Bradford Protein Assay Kit

Cat. No. KITG-073 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Bradford method for determining protein content is a dye-binding assay. Coomassie Brilliant Blue G-250 reaches equilibrium with proteins within 2-5 min, and the resulting complex remains stable for 1 h, enabling rapid, stable, and highly sensitive detection of protein content. Free Coomassie Brilliant Blue G-250 is brownish red and turns blue after binding to protein. The dye's maximum absorption peak shifts from 465 nm to 595 nm. Within a certain concentration range, the absorbance of the protein-dye complex is proportional to protein content; therefore, protein content can be quantified by measuring the change in absorbance at 595 nm.
Description	Pack Size: 500 tests (2,500 tests for the microplate method). For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit
Applications	Rapid, stable, and highly sensitive quantitative detection of protein content by measuring absorbance at 595 nm.
Notes	Instructions for Use 1. Spectrophotometer Method 1) Sample Dilution: Dilute the sample by an appropriate factor. It is recommended to test several dilution gradients, for example, 2-, 4-, and 8-fold dilutions. 2) Preparation of 1× G-250 Dye Solution: Invert the 5× G-250 Dye Solution 3-5 times to mix. Add 10 mL of 5× G-250 Dye Solution to 40 mL of double-distilled water and mix thoroughly. The prepared 1× G-250 Dye Solution may be stored at 4°C for one week. 3) Preparation of the Protein Standard Dilution: Add 100 µL of 5 mg/mL BSA Standard Solution to 2,400 µL of PBS

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Diluent to obtain a 0.2 mg/mL protein standard dilution. The diluent should be the same as the solution used for the protein sample; physiological saline may also be used as a reference diluent. Prepare the gradient standards as follows: Standard 1: 0 µL protein standard dilution + 500 µL PBS Diluent; final protein concentration 0 mg/mL. Standard 2: 100 µL protein standard dilution + 400 µL PBS Diluent; final protein concentration 0.04 mg/mL. Standard 3: 200 µL protein standard dilution + 300 µL PBS Diluent; final protein concentration 0.08 mg/mL. Standard 4: 300 µL protein standard dilution + 200 µL PBS Diluent; final protein concentration 0.12 mg/mL. Standard 5: 400 µL protein standard dilution + 100 µL PBS Diluent; final protein concentration 0.16 mg/mL. Standard 6: 500 µL protein standard dilution + 0 µL PBS Diluent; final protein concentration 0.20 mg/mL. 4) Reaction Setup in Centrifuge Tubes: Test tube: 100 µL diluted sample + 1,000 µL 1× G-250 Dye Solution. Standard tube: 100 µL protein standard dilution + 1,000 µL 1× G-250 Dye Solution. Mix thoroughly and allow to stand at room temperature for 3-5 min. Absorbance Measurement: Measure the absorbance of the test tube and standard tube at 595 nm and record the values as A_test and A_standard, respectively. Establishment and Use of the Standard Curve: Use 0, 0.04, 0.08, 0.12, 0.16, and 0.20 mg/mL as the x-axis values and the corresponding A_standard values as the y-axis values. Obtain the linear regression equation y = kx + b, substitute A_test into the equation, and calculate x (mg/mL). 5) Detection Timing: Measure the OD value after a 3 min reaction. To improve experimental accuracy, dye solution may be added to one tube every 2 min, and the OD value of one tube may be measured every 2 min. Use the following schedule: Tube 1: add dye solution at 0 min; measure OD at 3 min. Tube 2: add dye solution at 2 min; measure OD at 5 min. Tube 3: add dye solution at 4 min; measure OD at 7 min. Tube 4: add dye solution at 6 min; measure OD at 9 min. Tube 5: add dye solution at 8 min; measure OD at 11 min. Tube 6: add dye solution at 10 min; measure OD at 13 min. Tube 7: add dye solution at 12 min; measure OD at 15 min. Tube 8: add dye solution at 14 min; measure OD at 17 min. Continue at the same 2 min intervals as needed. 2. Microplate Reader Method 1) Sample Dilution:

