BCA Protein Colorimetric Assay Kit

Catalog No: E-BC-K318-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.0165 mg/mL

Detection range: 0.0165-1 mg/mL

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

General information

▲Intended use

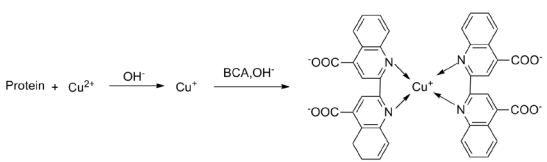
This kit can be used to measure Total Protein (TP) content in serum, plasma, culture cells, tissue and cells samples.

▲Background

The BCA protein concentration kit is an ideal protein quantification method which is superior to the Lowry method. This method is fast and sensitive, stable and reliable to different types of protein with small variation coefficient, which is greatly favored by professionals. The BCA method is not affected by the chemicals for most 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	Specification	Storage
Reagent 1	BCA Reagent	25 mL × 1 vial	RT, 12 months
Reagent 2	Copper Salt Solution	0.5 mL × 1 vial	RT, 12 months
Reagent 3	Protein BSA Standard	1 mg × 1 vial	RT, 12 months
Reagent 4	Standard Diluent	15 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.

Materials prepared by users

Instruments

Microplate reader (540-590 nm), Vortex mixer, Micropipettor, Incubator

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents:

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲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.

Pre-assay preparation

▲ Reagent preparation

1. Preparation of BCA working solution
   Mix the reagent 1 and reagent 2 fully at a ratio of 50:1. Prepare the needed amount solution before use. The prepared working solution can be stored at 4℃ for 24 h.
2. Preparation of 1 mg/mL standard solution
   Dissolve a vial of reagent 3 powder with 1 mL reagent 4 and mix fully before use. It is recommended to aliquot the prepared solution and it can be store at -20℃ for 3 months.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The sample should not contain chelating agents (EGTA, EDTA) and reductive substances (DTT, 2-mercaptoethanol).

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.0165-1 mg/mL).

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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	562 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A, blank wells; B-H, standard wells; S1-S80, sample wells.

▲Operating steps

The preparation of standard curve

Dilute 1 mg/mL BSA standard solution with normal saline 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.

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 to the wells of Step 1.

(3) Oscillate for 20 s to mix fully and incubate at 37°C for 30 min.

(4) Measure the OD value of each well at 562 nm with microplate reader.

▲Operation table

	Standard well	Sample well
Standard solution with different concentration (μL)	20	
Samples (μL)		20
BCA working solution (μL)	200	200
Oscillate for 20 s to mix fully and incubate at 37°C for 30 min. Measure the OD values of each well at 562 nm with microplate reader.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: $y = ax + b$.

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

Note:

y: The absolute OD value of standard

x: The concentration of Standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{562}: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before test.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.0165-1 mg/mL	Average intra-assay CV (%)	2.2
Sensitivity	0.0165 mg/mL	Average inter-assay CV (%)	4.5
Average recovery rate (%)	100		

▲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 table.

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, the calculation result is:

Protein content (mg/mL) =(1.100-0.087-0.03739) ÷ 0.88923 × 50 = 54.88 mg/mL

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (Heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Cells

Collect the cells and wash the cells with homogenization medium for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or normal saline
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.