

ProteinExt[®] Mammalian Total Protein Extraction Kit

Please read the manual carefully before use

Cat. No. DE101

Version No. Version 1.0

Storage: at -18°C or below for one year

Description

ProteinExt[®] 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
<i>ProteinSafe</i> [™] Protease Inhibitor Cocktail, EDTA-free (100×)	1 ml

Procedures

a. Cultured Cells

1. Collect $0.5\text{--}2 \times 10^7$ cells, discard the culture medium. Wash the cells with 1 ml of pre-chilled PBS. Centrifuge at $500 \times g$ for 5 minutes, and carefully discard the supernatant. Repeat the wash once.
2. Add 1 ml of TPEB to the cell pellet (adjust the volume according to cell number at a ratio of $0.5\text{--}2 \times 10^7$ cells/ml TPEB). Vortex vigorously for 15 seconds, then incubate on ice for 30 minutes with vortexing at every 10 minutes.
3. Centrifuge at $14,000 \times g$ for 10 minutes at 2–8°C.
4. Carefully collect the supernatant (total protein). The isolated total protein 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 $500 \times g$ 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 $14,000 \times g$ for 10 minutes at 2–8°C.
5. Gently collect the supernatant (total protein). The isolated total protein can be used for downstream applications or stored at -80°C.

Notes

- Add *ProteinSafe*[™] Protease Inhibitor Cocktail, EDTA-free (100×) to TPEB to achieve a $1 \times$ working concentration before use.
- Aliquot after initial thaw.
- All steps should be carried out on ice or at 2–8°C.
- For tissue extraction, prepare a 1–2 ml glass homogenizer.
- If protein quantification is needed, we suggest to use BCA method (*Easy II* Protein Quantitative Kit (BCA), Cat. No DQ111).

For research use only, not for clinical diagnosis.

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