Bradford Protein Assay Kit

Catalog No.: abx097194

Size: 96 tests (80 sample)

Detection Range: 0.046 mg/ml - 0.6 mg/ml

Sensitivity: 0.046 mg/ml

Storage: Store the Chromogenic Reagent in the dark at 2-8°C and all other components at room temperature for up to 6 months.

Application: For detection and quantification of protein concentration in serum, plasma and tissue homogenates.

Introduction

Abbexa's Bradford Protein Assay Kit is a quick, convenient, and sensitive method for measuring and calculating total protein concentration activity. Free Coomassie brilliant blue G-250 has a maximum absorbance at 465 nm. When the protein reacts with Coomassie brilliant blue, the resulting reaction product has a maximum absorbance at 595 nm. The absorbance value is directly proportional to the protein concentration which can then be calculated.

Kit components

1. 96-well microplate
2. Chromogenic Reagent: 6 ml
3. Standard (1 mg): 2 vials
4. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (595 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or Sonicating water bath
7. Centrifuge
8. 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:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

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 PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor (for reference only):

Sample Type	Dilution Factor
Human serum	90-110
Human plasma	90-110
Rabbit serum	90-110
Rat plasma	90-110
Chicken serum	90-110
10% Mouse kidney tissue homogenate	15-20
10% Mouse lung tissue homogenate	15-20
10% Rat spleen tissue homogenate	15-20
10% Rat heart tissue homogenate	15-20
10% Rat liver tissue homogenate	20-25

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 5-fold with double-distilled water. Prepare immediately before carrying out the assay. Unused Chromogenic reagent working solution can be stored in dark at 2-8°C for up to 7 days.
- **1 mg/ml Standard Solution:** Dissolve a vial of standard with 1 ml of normal saline and mix fully. Prepare immediately before carrying out the assay and mix fully. Unused aliquoted standard solution can be stored at -20°C for 3 months.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/ml. Dilute the 1 mg/ml standard solution with normal saline to concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/ml. The normal saline itself serves as the 0 mg/ml (blank) standard.

2. Chromogenic Reaction:

2.1. Set the Standard and sample wells.
2.2. Add 10 µl of prepared standards to the Standard wells.
2.3. Add 10 µl of sample to the Sample wells.
2.4. Add 250 µl of Chromogenic Reagent working solution to each well.
2.5. Mix fully using a microplate shaker then allow to stand at room temperature for 10 minutes.
2.6. Measure the OD values at 595 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{595} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The Protein concentration of the samples can be interpolated from the standard curve.

Total protein concentration in samples:

$$\mathrm{TP\ (mg/ml)} = \frac{\Delta A_{595} - b}{a} \times f$$

where:

TP	concentration of Total protein
ΔA_{595}	OD value of the sample ($OD_{Sample} - OD_{Blank}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay