Technical Manual

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

- **Catalogue Code: MAES0125**
- **Size: 100 Assays**
- **Research Use Only**

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

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.373-80 g/L

Sensitivity:

0.373 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:

The kit can be used to measure total protein content in serum, plasma,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 × 1 vial	2-8°C, 6 months
Alkali	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Protein Standard (50 g/L)	1.6 mL × 1 vial	-20°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (540 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:

1. If the protein is higher than 80g/L, please dilute the sample with 1 x PBS (0.01 M, pH 7.4) and re-test. If the protein content is lower than 5g/L. It is suggested to detect the sample with the BCA method (MAES0177) or coomassie brilliant blue method (MAES0127).
2. 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 100 mL of double distilled water. 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 200 mL of double distilled water. 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.

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 should 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.

Homogenized Method:

Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), put the tissue pieces into a glass homogenized tube. Add homogenized medium into the homogenized tube and place the tube into an ice bath. Using a glass tamping rod, grind up and down for 6-8 min.

Alternatively, place the tissue into a mortar and add liquid nitrogen to grind fully. Then, add the homogenized medium.

Mechanical Homogenate: Weigh the tissue in an EP tube. Add the homogenized medium to homogenize the tissue with an homogenizer instrument (60 Hz, 90s) in an ice bath. (For skin, muscle and plant tissue samples, prolong homogenization time accordingly). **Ultrasonication:** Treat the cells with an ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, total time is 5 min).

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 (0.373-80 g/L).

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

Sample Type:	Dilution Factor:
Porcine serum	2-4
Human serum	2-4
5% Mouse liver tissue homogenization	1
10% Epipremnum aureum tissue homogenization	1
5% Mouse heart tissue homogenization	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

9. Operation Steps:

1. **Blank tube:** Add 50 μL of normal saline (0.9% NaCl) into a 5 mL EP tube.
   Standard tube: Add 50 μL of **protein standard (50 g/L)** into a 5 mL EP tube.
   Sample tube: Add 50 μL of **sample** into a 5 mL EP tube.

2. Add 2500 μL of **biuret working solution** into each tube, mix fully with a vortex mixer.

3. Incubate the tubes at 37°C for 10 min, then cool the tubes with running water.

4. Set the spectrophotometer to zero with double distilled water and measure the absorbance at 540 nm with 1 cm optical path quartz cuvette.

Operation Table

	Blank tube	Standard tube	Sample tube
Normal saline (0.9% NaCl) (μL)	50		
Protein standard (50 g/L) (μL)		50	
Sample (μL)			50
Biuret working solution (μL)	2500	2500	2500
Mix fully with a vortex mixer, then incubate the tubes at 37°C for 10 min. Cool the tubes with running water. Set the spectrophotometer to zero with double distilled water and measure the absorbance at 540 nm with 1 cm optical path quartz cuvette.			

10. Calculations:

$$\text{Protein content (g/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

f: Dilution factor of sample before tested

c: Concentration of standard, 50 g/L

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11. Performance Characteristics:

Detection Range	0.373-80 g/L
Sensitivity	0.373 g/L
Average recovery rate (%)	99
Average inter-assay CV (%)	2.6
Average intra-assay CV (%)	1.2

Analysis

Take 50 μL of porcine serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the standard is 0.342, the average OD value of the blank is 0.119, the average OD value of the sample is 0.565, and the calculation result is:

$$\text{Protein content (g/L)} = \frac{0.565-0.119}{0.342-0.119} \times 50 \times 1$$

$$= 100 \text{ (g/L)}$$

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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