SUPPLEMENTAL PROTOCOL

Bradford Assay Protocol

Together we breakthrough™

A Bradford Assay is used to determine the concentration of a protein before or after modification. The Protein Assay Kit I (from BioRad) is recommended. A reference protocol is provided below.

A. Sample Preparation

1. Prepare 2 ml of Bradford working solution by adding 400 µl Bradford dye reagent to 1,600 µl ultrapure water (1:4 ratios).
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 11 µl of each standard (and the blank) into separate wells.
2. Add 10 µl of protein sample to 3 separate wells.
3. Add 100 µl of Bradford 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 at room temperature for 5–60 min.
2. Measure absorbance at 595 nm using pre-programmed BCA assay plate reader software.

Materials Required

Reagents	Equipment
Bradford Reagent	96-Well Plate
10X PBS	Plate Reader
Protein Standards	Microcentrifuge Tubes

6737 Mowry Avenue, Newark CA 94560
vectorlabs.com