MediRes Corp.

USER GUIDE **Bi2M-BCA-200**

BCA Protein Detection Kit

Product #: Bi2M-BCA-200
Quantity: 200 reactions

Product Description

- BCA Protein Quantitation Kit is widely used for determining protein concentration.
- **Principle:** Uses bicinchoninic acid (BCA) for colorimetric detection of total protein.
- **Reaction Mechanism:**
- Proteins reduce Cu2+ to Cu+ in an alkaline medium (biuret reaction).
    - Cu+ is detected by BCA, forming a purple-colored complex.
    - The complex shows strong absorbance at 562 nm, nearly linear with protein concentrations (20–2000 µg/mL).
- **Detergent Compatibility:** Effective with high concentrations of detergents such as 5% SDS, 5% Triton X-100, and 5% Tween-20, 60, 80.
- **Interference:**
    - Chelating agents (e.g., EGTA, high concentrations of EDTA).
    - Reducing agents (e.g., DTT, β-mercaptoethanol above 1 mM).
- **Recommendations:**
    - Ensure no EGTA in samples.
    - EDTA concentration should be below 10 mM.
    - For samples with chelating or reducing agents, use the Bradford protein quantitation kit (Cat #**Bi2M-BPA-250**).

Storage

- Store the protein standard solution at -20°C and utilize it within 6 months. Store the remaining reagents at room temperature.

Components Provided

BCA reagent	40 mL
Cupric sulfate solution	1.2 mL
Protein standard (BSA)	25 mg
Protein standard diluent	1.5 mL

Protocol

Preparation of BSA standard stock solution:

1. Add 1 mL of protein standard diluent solution to the protein standard tube (25 mg BSA).
2. Dissolve 25 mg of protein standard completely to obtain a protein standard stock solution with a concentration of 25 mg/mL.
3. Aliquot and store the standard protein storage solution at -20°C for long-term storage.

Preparation of protein standard working solution:

4. Dilute the 25 mg/mL protein standard storage solution 50 times with PBS or normal saline to obtain a protein standard working solution with a final concentration of 0.5 mg/mL. Use the 10 times gradient method for dilution to ensure accuracy.
5. Prepare a set of protein standards according to the following table for the Microplate Procedure:

Dilution Scheme for Microplate Procedure (Working Range = 0–500 µg/mL)		
Volume of Diluent (µL)	**Volume of 0.5 mg/mL Protein Standard Working Solution (µL)**	**Final BSA Concentration (µg/mL)**
A	20	0
B	19	25
C	18	50
D	16	100
E	12	200
F	8	300
G	4	400
H	0	500

Preparation of test sample:

6. Dilute the test-sample appropriately to ensure its protein concentration falls within the range of the standard curve. Pipette 20 µL of each test-sample into the 96-well plates. Dilute the test-sample and the protein standard working solution with the same solution.

Preparation of BCA working solution:

7. Mix 50 parts of BCA Reagent with 1 part of copper sulfate solution (50:1, BCA Reagent: copper sulfate solution). The BCA working solution can be stored at room temperature and used within 24 hours. Add 200 µL of the BCA working solution to each well. Prepare as required to avoid waste.

Detection:

8. Add 200 µL of the BCA working solution to each well and mix the plate thoroughly on a plate shaker for 30 seconds. Cover the plate and incubate at 37°C for 30 minutes. Use standard curve No. 0 as a reference to measure the absorbance at or near 562 nm on a plate reader. (Note: Alternatively, the reaction can be conducted at room temperature for 2 hours or at 60°C for 30 minutes. For low protein concentrations, it is recommended to react at 60°C.)

Calculation:

9. Plot the standard curve with the gradient protein content (µg/mL) as the abscissa and the absorption value as the ordinate. Determine the protein concentration (µg/mL) of the test-sample in the corresponding well based on the absorbance value. Multiply by the dilution factor of the sample to obtain the actual protein concentration of the test-sample.

Important Notes (PLEASE READ CAREFULLY)

1. Protein concentration determination using the BCA method is highly sensitive to variations in temperature and duration of the color reaction. The absorbance value may fluctuate with changes in time or temperature. If precise control over

the reaction conditions is challenging, it is advisable to construct a standard curve for each assay.

2. When preparing the protein standard storage solution, ensure complete dissolution of the protein. It is recommended to perform a 10-fold gradient dilution when preparing the protein standard working solution. Avoid direct 50-fold dilution at once to minimize potential errors.
3. To ensure accurate protein quantification, it is preferable to use the same buffer solution for both sample extraction and dilution of the protein standard. This ensures uniform detection conditions. If the buffer exhibits high background levels, consider alternative approaches.
4. Wear appropriate laboratory attire, including lab coats and disposable gloves, during all experimental procedures for safety and hygiene purposes.