PRODUCT INFORMATION

Mammalian Membrane Protein Extraction Kit Cat. No. 39242

Product Description:

General	Mammalian Membrane Protein Extraction Kit provides a simple and efficient method to extract membrane proteins from mammalian cells and tissue. The extracted proteins are suitable for SDS PAGE, Western blot, ELISA, and enzyme-activity assays.
Storage	Recommended temperature for long-term storage **Buffers**: + 2 °C to + 8 °C **Protease Inhibitor Mix M**: - 15 °C to - 25 °C
Compo-nents	50 ml Membrane Protein Extraction Buffer I (MPEB I) 7.5 ml Membrane Protein Extraction Buffer II (MPEB II) 15 ml Membrane Protein Extraction Buffer III (MPEB III) 1 vial Protease Inhibitor Mix M 1 ml DMSO

- Prior to use, add PMSF (0.1 – 1 µM) into MPEB I, II and III.
- All steps should be carried out on ice or at + 4 °C.
- Reconstitute 1 vial Protease Inhibitor Mix M with 1 ml DMSO to get a 100x solution.

Protocol – Cultured cells

(1) Harvest 0.5 - 2x10^7 cells, wash the cells with 1 ml pre-chilled PBS (centrifugation 1,000xg, 3 min) and discard the supernatant. Repeat the wash once.
(2) Add 750 µl MPEB I to the cell pellet and vortex for 15 s. Incubate on ice for 10 min and vortex every 2 min.
(3) Centrifuge at 16,000xg, + 4 °C, 15 min
(4) Gently transfer the supernatant (cytoplasmic proteins) to a new 1.5 ml-tube for immediate use or store at - 80 °C.
(5) Add 150 µl MPEB II to the pellet and resuspend it by vortexing 15 s. Incubate on ice for 30 min and vortex every 5 min.
(6) Add 300 µl of MPEB III to the pellet and vortex 5 s.
(7) Centrifuge at 16,000xg, + 4 °C, 15 min
(8) Gently transfer the supernatant (membrane proteins) to a new 1.5 ml-tube for immediate use or store at - 80 °C.

Protocol – Tissue

(1) Wash 20 - 60 mg tissue with 2 ml pre-chilled PBS, vortex briefly, gently discard the supernatant.
(2) Add 1 ml PBS, cut the tissue into small pieces and centrifuge 3 min at 500xg, gently discard the supernatant.
(3) Add 1 ml MPEB I and mix thoroughly by vortexing. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue by 6 – 10 strokes.
(4) Incubate on ice for 10 min and vortex every 2 min.
(5) Follow steps (3) – (8) as described in protocol for cultured cells.[7]

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