Technical Manual

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

- Catalogue Code: MAES0124
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric Method

Specification:

96T

Range:

0.58-100 g/L

Sensitivity:

0.58 g/L

Storage:

2-8°C and -20°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 tissue samples.

3. Detection Principle:

Any compound that contains two -CONH2 in the molecule can react with alkaline copper solution to form a purple complex, which is known as the biuret reaction. Many peptide bonds (-CONH-) in protein molecules can perform this reaction, and the color degree of all kinds of proteins are essentially the same.

4. Kit Components & Storage:

Item	Specification	Storage
Copper Reagent	Lyophilized × 5 vials	2-8°C, 6 months
Alkali	Lyophilized × 5 vials	2-8°C, 6 months, Avoid direct sunlight
Protein Standard (100 g/L)	1 mL × 5 vials	-20°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (520-580 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:

The time of incubation (37°C) should be accurately (10 min).

6. Reagent Preparation:

1. Preparation of **copper working solution:** Dissolve a vial of copper reagent with 10 mL of double distilled water fully. The prepared solution can be stored at 2-8°C for 3 months.

2. Preparation of **alkali working solution:** Dissolve a vial of alkali with 20 mL of double distilled water fully. The prepared solution can be stored at 2-8°C for 3 months; avoid direct sunlight.

3. Preparation of **biuret working solution:** Mix the copper working solution and alkali working solution at a ratio of 1:2. The prepared solution can be stored at 2-8°C for a day.
4. Take the protein standard (100 g/L) from -20°C and place on ice to thaw slowly (prevent repeated freezing and thawing).

7. Sample Preparation:

Sample requirements: The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge 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:
Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant), centrifuge at 700-1000 g 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 (0.58-100 g/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human serum	1
Human plasma	1
Rat serum	2-4
Mouse plasma	1
Rabbit serum	1
Chicken plasma	1
Horse serum	1-3
Porcine serum	1-3
Dog serum	2-4
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note: The diluent 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: 540 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 100 g/L protein standard with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 g/L.

The measurement of samples

1. **Standard well:** Add 7 μL of standard solution with different concentration to the well.
   Sample well: Add 7 μL of sample to the well.

2. Add 250 μL of biuret working solution to each well.

3. Mix fully with microplate reader for 5s and incubate the microplate at 37°C for 10 min accurately.

4. Measure the OD value at 540 nm with microplate reader.

Operation Table

	Standard well	Sample well
Standard solution with different concentration (μL)	7	
Sample (μL)		7
Biuret working solution (μL)	250	250
Mix fully with microplate reader for 5s and incubate the microplate at 37°C for 10 min accurately. Measure the OD value at 540 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.

The standard curve is: y= ax + b.

$$\text{TP content (g/L)} = (\Delta A_{540} - b) \div a \times f$$

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 tested.

ΔA_{540}: $OD_{Sample} - OD_{Blank}$.

Email: info@assaygenie.com **Web:** www.assaygenie.com

11. Performance Characteristics:

Detection Range	0.58-100 g/L
Sensitivity	0.58 g/L
Average recovery rate (%)	98
Average inter-assay CV (%)	6.5
Average intra-assay CV (%)	4.0

Analysis

Take 7 μL of human serum, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0052 x - 0.0057, the average OD value of the sample is 0.497, the average OD value of the blank is 0.121, and the calculation result is:

$$\text{TP content (g/L)} = (0.497 - 0.121 + 0.0057) \div 0.0052$$

$$= 73.40 \text{g/L}$$

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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