PRODUCT INFORMATION & MANUAL

Bradford Protein Assay Kit (Colorimetric)

NBP3-25896

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Bradford Protein Colorimetric Assay Kit

Catalog No: NBP3-25896

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 0.046 mg/mL

Detection range: 0.046-0.6 mg/mL

Average intra-assay CV (%): 3.2

Average inter-assay CV (%): 8.2

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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, animal tissue and cell samples.

▲Detection principle

Coomassie brilliant blue G-250 is red under the free state, and it has the maximum absorbance at 465 nm. When the Coomassie brilliant blue G-250 combined to protein, the compound will have the maximum at 595 nm.
The absorbance value is directly proportional to the protein content, so the concentration of total protein can be calculated directly by measuring the OD value at 595 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent Stock Solution	6 mL× 1 vial	2-8℃ , 12 months, shading light
Reagent 2	1 mg Standard	1 mg × 2 vials	RT, 12 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 (550 nm-630 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

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 point of the assay

Prevent the formulation of bubbles when adding the liquid to the microplate.

Pre-assay preparation

▲Reagent preparation

1. Preparation of chromogenic agent

Dilute the reagent 1 with double distilled water for 5 times. The prepared solution can be stored at 2-8℃ for 7 days with shading light.

2. Preparation of 1 mg/mL standard solution

Dissolve a vial of reagent 2 with 1 mL of normal saline and mix fully. Prepare the fresh solution before use. It is recommended to aliquot the prepared solution and it can be store at -20℃ for 3 months. Avoid repeated freezing and thawing.

▲Sample preparation

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

▲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 (0.046-0.6 mg/mL).

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

Sample type	Dilution factor
Human serum	90-110
Human plasma	90-110
Rabbit serum	90-110
Rat plasma	90-110
Chicken serum	90-110
10% Mouse kidney tissue homogenate	15-20
10% Mouse lung tissue homogenate	15-20
10% Rat spleen tissue homogenate	15-20
10% Rat heart tissue homogenate	15-20
10% Rat liver tissue homogenate	20-25
293T	1
HL-60	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

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.

▲Detailed operating steps

The preparation of standard curve

Dilute 1 mg/mL standard solution with normal saline (0.9% NaCl) to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/mL. Reference is as follows:

Number	Standard concentrations (mg/mL)	1 mg/mL Standard (μL)	Normal saline (μL)
A	0	0	100
B	0.05	5	95
C	0.1	10	90
D	0.2	20	80
E	0.3	30	70
F	0.4	40	60
G	0.5	50	50
H	0.6	60	40

The measurement of samples

1) Standard well: Add 10 μL of standard solution with different concentration to the well.
   Sample well: Add 10 μL of sample to the well.
2) Add 250 μL of chromogenic agent to each well.
3) Mix fully with microplate reader for 10 s and stand at room temperature for 10 min.
4) Measure the OD value at 595 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentration (μL)	10	
Sample (μL)		10
Chromogenic agent (μL)	250	250
Mix fully and stand at room temperature for 10 min. Measure the OD value at 595 nm.		

▲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.

$$\text{TP content (mg/mL)} = (\Delta A_{595} - b) \div a \times f$$

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_{595}: OD_{Sample} – OD_{Blank}.

Appendix I Data

▲Example analysis

Dilute human serum with normal saline (0.9% NaCl) for 100 times, take 10 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.3727 x + 0.0043, the average OD value of the sample is 0.550, the average OD value of the blank is 0.319, and the calculation result is:

$$\text{TP content (mg/mL)} = \frac{0.550 - 0.319 - 0.0043}{0.3727} \times 100 = 60.83\text{mg/mL}$$

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.

▲ Cells

Collect the 1×10^6 cells, add 300-500 μL normal saline (0.9% NaCl) and PBS (0.01 M, pH 7.4) . Homogenize the cells sample with homogenizer on ice. Centrifuge the homogenized cells at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

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.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 10 min).