# Bradford Protein Colorimetric Assay Kit

Catalog No: E-BC-K168-S

Method: Colorimetric method

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

Instrument: Spectrophotometer

Sensitivity: 0.026 mg/mL

Detection range: 0.026-1.2 mg/mL

- 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 the total protein in serum, plasma and animal tissue 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 | 35 mL × 2 vials | 2-8℃ , 6 months, shading light |
| Reagent 2 | 0.563 mg BSA Standard | 0.563 mg × 2 vials | RT, 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 (595 nm), Micropipettor, Vortex mixer

### Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 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 point of the assay

When measuring the absorbance with spectrophotometer, the glass cuvette should be used.

# Pre-assay preparation

## ▲ Reagent preparation

1. Preparation of chromogenic agent working solution:

   Dilute the reagent 1 with double distilled water at a ratio of 1:4 and mix fully. Prepare the fresh solution before use. It can be stored at 2-8℃ for 7 days with shading light.

2. The preparation of 0.563 mg/mL standard solution:

   Dissolve a vial of standard powder with 1 mL PBS (0.01 M, pH 7.4) 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 (0.026-1.2 mg/mL).

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

| Sample type | Dilution factor |
|---|---|
| Human serum | 40-60 |
| 10% Mouse liver tissue homogenization | 8-12 |
| Rat serum | 40-60 |

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 | 595 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

### The measurement of samples

1 Blank tube: add 3000 μL of chromogenic agent working solution and 50 μL of PBS (0.01 M, pH 7.4) to the 5 mL EP tube and mix fully.
Standard tube: add 3000 μL of chromogenic agent working solution and 50 μL of 0.563 mg/mL standard solution to the 5 mL EP tube and mix fully.
Sample tube: add 3000 μL of chromogenic agent working solution and 50 μL of sample to the 5 mL EP tube and mix fully.

2 Stand the tubes at room temperature for 10 min. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 595 nm with 1 cm optical path glass cuvette.

## ▲Operation table

| | Blank tube | Standard tube | Sample tube |
|---|---|---|---|
| Chromogenic agent working solution (μL) | 3000 | 3000 | 3000 |
| PBS (0.01 M, pH 7.4) (μL) | 50 | | |
| 0.563 mg/mL standard solution (μL) | | 50 | |
| Sample (μL) | | | 50 |
| Mix fully and stand the tubes at room temperature for 10 min. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 595 nm with 1 cm optical path glass cuvette. | | | |

## ▲Calculation

$$\text{Total protein concentration (mg/mL)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

**Note:**

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

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

c: Concentration of standard (0.563 mg/mL)

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 | 0.026-1.2 mg/mL | Average intra-assay CV (%) | 1.8 |
| Sensitivity | 0.026 mg/mL | Average inter-assay CV (%) | 2.3 |
| Average recovery rate (%) | 97 | | |

## ▲Example analysis

Dilute human serum with PBS (0.01 M, pH 7.4) for 50 times, then take 0.05 mL of human serum, and carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.723, the average OD value of the blank is 0.419, the average OD value of the standard is 0.581, and the calculation result is:

Total protein concentration(mg/mL) = (0.723-0.419)÷(0.581-0.419)×0.563×50
=52.825(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, 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 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: 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 5 min).

## ▲ 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.