

# ProteinExt<sup>®</sup> Mammalian Total Protein Extraction Kit

Cat. No. DE101

Storage: at -20°C for one year

## Description

ProteinExt<sup>®</sup> Mammalian Total Protein Extraction Kit provides a fast and efficient method to extract total proteins (cytoplasmic, membrane and nuclear proteins) from mammalian cells and tissues without ultracentrifugation. The extracted proteins are suitable for SDS-PAGE, Western blot, ELISA, and other functional assays.

## Kit Content

Component	DE101-01
Total Protein Extraction Buffer (TPEB)	100 ml
ProteinSafe <sup>™</sup> Protease Inhibitor Cocktail, EDTA-free (100×)	1 ml

## Procedures

### a. Cultured Cells

1. Harvest  $0.5-2 \times 10^7$  cells. Centrifuge at 500xg for 5 minutes to pellet the cells. Wash the pellet with 1 ml of pre-chilled PBS. Centrifuge at 500xg for 5 minutes. Repeat the wash once.
2. Add 1 ml of TPEB to the pellet. Mix thoroughly by vortexing, and incubate on ice for 30 minutes with vortexing at every 10 minutes.
3. Centrifuge at 14,000×g, 2-8°C for 10 minutes.
4. Gently collect the supernatant (total protein) to a new 1.5 ml microcentrifuge tub. The isolated total proteins can be used for downstream applications or stored at -80°C.

### b. Tissues

1. Cut 20-100 mg of tissues into small pieces, wash minced tissues with 1 ml of pre-chilled PBS and centrifuge at 500×g for 5 minutes, gently discard the supernatant. Repeat the wash once.
2. Add 1 ml of TPEB to tissues and mix thoroughly by vortexing. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue by 6-10 strokes.
3. Gently transfer the suspension to a new 1.5 ml microcentrifuge tube, incubate on ice for 30 minutes with vortexing at every 10 minutes.
4. Centrifuge at 14,000×g, 2-8°C for 10 minutes.
5. Gently collect the supernatant (total proteins). The isolated total proteins can be used for downstream applications or stored at -80°C.

## Notes

- Prior to use, add PMSF into TPEB.
- Aliquoting TPEB after first use.
- All steps should be carried out on ice or at 2-8°C.
- If protein quantification is needed, we suggest to use BCA method (*Easy II Protein Quantitative Kit* (BCA), Cat. No DQ111).

FOR RESEARCH USE ONLY