



TECHNICAL MANUAL

BCA Protein Colorimetric Assay Kit

- **SKU CODE:** MAES0177
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. General information

Method: Colorimetric method

Instrument: Microplate reader (540-590 nm)

Specification: 96T/500 Assays

Note:

① The samples should not contain chelating agents such as EGTA or EDTA, nor reducing substances such as DTT or mercaptoethanol.

② Protein concentration: When the sample background value is high, the Bradford protein colorimetric assay kit is recommended (MAES0402); When the protein concentration is high, the biuret protein colorimetric assay kit is recommended (MAES0401).

2. Intended use

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

3. Detection range and sensitivity

Detection range: 0.0165-1 mg/mL

Sensitivity: 0.0165 mg/mL

4. Detection principle

Cu^{2+} can be reduced to Cu^{+} by protein under alkaline conditions. Cu^{+} can combine with BCA reagent to form a 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.

5. 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

Item	Component	Size 1(96 T)	Size 2 (500 Assays)	Storage
Reagent 4	Standard Diluent	15 mL × 1 vial	30 mL × 1 vial	RT, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

6. 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)

7. Reagent preparation

- 1. Preparation of 1 mg/mL standard solution:** Dissolve a vial of standard with 1 mL standard diluent and mix fully. Store aliquots at -20°C for 3 months.
- 2. 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 hours.
- 3. 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 create 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.

8. Standard dilution table

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

Item	1	2	3	4	5	6	7	8
Standard Diluent (μL)	200	180	160	140	120	80	40	0

9. Operation steps

1. Standard well: add 20 μL of standard solution with different concentrations.
Sample well: add 20 μL of tested samples.
2. Add 200 μL of BCA working solution into each well.
3. Oscillate for 20 seconds to mix fully and incubate at 37°C for 30 minutes.
4. Measure the OD values of each well at 562 nm with microplate reader.

10. 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 absolute OD value.
3. Plot the standard curve by using the absolute 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 ($OD_{\text{Sample}} - OD_{\text{Blank}}$).

f: Dilution factor of sample before test.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.