Bradford Protein Colorimetric Assay Kit

Catalog No:	E-BC-K168-S	**Method:**	Colorimetric method
Instrument:	Spectrophotometer (595 nm)	**Specification:**	100Assays/500Assays

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

General information

Intended use

This kit can be used to measure the total protein in serum, plasma and animal tissue samples.

Detection range and sensitivity

Detection range: 0.026-1.2 mg/mL
Sensitivity: 0.026 mg/mL

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	Size 1 (100 Assays)	Size 2 (500 Assays)	Storage
Reagent 1	Chromogenic Agent Stock Solution	35 mL × 2 vials	60 mL × 6 vials	2-8℃, shading light, 12 months
Reagent 2	0.563 mg BSA Standard	0.563 mg × 2 vials	0.563 mg × 8 vials	RT, 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 (595 nm), Micropipettor, Vortex mixer

Reagents:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② Preparation of chromogenic working solution:

For each well, prepare 3000 μL of chromogenic working solution (mix well 600 μL of chromogenic agent stock solution and 2400 μL of double distilled water). Store at 2-8°C for 7 days protected from light.

③ 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 well. The 0.563 mg/mL standard solution should be prepared on spot. Aliquoted storage at -20°C for 3 months, and avoid repeated freeze/thaw cycles is advised.

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.

Operation steps

① **Blank tube:** add 3000 μL of chromogenic 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 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 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.

Calculation

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

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard (0.563 mg/mL)

f: Dilution factor of sample before test.

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:

$$\text{Total protein concentration (mg/mL)} = \frac{0.723-0.419}{0.581-0.419} \times 0.563 \times 50 = 52.825 \text{ (mg/mL)}$$

Detect human serum (dilute for 50 times), 10% mouse liver tissue homogenate (dilute for 20 times) according to the protocol, the result is as follows: