Technical Manual

Total Protein (TP) Colorimetric Assay Kit (Coomassie Brilliant Blue Method)

- Catalogue Code: MAES0126
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.046-0.6 mg/mL

Sensitivity:

0.046 mg/mL

Storage:

2-8°C for 6 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 6 months.

Do not use components from different batches of kit.

2. Intended Use:

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

3. 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.

4. Kit Components & Storage:

Item	Specification	Storage
Chromogenic Agent Stock Solution	6 mL× 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard (1 mg)	1 mg × 2 vials	RT, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (550-630 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)

5. Assay Notes:

Prevent the formation of bubbles when the reagents are added into the microplate.

6. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. **Preparation of chromogenic agent:** Dilute the chromogenic agent stock solution with double distilled water for 5 times. The prepared solution can be stored at 2-8°C for 7 days; avoid direct sunlight.

3. **Preparation of standard solution (1 mg/mL):** Dissolve a vial of standard (1 mg) with 1 mL of normal saline and mix fully. Prepare the fresh solution before use. It is recommended to aliquot the prepared solution and it can be store at -20°C for 3 months. Prevent repeated freezing and thawing.

7. Sample Preparation

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 Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (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.

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 (0.046-0.6 mg/mL).

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

Note: The diluent of is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 595 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-H, standard wells; S1-S80, sample wells.

9. Operation Steps:

The preparation of standard curve

Dilute 1 mg/mL standard solution with normal saline (0.9% NaCl) 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.

The measurement of samples

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

Operation Table

	Standard well	Sample well
Standard solution with different concentration (µL)	10	
Sample (µL)		10
Chromogenic agent (µL)	250	250
Mix fully with microplate reader for 10 s and stand at room temperature for 10 min. Measure the OD value at 595 nm with microplate reader.		

10. 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.

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

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.

ΔA595: $OD_{Sample} - OD_{Blank}$

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11. Performance Characteristics:

Detection Range	0.046-0.6 mg/mL
Sensitivity	0.046 mg/mL
Average recovery rate (%)	104
Average inter-assay CV (%)	8.2
Average intra-assay CV (%)	3.2

Analysis

Dilute human serum with normal saline (0.9% NaCl) for 100 times, take 10 μL of diluted sample, carry the assay according to the operation table.

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:

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

$$= 60.83 \text{ mg/mL}$$

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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