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(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Total Protein (TP) Colorimetric Assay Kit (Coomassie Brilliant Blue Method)

Catalog No: E-BC-K168-S

Method: Colorimetric method

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

Measuring instrument: Spectrophotometer

Sensitivity: 0.026 mg/mL

Detection range: 0.026-1.2 mg/mL

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

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

Application

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

	Component	Specification	Storage
Reagent 1	Chromogenic Agent Stock Solution	35 mL × 2 vials	2-8°C, 6 months, shading light
The preparation of reagent 1 application solution: dilute the reagent 1 with double distilled water at a ratio of 1:4 and mix fully. Prepare the fresh solution before use.			
Reagent 2	0.563 mg BSA Standard	0.563 mg × 2 vial	-20°C, 6 months
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°C for 3 months. Avoid repeated freezing and thawing.			

Experimental instrument

Micropipette, Vortex mixer, Centrifuge, Spectrophotometer (595 nm)

Sample preparation

1. **Serum (plasma) samples:** detect directly. If the concentration of sample is beyond the linear range, please dilute the sample with PBS (0.01 M, pH 7.4) and then carry the assay.
2. **Tissue samples:** Weigh 0.02-1 g of tissue accurately. Add 9 times the volume of PBS (0.01 M, pH 7.4) according to the proportion of Weight (g): Volume (mL) =1:9. Mechanically homogenize the sample in ice water bath. Centrifuge at 1500 g for 10 min. Take the supernatant and preserve it on ice for detection.

Operation steps

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

1. **Blank tube:** add 3000 μL of reagent 1 application solution and 50 μL of double distilled water into a 5 mL EP tube and mix fully.

Standard tube: add 3000 μL of reagent 1 application solution and 50 μL of Standard solution into a 5 mL EP tube and mix fully.

Sample tube: add 3000 μL of reagent 1 application solution and 50 μL of sample into a 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 diameter cuvette.

Note: It can be refer to the following operating table.

	Blank tube	Standard tube	Sample tube
Reagent 1 application solution (μL)	3000	3000	3000
Double distilled water (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 diameter cuvette.			

Calculation of results

Total protein concentration (mg/mL)

$$= \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times \text{the concentration of standard} \times \text{Dilution factor of sample before tested}$$

Technical parameters

1. The sensitivity of the kit is 0.026 mg/mL .
2. The intra-assay CV is 1.8% and the inter-assay CV is 2.3%.
3. The recovery of the kit is 97%.
4. The linear range of the kit is 0.026-1.2 mg/mL .

Notes

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

Appendix: Preparation of Standard Curve

(This is for reference only)

Dilution of Standard

Prepare the 2.0 mg/mL standard solution, then dilute the 2.0 mg/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 1.2, 1.0, 0.8, 0.6, 0.4, 0.2, 0 mg/mL.

Operation table

	Standard tube
Reagent 1 application solution (μL)	3000
Standard solution (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 diameter cuvette.	

Standard curve

