SUPPLEMENTAL PROTOCOL

BCA Protein Assay Protocol

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A BCA Protein Assay is used to determine the concentration of a protein before or after modification. A reference protocol is provided below.

Materials Required:

Reagents	Equipment
Pierce™ BCA Protein Assay Kit	96-Well Plate
10x PBS	Plate Reader
Protein Standards	Microcentrifuge Tubes

A. Sample Preparation

1. Prepare 5 ml of BCA working solution by adding 100 µl BCA reagent B to 5 ml reagent A to form a green solution.
2. Prepare bovine IgG standards (or other appropriate standard) and a blank in 1.5 ml tubes as follows:
    a. Add 100 µl 2 mg/ml bovine IgG standard to 300 µl PBS (0.5 mg/ml standard)
    b. Add 200 µl 0.5 mg/ml standard to 200 µl PBS (0.25 mg/ml standard)
    c. Add 200 µl 0.25 mg/ml standard to 200 µl PBS (0.125 mg/ml standard)
    d. Add 200 µl 0.125 mg/ml standard to 200 µl PBS (0.0625 mg/ml standard)
    e. 100 µl PBS (buffer blank)
3. Dilute the protein sample to approximately 0.25 mg/ml with PBS to fall within the standard curve. Note the dilution factor used.

B. Well Loading

1. In a flat-bottom 96-well plate, prepare standards by pipetting 20 µl of each standard (and the blank) into separate wells.
2. Add 20 µl of protein sample to 3 separate wells.
3. Add 150 µl of BCA working solution to each well using a multi-channel pipette.
4. Seal the plate with adhesive tape and shake for 15 seconds using a plate reader to mix.

C. Plate Reading

1. Incubate the plate in a water bath at 37°C for 15 minutes.
2. Measure absorbance at 562 nm using pre-programmed BCA assay plate reader software.

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