Biuret Protein Colorimetric Assay Kit

Catalog No:	E-BC-K165-S	**Method:**	Colorimetric method
Instrument:	Spectrophotometer (540 nm)	**Specification:**	100Assays/500Assays

Note:

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 (E-BC-K318-M) or coomassie brilliant blue method (E-BC-K168-S).
2. The time and temperature of incubation must be controlled strictly.

General information

Intended use

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

Detection range and sensitivity

Detection range: 0.373-80 g/L

Sensitivity: 0.373 g/L

Detection principle

Any compound that contains two -$CONH_2$ 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.

Kit components & storage

Item	Component	Size 1 (100 Assays)	Size 2 (500 Assays)	Storage
Reagent 1	Copper Reagent	Powder × 1 vial	Powder × 2 vial	2-8°C, 12 months
Reagent 2	Alkali	Powder × 1 vial	Powder × 2 vial	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

Note: The reagents must be stored strictly according to the preservation conditions in the above table.

The reagents in different kits cannot be mixed with each other.

Materials prepared by users

Instruments:

Spectrophotometer (540 nm), Micropipettor, Incubator

Reagents:

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

Reagent preparation

For 100Assays:

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 500Assays:

① Keep 50 g/L protein standard on ice during use. Equilibrate other reagents to room temperature before use.

② The 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 The 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 The 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.

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 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

The key points of the assay

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

Operation table

① **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.

Calculation

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

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 50 g/L

f: Dilution factor of sample before test

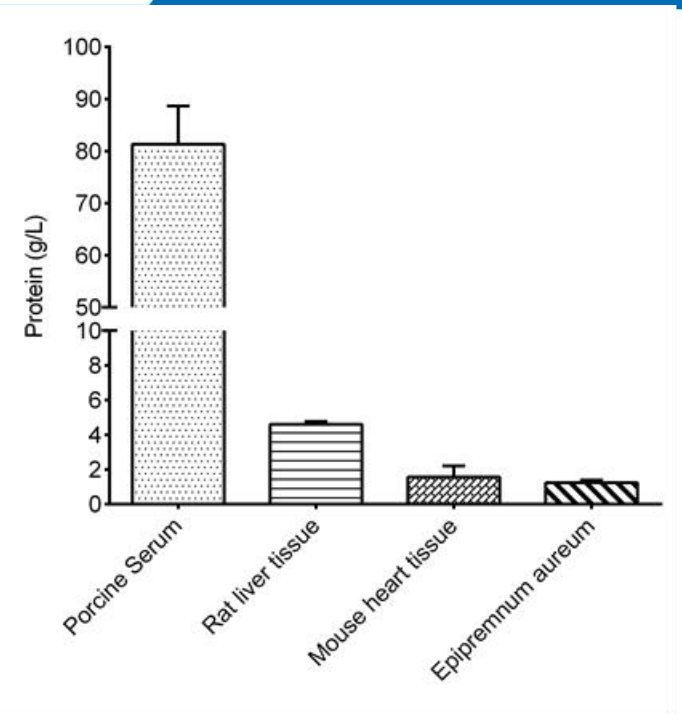
Example analysis

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:

$$\text{Protein content (g/L)} = \frac{0.565 - 0.119}{0.342 - 0.119} \times 50 \times 1 = 100\ (\text{g/L})$$

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


Protein (g/L)
100
90
80
70
60
50
10
8
6
4
2
0
Porcine Serum
Rat liver tissue
Mouse heart tissue
Epipremnum aureum