MediRes Corp.

USER GUIDE **Bi2M-BPA-250**

Bradford Protein Assay Kit

Product #: Bi2M-BPA-250
Quantity: 250 mL

Product Description

- The Bradford assay is widely used for determining protein concentration.
  - Based on the binding of Coomassie Brilliant Blue G250 to proteins.
  - Free dye has maximum absorption at 488 nm.
  - When bound to protein, maximum absorption shifts to 595 nm.
  - Absorbance at 595 nm is proportional to protein concentration.
  - Effective for most samples, tolerating up to 1 mM β-mercaptoethanol and 5 mM dithiothreitol.
  - Sensitive to descaling agents: ensure SDS < 0.01%, Triton X-100 < 0.05%, and Tween-20/60/80 < 0.015%.
  - Use BCA Protein Quantitative Detection Kit (G2026) for samples with descaling agents.
- **Advantages of Bradford Method:**
  - Quick and easy to perform.
  - High sensitivity.
  - Fast detection, suitable for protein concentrations of 25-1000 µg/mL.

Storage

- Store BSA standard ampules at 4°C.
- Store protein standard solution (prepared with BSA standard ampules and standard ampules dilution) at -20°C.

Components Provided

Coomassie G-250 dye	250 mL
Bovine Serum Albumin (BSA) Standard Ampules	25 mg
Standard Ampules Dilution	1.5 mL

Important Notes (PLEASE READ CAREFULLY)

1. **Coomassie G-250 Dye**:
   - Bring to room temperature before use.
   - Mix by inverting to maintain assay sensitivity.
2. **Protein Standard Preparation**:
   - Ensure the protein standard stock solution is fully dissolved.
   - Dilute the working solution in 10-fold gradients.
   - Avoid 50-fold dilutions to minimize errors.
3. **Safety Precautions**:
   - Wear safety glasses.
   - Use gloves.
   - Wear protective clothing.

Protocol

1. **Preparation of Protein Standard Stock Solution:**
   1. Dissolve 25 mg Bovine Serum Albumin (BSA) in 1 mL Standard Ampules Dilution.
   2. Final concentration: 25 mg/mL.
   3. Store at -20°C.

2. **Preparation of Protein Standard Working Solution:**
   1. Dilute the 25 mg/mL Protein Standard Stock Solution 50 times with PBS or normal saline.
   2. Final concentration: 0.5 mg/mL.
   3. Use a 10-fold gradient dilution method for accuracy.

3. **Preparation of Standard Curve (Microplate Reader Method):**
   1. Add protein standard working solution to 96-well plate: 0, 1, 2, 4, 8, 12, 16, 20 µL.
   2. Add PBS or normal saline: 20, 19, 18, 16, 12, 8, 4, 0 µL to each well.

3. Final concentrations: 0, 25, 50, 100, 200, 300, 400, 500 µg/mL.

4. Preparation of Samples:

1. Dilute samples appropriately to fall within the standard curve range.
2. Add 20 µL of each sample to a 96-well plate.
3. Use the same diluent as the protein standard.

5. Microplate Procedure:

1. Add 200 µL of Coomassie G-250 dye to each well.
2. Shake plate for 30 seconds.
3. Incubate at room temperature for 3-5 minutes.
4. Measure absorbance at 595 nm using a microplate reader.
5. Plot the standard curve and calculate sample protein concentration from the absorbance values.

6. Spectrophotometer Procedure:

1. Adjust the gradient standard reaction volume to 1 mL.
2. Add 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mL of 0.5 mg/mL protein standard working solution to glass test tubes.
3. Add 1.0, 0.95, 0.9, 0.8, 0.6, 0.4, 0.2, 0 mL of PBS or saline to make up to 1 mL.
4. Mix thoroughly to obtain gradient concentrations of 0, 25, 50, 100, 200, 300, 400, 500 µg/mL.

7. Sample Preparation for Spectrophotometer:

1. Dilute protein samples as needed.
2. Add 1 mL of diluted sample to glass test tubes.

8. Colorimetric Detection (Spectrophotometer):

1. Add 3 mL of Coomassie G-250 dye to each standard curve and sample tube.

2. Mix thoroughly and let stand for 3-5 minutes.
3. Measure absorbance at 595 nm.
4. Use standard curve to determine protein concentration in samples.

9. Calculation:

1. Plot gradient protein content (µg/mL) against absorbance.
2. Determine sample protein concentration from the standard curve.
3. Multiply by the dilution factor for actual protein concentration.