liver tissue homogenate (dilute for 6 times), 5% daucus carota tissue homogenate (dilute for 2 times) and HepG2 cells (dilute for 3 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.