Technical Manual

Total Protein (TP) Colorimetric Assay Kit (BCA Method)

- Catalogue Code: MAES0177
- Size: 48T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

48T

Range:

0.0165-1 mg/mL

Sensitivity:

0.0165 mg/mL

Storage:

RT for 12 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 12 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. Detection Principle

Cu2+ 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.

5. Kit Components & Storage

Item	Specification	Storage
BCA Reagent	12.5 mL × 1 vial	RT, 12 months
Copper Salt Solution	0.25 mL × 1 vial	RT, 12 months
Protein BSA Standard	1 mg × 1 vial	RT, 12 months
Standard Diluent	15 mL × 1 vial	RT, 12 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Vortex mixer
- Microplate Reader (540-590 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. The time of incubation should be accurate.

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.

7. Reagent Preparation:

1. Preparation of **BCA working solution:** Mix the BCA reagent and copper salt solution fully at a ratio of 50:1. Prepare the needed amount solution before use. The prepared working solution can be stored at 4°C for 24 h.

2. Preparation of **standard solution (1 mg/mL):** Dissolve a vial of protein BSA standard powder with 1 mL standard diluent and mix fully before use. It is recommended to aliquot the prepared solution and it can be store at -20°C for 3 months.

8. Sample Preparation

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

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) or normal saline (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

4. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) 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 (4×10^6): PBS (0.01 M, pH 7.4) or normal saline (μL) =1: 400. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 562 nm

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.

10. Operation 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 tube:** add 20 μL of standard solution with different concentration.
   Sample tube: 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.		

11. Calculations

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.

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

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA_{562}: OD_{Sample} – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0)
f: Dilution factor of sample before test.

12. Performance Characteristics

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

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:

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

$$= 54.88 \text{ mg/m}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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