SERVA Electrophoresis

PRODUCT INFORMATION

Mammalian Nuclear and Cytoplasmic Protein Extraction Kit

Cat. no. 39243

Product Description:

General

Mammalian Nuclear and Cytoplasmic Protein Extraction Kit provides a fast and efficient method to extract nuclear and cytoplasmic proteins from mammalian cells and tissue. The extracted proteins are suitable for SDS PAGE, Western blot, ELISA, immunoprecipitation, transcription factor- 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

Components

- 75 ml Cytoplasmic Protein Extraction Buffer I (CPEB I)
- 3 ml Cytoplasmic Protein Extraction Buffer II (CPEB II)
- 25 ml Nuclear Protein Extraction Buffer (NPEB)
- 1 vial Protease Inhibitor Mix M
- 1 ml DMSO

- Reconstitute 1 vial Protease Inhibitor Mix M with 1 ml DMSO to get a 100x solution.
- Prior to use, add Protease Inhibitor Mix M into CPEB I and NPEB.
- All steps should be carried out on ice or at + 4 °C.

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Protocols

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 1 ml CPEB I to the cell pellet and vortex for 15 s. Incubate on ice for 10 min and vortex every 2 min.
(3) Add CPEP II (maintain the volume ratio CPEP I:CPEP II = 200:11) to the pellet and vortex 5 s. Incubate on ice for 1 min.
(4) Centrifuge at 16,000xg, + 4 °C, 15 min
(5) Gently transfer the supernatant (cytoplasmic proteins) to a new 1.5 ml-tube for immediate use or store at - 80 °C.
(6) Add 500 µl CPEB I to the pellet and resuspend it by vortexing 5 s on highest setting. Incubate on ice for 30 min and vortex every 5 min.
(7) Centrifuge at 16,000xg, + 4 °C, 15 min and discard the supernatant.
(8) Add 500 µl of NPEB (use less volume for higher nuclear protein concentration) to the pellet and vortex 15 s. Incubate on ice for 30 min and vortex for 15 s every 5 min.
(9) Centrifuge at 16,000xg, + 4 °C, 15 min
(10) Gently transfer the supernatant (nuclear proteins) to a new 1.5 ml-tube for immediate use or store at - 80 °C.

Protocol – Tissue

(1) Cut 50 – 100 mg tissue in small pieces, wash the minced tissue with 1 ml of pre-chilled PBS and centrifuge 3 min at 500xg, gently discard the supernatant. Repeat the wash once.
(2) Add 1 ml CPEB I and mix thoroughly by vortexing. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue by 6 – 10 strokes. Avoid over-homogenization.
(3) Gently transfer the supernatant (membrane proteins) to a new 1.5 ml-tube and vortex for 15 s.
(4) Incubate on ice for 10 min and vortex every 2 min.
(5) Follow steps (3) – (10) as described in protocol for cultured cells.

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