# BCA Protein Colorimetric Assay Kit

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|---|---|---|---|
| **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 | | |
| | 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 ℃ 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 ℃ 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 ℃ 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:**

$\Delta A_{562}$: Absolute OD ($OD_{Sample}$ - $OD_{Blank}$).

f: Dilution factor of sample before test.