Protein Content Assay Kit (Bradford Method)

Cat. No. KITG-152 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Method: Microplate method | Size: 96 samples. In acidic solution, Coomassie Brilliant Blue G-250 binds to proteins to form a blue complex. Spectroscopic scanning shows that this blue complex has a maximum absorption peak at 600 nm. Within a certain protein concentration range (1–1000 g/mL), the color intensity is directly proportional to the protein content.
Description	Description In acidic solution, Coomassie Brilliant Blue G-250 binds to proteins to form a blue complex. Spectroscopic scanning shows that this blue complex has a maximum absorption peak at 600 nm. Within a certain protein concentration range (1–1000 g/mL), the color intensity is directly proportional to the protein content. Scientific Background In acidic solution, Coomassie Brilliant Blue G-250 binds to proteins to form a blue complex. Spectroscopic scanning shows that this blue complex has a maximum absorption peak at 600 nm. Within a certain protein concentration range (1–1000 g/mL), the color intensity is directly proportional to the protein content. Kit Components Reagent 1: Liquid, 20 mL × 1 bottle; store at 4 °C. Standard: Liquid, 1 mL × 1 tube; store at 4 °C. Note: if the standard curve needs to be re-made, this reagent is required. Materials Required but Not Supplied Microplate reader, 96-well plate, centrifuge, adjustable pipette, mortar and pestle, and distilled water.
Notes	It is recommended to select 2 samples for a pre-determination before the formal experiment, to understand the conditions of this batch of samples and become familiar with the experimental procedure, so as to avoid wasting experimental samples and reagents. Note: This operating procedure is suitable for most routine

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	sample tests; experimental conditions can be appropriately adjusted according to the actual state of the samples. For special types of samples, our technical support can provide dedicated optimization suggestions. Size: 96 samples
Storage	Store Reagent 1 and the Standard at 4 °C.
Reaction conditions	Microplate method; absorbance at 600 nm.
Usage	Sample Preparation Sample preparation: 1. Tissue samples: Weigh approximately 0.1 g of tissue, add 1 mL of extraction solution (the extraction solution can be enzyme extraction buffer, distilled water, or normal saline), homogenize on ice, centrifuge at 12,000 rpm at 4 °C for 10 min, and collect the supernatant as the sample to be tested. Note: Based on experience, the crude extract generally needs to be diluted to an appropriate multiple (e.g., 10-fold) before measurement. Before the experiment, two samples can be tested first to determine the appropriate dilution factor for this experiment. 2. Bacterial or cell samples: First collect the bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant; take 5 million bacteria or cells and add 1 mL of extraction solution; sonicate the bacteria or cells (on ice, power 20% or 200 W, sonicate for 3 s, with an interval of 10 s, repeated 30 times), centrifuge at 12,000 rpm at 4 °C for 10 min, collect the supernatant, and keep it on ice for testing. Note: Based on experience, the crude extract generally needs to be diluted to an appropriate multiple (e.g., 10-fold) before measurement. Before the experiment, two samples can be tested first to determine the appropriate dilution factor for this experiment. 3. Liquid samples: Clear and colorless liquid samples can be measured directly. If turbid, centrifuge and use the supernatant for testing. Note: Based on experience, the sample generally needs to be diluted to an appropriate multiple (e.g., 10-fold) before measurement. Before the experiment, two samples can be tested first to determine the appropriate dilution factor for this experiment. Instrument Detection Instrument detection: 1. Preheat the microplate reader for more than 30 min and set the wavelength to 600 nm. 2. Add the following sequentially to

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the wells of a 96-well plate: Test well: 40 µ of the sample to be tested + 200 µ of Reagent 1. Blank well (perform only once): 40 µ of distilled water + 200 µ of Reagent 1. Mix well, let stand at room temperature (25 °C) for 10 min, measure the absorbance A at 600 nm (complete the colorimetry within 5–15 min), ΔA = A(test well) − A(blank well). Assay Time Let stand at room temperature (25 °C) for 10 min; complete the colorimetric reaction within 5–15 min. Analysis Result calculation: 1. Standard curve: y = 3.8457x + 0.0136, where x is the standard concentration (mg/mL) and y is ΔA. The standard curve is obtained by measuring the standards; for the specific preparation method, refer to the instructions provided with the product or contact technical support. 2. Protein content (mg/g) = (ΔA − 0.0136) × D × V / (3.8457 × W × V1) where V = extraction solution volume: 1 mL; W = sample mass (g); V1 = volume of crude extract added: 0.04 mL; D = dilution factor (if undiluted, D = 1). Sensitivity 1–1000 µg/mL

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