Biuret Protein Colorimetric Assay Kit

Catalog No: E-BC-K165-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.58 g/L

Detection range: 0.58-100 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

This kit can be used to measure total protein (TP) content in serum, plasma and 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	100 g/L Protein Standard	1 mL × 1 vial	-20℃ , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader (520-580 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL)

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. The time of incubation (37℃) should be accurately (10 min).
2. Prevent the formulation of bubbles when adding the liquid to the microplate.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 with 10 mL of double distilled water fully. 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 with 20 mL of double distilled water fully. 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 a day.
4. Take the reagent 3 from -20℃ and place on ice to thaw slowly (avoid repeated freezing and thawing).

▲Sample preparation

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

Sample requirements

The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

▲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 (0.58-100 g/L).

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

Sample type	Dilution factor
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).

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

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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

1. The preparation of standard curve

Dilute 100 g/L protein standard with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 g/L.

2. The measurement of samples

1) Standard well: Add 7 μL of standard solution with different concentration to the well.
   Sample well: Add 7 μL of sample to the well.
2) Add 250 μL of biuret working solution to each well.
3) Mix fully with microplate reader for 5 s and incubate the microplate at 37℃ for 10 min accurately.
4) Measure the OD value at 540 nm with microplate reader.

▲Operation table

	Standard well	Sample well
Standard solution with different concentration (μL)	7	
Sample (μL)		7
Biuret working solution (μL)	250	250
Mix fully with microplate reader for 5 s and incubate the microplate at 37℃ for 10 min accurately. Measure the OD value at 540 nm with microplate reader.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

$$\text{TP content (g/L)} = (\Delta A_{540} - b) \div a \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$.($OD_{Blank}$ is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

ΔA_{540}: $OD_{Sample} - OD_{Blank}$.

▲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

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Detection range	0.58-100 g/L	Average intra-assay CV (%)	4.0
Sensitivity	0.58 g/L	Average inter-assay CV (%)	6.5
Average recovery rate (%)	98		

▲Example analysis

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

Standard curve: y = 0.0052 x - 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:

$$\text{TP content (g/L)} = \frac{0.497 - 0.121 + 0.0057}{0.0052} = 73.40\text{g/L}$$

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 (Heparin is used as 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 PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water 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: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).
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.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.