Bicinchoninic Acid Protein Assay Kit

Catalog No.: abx097193

Size: 96 tests (80 sample)

Detection range: 0.0165 mg/ml -1 mg/ml

Sensitivity: 0.0165 mg/ml

Storage: Store all components at room temperature for up to 12 months.

Application: For detection and quantification of Total Protein in serum, plasma, tissue, cell lysates, cell culture supernatants and other biological fluids.

Introduction

Abbexa's Bicinchoninic Acid Protein Assay Kit is a quick, convenient, and sensitive method for measuring and calculating total protein concentration. The reduction of copper by protein in alkaline condition and its further reaction with BCA reagent leads to the formation of a purple-colored complex, the resulting reaction product has an absorbance maximum at 562 nm. The absorbance value is directly proportional to the protein content which can then be calculated.

Kit components

1. 96-well microplate
2. BCA reagent: 25 ml
3. Copper salt solution: 0.5 ml
4. Protein BSA standard: 1 vial
5. Standard diluent: 15 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (562 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vials/tubes
7. Incubator or Sonicating water bath
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:** 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 (PBS or normal saline). 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.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10,000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately, or aliquot and store at -80°C for up to 1 month. 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, chelating agents such as EGTA and EDTA 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 different samples is as follows (for reference only):

Sample Type	Dilution factor
Human serum	100-200
Rat serum	100-200
10% Mouse brain tissue homogenate	8-12
10% Mouse kidney tissue homogenate	8-12
10% Rat liver tissue homogenate	15-20
10% Mouse heart 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 at room temperature or on ice for cell lysates and tissue homogenates.

2. Reagents

- **BCA working solution:** Mix the Copper salt solution 1:50 with BCA reagent. Prepare immediately before carrying out the assay and mix fully. Unused working solution can be stored at 4°C for up to 24 hrs.
- **1 mg/ml standard solution:** Dissolve a vial of Protein BSA standard vial with 1 ml of standard diluent. 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, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.9, and 1 mg/ml. Dilute the 1 mg/ml standard solution with normal saline to concentrations of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.9, and 1 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 20 µl of prepared standards to the Standard wells.
2.3. Add 20 µl of samples to the Sample wells.
2.4. Add 200 µl of BCA working solution to each well.
2.5. Mix fully using a microplate shaker and then incubate at 37°C for 30 min.
2.6. Measure the OD values at 562 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{562} 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.

$$\mathrm{TP\ (mg/ml)} = \frac{\Delta A_{562} - b}{a} \times f$$

where:

TP	Total protein concentration
ΔA_{562}	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