Total Protein Extraction Kit

Catalog No.: abx294059

Size: 100 reactions

Storage: Store the Lysis Buffer at 4°C for up to 12 months. Store all other components at -20°C for up to 12 months in the dark.

Application: For the extraction of Total Protein in tissue homogenates and cell lysates.

Introduction

Abbexa's Total Protein Extraction Kit is a quick and convenient method for extracting Total Protein. The cell and tissue samples are treated with protease inhibitors and phosphatase inhibitors to prevent enzymes in the sample from hydrolyzing or dephosphorylating the protein content due to the disruption of the membrane system.

Kit components

1. Lysis Buffer: 2 x 60 ml
2. Phosphatase Inhibitor: 1.2 ml
3. Protease Inhibitor: 1.2 ml
4. Phenylmethylsulfonyl Fluoride: 1.2 ml

Materials required but not provided

1. 5 ml Glass Homogenizer
2. High-speed freezing centrifuge
3. Double distilled water
4. PBS (0.01 M, pH 7.4)

Protocol

A. Preparation of reagents

Reagents

- Place all reagents in ice water for pre-cooling for at least 15 minutes until fully thawed before use.
- **Lysis Working Solution:** Mix Lysis Buffer, Phosphatase Inhibitor and Protease Inhibitor with a ratio of 1000:10:10 and keep on ice in the dark. Once prepared, the solution should be used within 20 minutes.

B. Assay Procedure

Total Protein Extraction of Tissue

1. Weigh 0.1 g of tissue and wash with PBS (0.01 M, pH 7.4) at 4°C to remove blood from the sample. Blot dry with absorbent paper.
2. Cut the tissue into pieces and transfer into a pre-cooled 5 ml glass homogenizer.
3. Add 1 ml of pre-cooled Lysis Working Solution and 10 µl of pre-cooled Phenylmethylsulfonyl Fluoride.
4. On ice, homogenize the tissue sample with approximately 30 up/down cycles.
5. Transfer tissue homogenate to a 2 ml pre-cooled centrifuge tube.
6. Stand the tube in an ice bath for 15 minutes.
7. Centrifuge at 12,000 × g for 15 minutes at 4°C, then collect the supernatant and store on ice for detection.
8. The prepared total protein extract can be stored at -70°C for long-term storage, avoid repeated freeze-thaw cycles.

Total Protein Extraction of Cells

a) Cell collection

Suspension cells:

1. Transfer cell suspension to pre-cooled centrifuge tubes.
2. Centrifuge at 1000 × g at 4°C for 10 minutes to remove the supernatant.
3. Wash with PBS (0.01 M, pH 7.4) at 4°C.
4. Centrifuge at 1000 × g for 10 minutes at 4°C and discard the supernatant, leaving the pellet for extraction.

Adherent cells:

1. Discard the cell culture solution and wash the cells twice with PBS (0.01 M, PH 7.4) at 4°C.
2. Scrape down the cells with a cell scraper, or treat with EDTA.
3. Detach the cells using the air pressure from a pipette.
4. Transfer the cell suspension to a pre-cooled centrifuge tube.
5. Centrifuge at 1000 × g for 10 minutes at 4°C to remove the supernatant.
6. Wash with PBS (0.01 M, pH 7.4) at 4°C.
7. Centrifuge at 1000 × g for 10 minutes at 4°C and discard the supernatant, leaving the pellet for extraction.

b) Cell extraction

1. Collect 5×10^6 cells.

2. Add 0.5 ml of pre-cooled Lysis Working Solution and 10 µl of pre-cooled Phenylmethylsulfonyl Fluoride to the cells.

3. Stand on ice for 15 minutes. Vortex mix for 10 seconds every 5 minutes.

4. Centrifuge at 12,000 × g for 15 minutes at 4°C, then collect the supernatant and store on ice for detection.

5. The prepared total protein extract can be stored at -70°C for long-term storage, avoid repeated freeze-thaw cycles.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.