Mammalian Total Protein Extraction Kit

Catalog No.: abx098853

Size: 100 ml

Storage: Store at -20°C for up to one year.

Introduction: Abbexa's Mammalian Total Protein Extraction Kit is a fast and efficient way to extract total protein (including cytoplasmic, membrane and nuclear proteins) from mammalian cells and tissues without ultracentrifugation. The extracted proteins can be analyzed by SDS-PAGE, ELISA, WB and other functional assays.

Kit components

1. Total Protein Extraction Buffer (TPEB): 100 ml
2. 100X EDTA-free Protease Inhibitor Cocktail: 1 ml

Materials Required But Not Provided

1. Phosphate-Buffered Saline (PBS)
2. Phenylmethanesulfonyl Fluoride (PMSF)
3. Distilled water
4. High-precision pipette and sterile pipette tips
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice
8. Vortexer
9. Homogenizer

Notes

- Before use, add PMSF (not provided in this kit) to the Total Protein Extraction Buffer (TPEB). Mix thoroughly and aliquot after first use.
- All steps should be carried out on ice or at 2-8 °C.
- A BCA assay is recommended for protein quantification.

Procedure

A. Cultured cells

1. Harvest 0.5-2 × 10^7 cells. Centrifuge at 500 × g for 5 minutes to pellet the cells. Wash the pellet with 1 ml of pre-chilled PBS and centrifuge at 500 × g for 5 minutes. Carefully discard the supernatant and repeat the wash one more time.
2. Add 1 ml of TPEB to the pellet. Mix thoroughly by vortexing, and allow to stand on ice for 30 minutes, vortexing every 10 minutes.
3. Centrifuge at 14,000 × g at 4 °C for 10 minutes.
4. Carefully transfer the supernatant (which contains the total protein) to a new 1.5 ml microcentrifuge tube. The isolated total proteins can be analyzed immediately or stored at -80°C.

B. Tissues

1. Cut and mince 20-100 mg of tissue into small pieces. Wash with 1 ml of pre-chilled PBS and centrifuge at 500 × g for 5 minutes. Carefully discard the supernatant and repeat the wash one more time.
2. Add 1 ml of TPEB to the tissue. Mix thoroughly by vortexing. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue with 6-10 strokes.
3. Carefully transfer the suspension to a new 1.5 ml microcentrifuge tube. Allow to stand on ice for 30 minutes, vortexing every 10 minutes.
4. Centrifuge at 14,000 × g at 4 °C for 10 minutes.
5. Carefully transfer the supernatant (which contains the total protein) to a new 1.5 ml microcentrifuge tube. The isolated total proteins can be analyzed immediately or stored at -80°C.