BCA Protein Colorimetric Assay Kit

Catalog No:	E-BC-K318-M	**Method:**	Colorimetric method
Instrument:	Microplate reader(540-590 nm)	**Specification:**	96T/ 500Assays

Note:
① The samples should not contain EGTA, EDTA and other chelating agents, and should not contain DTT, mercaptoethanol and other reducing substances.
② Protein concentration: When the sample background value is high, the bradford protein colorimetric assay kit is recommended (E-BC-K168-S); When the protein concentration is high, the biuret protein colorimetric assay kit is recommended (E-BC-K165-S).

General information

Intended use

This kit can be used to measure total protein (TP) content in serum, plasma, culture cells, tissue and cells samples.

Detection range and sensitivity

Detection range: 0.0165-1 mg/mL
Sensitivity: 0.0165 mg/mL

Detection principle

Cu^{2+} can be reduced to Cu^{+} by protein in alkaline condition. Cu^{+} can combine with BCA reagent and form purple complex, which has a maximum absorption peak at 562 nm. The absorbance value is proportional to the protein concentration. Therefore, the protein concentration can be calculated according to the OD value.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	BCA Reagent	25 mL × 1 vial	50 mL × 2 vials	RT, 12 months
Reagent 2	Copper Salt Solution	0.5 mL × 1 vial	3 mL × 1 vial	RT, 12 months
Reagent 3	Standard	1 mg × 1 vial	1 mg × 5 vials	RT, 12 months
Reagent 4	Standard Diluent	15 mL × 1 vial	30 mL × 1 vial	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 (540-590 nm), Vortex mixer, Micropipettor, Incubator

Reagents:

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

Reagent preparation

① The preparation of 1 mg/mL standard solution:

Dissolve a vial of standard with 1 mL standard diluent and mix fully. Aliquoted storage at -20°C for 3 months.

② The preparation of BCA working solution:

For each well, prepare 200 μL of BCA working solution (mix well 4 μL of copper salt solution and 196 μL of BCA reagent). Store at 2-8°C for 24 h.

③ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mg/mL standard solution with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mg/mL. Reference is as follows.

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
1 mg/mL standard solution (μL)	0	20	40	60	80	120	160	200
Standard Diluent (μL)	200	180	160	140	120	80	40	0

Operation steps

① Standard well: add 20 μL of standard solution with different concentration.

Sample well: add 20 μL of tested samples.

② Add 200 μL of BCA working solution into each tube.

③ Oscillate for 20 s to mix fully and incubate at 37°C for 30 min.

④ Measure the OD values of each well at 562 nm with microplate reader.

Note: When the reagent is added to the wells, it should be added to the bottom of the enzyme well; Add the sample slowly to avoid bubbles (Bubble influence test results).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

$$\text{Protein content (mg/mL)} = (\Delta A_{562} - b) \div a \times f$$

Note：

ΔA_{562}: Absolute OD (OD_{Sample} - OD_{Blank}).

f: Dilution factor of sample before test.