BCA Protein Assay Protocol

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

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 pipet.
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.

Materials Required

Reagents	Equipment
Pierce™ BCA Protein Assay Kit (from ThermoFisher Scientific)	96-Well Plate
10x PBS	Plate Reader
Protein Standards	Microcentrifuge Tubes