ProteinExt® Mammalian Nuclear and Cytoplasmic Protein Extraction Kit

Cat. No. DE201

Storage: *ProteinSafe*™ Protease Inhibitor Cocktail, EDTA-free (100×) at -20°C for one year, others at 2-8°C for one year

Description

ProteinExt® Mammalian Nuclear and Cytoplasmic Protein Extraction Kit provides a fast and efficient method to extract nuclear and cytoplasmic proteins from mammalian cells and tissues. Native proteins can be obtained within 80 minutes without ultracentrifugation. The extracted proteins are suitable for a variety of downstream applications, including SDS-PAGE, Western blot, ELISA, enzyme-activity assays, immunoprecipitation and transcription factor activity analysis.

Kit Contents

Component	DE201-01 (50 rxns)
Cytoplasmic Protein Extraction Buffer I (CPEB I)	75 ml
Cytoplasmic Protein Extraction Buffer II (CPEB II)	3 ml
Nuclear Protein Extraction Buffer (NPEB)	25 ml
ProteinSafe™ Protease Inhibitor Cocktail, EDTA-free (100×)	1 ml

Procedures

a. Cultured Cells

1. Harvest 0.5-2×10^7 cells and wash the cells with 1 ml of pre-chilled PBS. Centrifuge at 1,000×g for 3 minutes. Discard the supernatant. Repeat the wash once.
2. Add 1 ml of CPEB I to the pellet. Mix thoroughly by vortexing for 15 seconds, and incubate on ice for 10 minutes with vortexing at every 2 minutes.
3. Add 55 µl of CPEB II to the tube, vortex the tube for 5 seconds. Incubate on ice for 1 minute.
4. Centrifuge at 16,000×g, 2-8°C for 15 minutes.
5. Gently collect the supernatant (cytoplasmic proteins). Placed on ice.
6. Add 500 µl of CPEB I to the pellet and resuspend the pellet with vortexing on the highest setting for 5 seconds.
7. Centrifuge at 16,000×g, 2-8°C for 5 minutes and gently discard the supernatant.
8. Add 500 µl of NPEB to the pellet (use less volume for higher nuclear protein concentration). Vortex for 15 seconds to resuspend the pellet. Incubate on ice for 30 minutes, vortex for 15 seconds every 5 minutes.
9. Centrifuge at 16,000×g, 2-8°C for 10 minutes.
10. Gently collect the supernatant (nuclear proteins). The isolated nuclear proteins can be used for downstream applications or stored at -80°C.

b. Tissues

1. Cut 50-100 mg of tissues into small pieces, wash minced tissues with 1 ml of pre-chilled PBS and centrifuge at 500×g for 3 minutes, gently discard the supernatant. Repeat the wash once.
2. Add 1 ml of CPEB I to tissues and mix thoroughly by vortexing. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissues (6-10 strokes, avoid over-homogenization).
3. Gently transfer the suspension to a new 1.5 ml microcentrifuge tube, vortex for 15 seconds. Incubate on ice for 10 minutes. Vortex every 2 minutes.
4. Following steps are the same as the steps 3-10 described in “Cultured Cells” section.

Notes

- Prior to use, *ProteinSafe*™ Protease Inhibitor Cocktail, EDTA-free (100×) should be added into CPEB I and NPEB to make its concentration of 1×.
- All steps should be carried out on ice or at 2-8ºC.
- If protein quantification is needed, we suggest to use BCA method (*Easy* II Protein Quantitative Kit (BCA), Cat. No DQ111).
- When extracting tissue samples, please self-prepare glass homogenizer

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