# TECHNICAL MANUAL

# Biuret Protein Colorimetric Assay Kit

- **SKU CODE:** MAES0401
- **SIZE:** 500 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Intended use

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

# 2. Detection range and sensitivity

Detection range: 0.373-80 g/L

Sensitivity: 0.373 g/L

# 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 kinds of proteins is essentially the same.

# 4. Kit components & storage

| Item | Component | Size 1 (100 Assays) | Size 2 (500 Assays) | Storage |
|---|---|---|---|---|
| Reagent 1 | Copper Reagent | Powder × 1 vial | Powder × 2 vials | 2-8°C, 12 months |
| Reagent 2 | Alkali | Powder × 1 vial | Powder × 2 vials | 2-8°C, 12 months, shading light |
| Reagent 3 | 50 g/L Protein Standard | 1.6 mL × 1 vial | 5 mL × 1 vial | -20°C, 12 months |

# 5. Materials prepared by users

**Instruments:** Spectrophotometer (540 nm), Micropipette, Incubator

**Reagents:** Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

# 6. Reagent preparation

For 100 Assays:

1. Keep 50 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 100 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 200 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 3 months protected from light.

4. Preparation of biuret working solution: For each well, prepare 2500 µL of biuret working solution (mix well 850 µL of copper working solution and 1700 µL of alkali working solution). Store at 2-8°C for 3 months protected from light.

For 500 Assays:

1. Keep 50 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 250 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 500 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 3 months protected from light.

4. Preparation of biuret working solution: For each well, prepare 2500 µL of biuret working solution (mix well 850 µL of copper working solution and 1700 µL of alkali working solution). Store at 2-8°C for 3 months protected from light.

# 7. Sample preparation

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

**Tissue sample:**

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×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

# 8. The key points of the assay

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

# 9. Operation procedure

1. **Tube preparation:** Blank tube: Add 50 μL of PBS into a 5 mL EP tube. Standard tube: Add 50 μL of 50 g/L protein standard 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.

# 10. Calculation

Protein content (g/L) = ΔA1/ΔA2 × c × f

**Note:**

ΔA1: $OD_{Sample}$ - $OD_{Blank}$

ΔA2: $OD_{Standard}$ - $OD_{Blank}$

c: Concentration of standard, 50 g/L

f: Dilution factor of sample before test

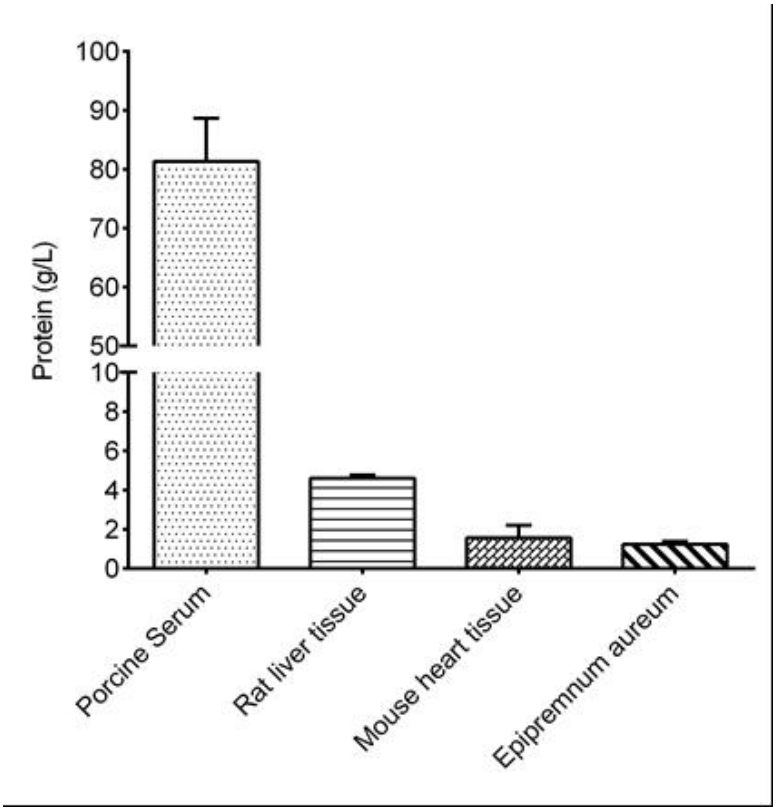
# 11. Example analysis

Take 50 μL of porcine serum, carry out the assay according to the operation procedure. 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:

Protein content (g/L) = (0.565 - 0.119)/(0.342 - 0.119) × 50 × 1 = 100 (g/L)

Detect porcine serum, 5% rat liver tissue homogenate, 5% mouse heart tissue homogenate, and 10% leaves tissue homogenate of epipremnum aureum according to the protocol. The result is as follows:




## 12. Notes

1. If the protein is higher than 80 g/L, please dilute the sample with 1×PBS (0.01 M, pH 7.4) and re-test. If the protein content is lower than 5 g/L, it is suggested to detect the sample with BCA method (MAES0177) or Coomassie brilliant blue method (MAES0402).
2. The time and temperature of incubation must be controlled strictly.
3. The reagents must be stored strictly according to the preservation conditions in the table. The reagents in different kits cannot be mixed with each other.

## 13. 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 pre-test 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.

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