Bradford Protein Assay Kit

Catalog No.: abx090644

Size: 100 assays

Detection Range: 0.026 mg/ml – 1.2 mg/ml

Sensitivity: 0.026 mg/ml

Storage: Store the Chromogenic Reagent at 4°C in the dark. Store the Standard at room temperature. All components can be stored for up to 12 months.

Application: For detection and quantification of total protein concentration in serum, plasma, and animal tissues.

Introduction

Abbexa's Bradford Protein Assay Kit is a quick, convenient, and sensitive method for measuring and calculating total protein concentration. This kit contains Brilliant Blue G-250 dye, which binds non-covalently with the carboxylic acid groups on peptides to form a green-blue complex with an absorbance maximum at 595 nm. The intensity of the color is proportional to the total protein concentration, which can then be calculated.

Kit components

1. Chromogenic Reagent: 2 × 35 ml
2. Standard (0.563 mg): 2 vials

Materials required but not provided

1. Spectrophotometer (595 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Normal saline (0.9% NaCl)
5. Pipette and pipette tips
6. Microcentrifuge tubes
7. Cuvettes
8. Centrifuge
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh up to 1 g of tissue, and wash with PBS (0.01 M, pH 7.4) at 4°C. Dry with absorbent filter paper, and then add the tissue into PBS on ice in a ratio of 1:9 mass to volume (i.e. for each g of tissue, add 9 ml of PBS). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Centrifuge the homogenate at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant and assay immediately, or aliquot and store at -80°C for up to 1 month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or 1X PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	40 – 60
Rat serum	40 – 60
10% Mouse liver tissue homogenate	8 – 12

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic Reagent Working Solution**: Dilute the Chromogenic Reagent with double-distilled water in a ratio of 1:4 (i.e. for 35 ml of Chromogenic Reagent, add 140 ml distilled water), and mix fully. Any unused Chromogenic Reagent Working Solution can be stored for up to 7 days at 4°C in the dark.
- **Standard Solution:** Reconstitute the lyophilized Standard with 1 ml PBS (0.01 M, pH 7.4) and mix fully by gently pipetting the solution up and down to create the 0.563 mg/ml Standard Solution. Any unused Standard Solution can be stored for up to 3 months at -20°C. Avoid repeated freeze-thaw cycles.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Prepare the reagents just before use.

B. Assay Procedure

1. Mark microcentrifuge tubes for the standard, blank, and each sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 3000 µl of Chromogenic Reagent Working Solution to all tubes.
3. Add 50 µl of PBS (0.01 M, pH 7.4) to the blank tube, and mix fully.
4. Add 50 µl of Standard Solution to the standard tube, and mix fully.
5. Add 50 µl of sample to each corresponding sample tube, and mix fully.
6. Stand all tubes for 10 minutes at room temperature.
7. Zero the spectrophotometer using double-distilled water, and measure the OD of the contents of each tube in glass cuvettes.

C. Calculation of Results

The concentration of total protein in each tube can be derived with the following formula:

1. All samples

$$\text{Total Protein (mg/ml)} = F \times C_{Standard} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

OD_{Sample}	OD value of sample tube
$OD_{Standard}$	OD value of standard tube
OD_{Blank}	OD value of blank tube
$C_{Standard}$	Concentration of protein in the standard (= 0.563 mg/ml)
F	The dilution factor of sample