TECHNICAL MANUAL

Biuret Protein Colorimetric Assay Kit

- **SKU CODE:** MAES0124
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Standard preparation
- Add standards and samples
- Add biuret working solution
- Incubate at 37°C
- Measure OD at 540 nm

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 groups 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 intensity of all types of proteins is essentially the same.

4. Kit components & storage

Item	Component	Size 1(96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Copper Reagent	Powder x 1 vial	Powder x 5 vials	2-8°C, 12 months
Reagent 2	Alkali	Powder x 1 vial	Powder x 5 vials	2-8°C, 12 months, protect from light
Reagent 3	100 g/L Protein Standard	1 mL x 1 vial	1 mL x 5 vials	-20°C, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (520-580 nm, optimal wavelength: 540 nm), micropipettor, incubator, vortex mixer, centrifuge.

Reagents:

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

6. Reagent preparation

1. Keep 100 g/L protein standard on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of copper working solution::** Dissolve one vial of copper reagent with 10 mL of double distilled water, mix well. Store at 2-8°C for 3 months.
3. **Preparation of alkali working solution::** Dissolve one vial of alkali with 20 mL of double distilled water, mix well. Store at 2-8°C for 3 months.
4. **Preparation of biuret working solution::** Before testing, prepare sufficient biuret working solution according to the number of test wells. For example, to prepare 300 µL of working solution, thoroughly mix 100 µL of copper working solution and 200 µL of alkali working solution. Store at 2-8°C for 1 day.
5. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 g/L protein standard with normal saline to serial concentrations. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 g/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 x g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): 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). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The incubation time (37°C) should be accurate (10 min).

2. Prevent bubble formation when adding liquid to the microplate.

9. Operating steps

1. Standard wells: Add 7 µL of standard solution with different concentrations to each well. Sample wells: Add 7 µL of sample to each well.

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

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

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

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

For sample:

TP content (g/L) = (ΔA540 - b) ÷ a × f

[Note]

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

ΔA540: $OD_{Sample} - OD_{Blank}$

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

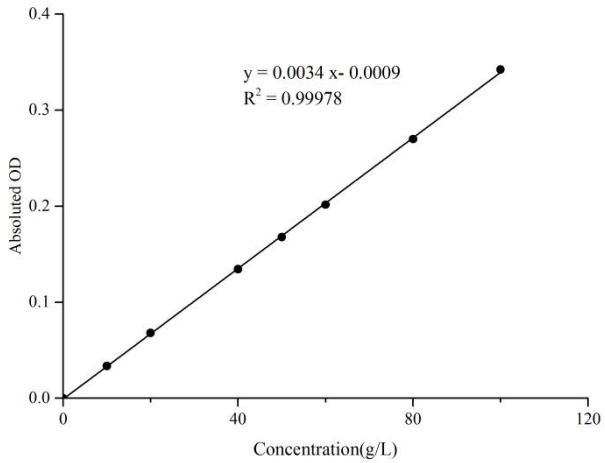
Three samples of high, middle, and low concentrations were tested in parallel 6 times for each concentration to obtain an average recovery rate of 98%.

Sensitivity

The analytical sensitivity of the assay is 0.58 g/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only.



11. Appendix II Example Analysis

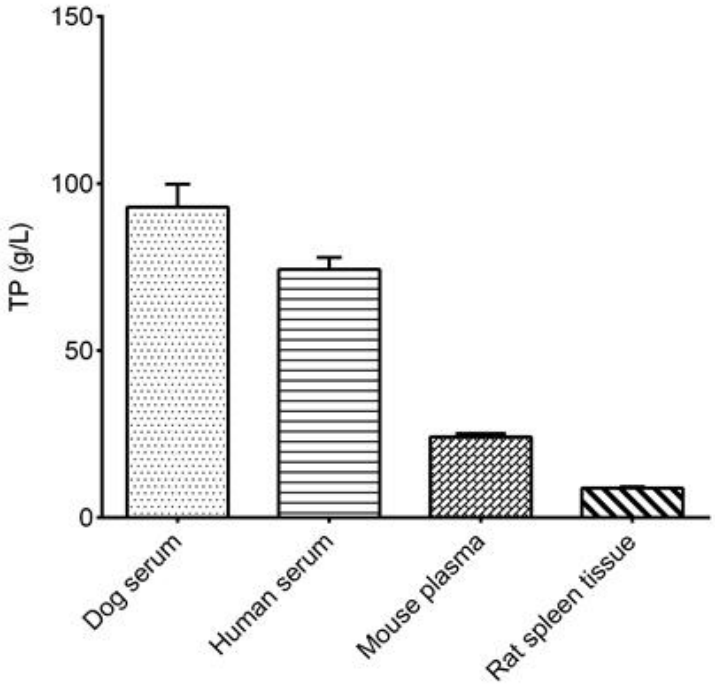
Example analysis:

Take 7 μL of human serum and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0052x - 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:

TP content (g/L) = (0.497 - 0.121 + 0.0057) ÷ 0.0052 = 73.40 g/L

Detect dog serum (diluted 3 times), human serum, mouse plasma, and 10% rat spleen tissue homogenate according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.