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Dilute the sample by an appropriate factor. It is recommended to test several dilution gradients, for example, 2-, 4-, and 8-fold dilutions. 2) Preparation of 1× G-250 Dye Solution: Invert the 5× G-250 Dye Solution 3-5 times to mix. Add 1 mL of 5× G-250 Dye Solution to 4 mL of double-distilled water and mix thoroughly. The prepared 1× G-250 Dye Solution may be stored at 4°C for one week. 3) Preparation of the Protein Standard Dilution: Add 10 µL of 5 mg/mL BSA Standard Solution to 240 µL of PBS Diluent to obtain a 0.2 mg/mL protein standard dilution. The diluent should be the same as the solution used for the protein sample; physiological saline may also be used as a reference diluent. Prepare the gradient standards as follows: Standard 1: 0 µL protein standard dilution + 20 µL PBS Diluent; final protein concentration 0 mg/mL. Standard 2: 2 µL protein standard dilution + 18 µL PBS Diluent; final protein concentration 0.02 mg/mL. Standard 3: 4 µL protein standard dilution + 16 µL PBS Diluent; final protein concentration 0.04 mg/mL. Standard 4: 6 µL protein standard dilution + 14 µL PBS Diluent; final protein concentration 0.06 mg/mL. Standard 5: 8 µL protein standard dilution + 12 µL PBS Diluent; final protein concentration 0.08 mg/mL. Standard 6: 12 µL protein standard dilution + 8 µL PBS Diluent; final protein concentration 0.12 mg/mL. Standard 7: 16 µL protein standard dilution + 4 µL PBS Diluent; final protein concentration 0.16 mg/mL. Standard 8: 20 µL protein standard dilution + 0 µL PBS Diluent; final protein concentration 0.20 mg/mL. 4) Reaction Setup in a 96-Well Plate: Test well: 20 µL diluted sample + 200 µL 1× G-250 Dye Solution. Standard well: 20 µL protein standard dilution + 200 µL 1× G-250 Dye Solution. Mix thoroughly and allow to stand at room temperature for 3-5 min. Absorbance Measurement: Measure the absorbance of the test well and standard well at 595 nm and record the values as A_test and A_standard, respectively. Establishment and Use of the Standard Curve: Use 0, 0.02, 0.04, 0.06, 0.08, 0.12, 0.16, and 0.20 mg/mL as the x-axis values and the corresponding A_standard values as the y-axis values. Obtain the linear regression equation y = kx + b, substitute A_test into the equation, and calculate x (mg/mL). Note: Errors caused by pipetting small liquid volumes may affect the low-concentration points of the standard curve.

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	Sample points should fall above the midpoint of the standard curve. Precautions 1) Bradford protein concentration measurement is not affected by most chemicals in samples. The concentration of β-mercaptoethanol in the sample may be as high as 1 M, and the concentration of dithiothreitol may be as high as 5 mM. However, the assay is affected by slightly higher detergent concentrations. Ensure that the SDS concentration in the sample is below 0.1%, Triton X-100 is below 0.1%, and Tween 20, 60, or 80 is below 0.06%. 2) To ensure accurate results and avoid reagent loss, carefully read the instructions before the assay, confirm that reagent storage and preparation are adequate, and ensure that the operating steps are clear. Pretest 2-3 samples expected to show substantial differences.
Storage	Kit Components and Storage: 5× G-250 Dye Solution: liquid, 100 mL × 1 bottle; store at 4°C. PBS Diluent: liquid, 30 mL × 1 bottle; store at 4°C. BSA Standard Solution (5 mg/mL BSA): liquid, 1 mL × 1 tube; store at -20°C. The kit storage temperature stated in the instructions is -20°C.
Reconstitution	Preparation for the Spectrophotometer Method: 1× G-250 Dye Solution: Invert the 5× G-250 Dye Solution 3-5 times to mix. Add 10 mL of 5× G-250 Dye Solution to 40 mL of double-distilled water and mix thoroughly. The prepared 1× G-250 Dye Solution may be stored at 4°C for one week. 0.2 mg/mL Protein Standard Dilution: Add 100 µL of 5 mg/mL BSA Standard Solution to 2,400 µL of PBS Diluent. The diluent should be the same as the solution used for the protein sample; physiological saline may also be used as a reference diluent. Preparation for the Microplate Reader Method: 1× G-250 Dye Solution: Invert the 5× G-250 Dye Solution 3-5 times to mix. Add 1 mL of 5× G-250 Dye Solution to 4 mL of double-distilled water and mix thoroughly. The prepared 1× G-250 Dye Solution may be stored at 4°C for one week. 0.2 mg/mL Protein Standard Dilution: Add 10 µL of 5 mg/mL BSA Standard Solution to 240 µL of PBS Diluent. The diluent should be the same as the solution used for the protein sample; physiological saline may also be used as a reference diluent.

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Reaction conditions	Incubate at room temperature for 3-5 min and measure absorbance at 595 nm.

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