# Biuret Protein Colorimetric Assay Kit

Catalog No: E-BC-K165-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.373 g/L

Detection range: 0.373-80 g/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

# General information

## ▲Intended use

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

## ▲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 | Specification | Storage |
|---|---|---|---|
| Reagent 1 | Copper Reagent | Powder × 1 vial | 2-8℃ , 6 months |
| Reagent 2 | Alkali | Powder × 1 vial | 2-8℃ , 6 months, shading light |
| Reagent 3 | 50 g/L Protein Standard | 1.6 mL × 1 vial | -20℃ , 6 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

### Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (5 mL)

### Reagents

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

## ▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

## ▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

## ▲The key points of the assay

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.

# Pre-assay preparation

## ▲Reagent preparation

1. Preparation of reagent 1 working solution:

   Dissolve a vial of reagent 1 powder with 100 mL double distilled water. The prepared solution can be stored at 2-8℃ for 3 months.

2. Preparation of reagent 2 working solution:

   Dissolve a vial of reagent 2 powder with 200 mL double distilled water. The prepared solution can be stored at 2-8℃ for 3 months with shading light.

3. Preparation of biuret working solution:

   Mix the reagent 1 working solution and reagent 2 working solution at a ratio of 1:2. The prepared solution can be stored at 2-8℃ for 3 months with shading light.

## ▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

### Sample requirements

The sample should not contain chelating agents such as EGTA and EDTA and reducing substances such as DTT and mercaptoethanol.

## ▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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).

| Assay protocol | |
|---|---|
| Ambient temperature | 25-30℃ |
| Optimum detection wavelength | 540 nm |

## Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

# Assay protocol

## ▲Operating steps

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℃ 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 |
|---|---|---|---|
| PBS (μL) | 50 | | |
| 50 g/L Protein standard (μL) | | 50 | |
| Sample (μL) | | | 50 |
| Biuret working solution (μL) | 2500 | 2500 | 2500 |
| Mix fully with a vortex mixer, then incubate the tubes at 37℃ 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. | | | |

## Calculation

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

**Note:**

$\Delta A_1$: $OD_{Sample} - OD_{Blank}$

$\Delta A_2$: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 50 g/L

f: Dilution factor of sample before test.

## Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

# Appendix I Performance characteristics

| Appendix I Performance characteristics | | | |
|---|---|---|---|
| Detection range | 0.373-80 g/L | Average intra-assay CV (%) | 1.2 |
| Sensitivity | 0.373 g/L | Average inter-assay CV (%) | 2.6 |
| Average recovery rate (%) | 99 | | |

## ▲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:

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

# Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

## ▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

## ▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

## ▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the liquid with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃ ) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

**Note:**

1. Homogenized medium: PBS (0.01 M, pH 7.4) or normal saline (0.9% NaCl).
2. Homogenized method:

   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 $mm^3$), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

   (2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

## ▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.