TECHNICAL NOTES

Mammalian Protein Extraction Kits

SERVA
serving scientists

Fast and Simple Isolation of Native Nuclear, Cytoplasmic and Membrane Proteins

The mammalian protein extraction kits provide a fast and efficient method to extract total proteins or cytoplasmic, nuclear and membrane protein fractions from mammalian cells or tissues. The lysis buffers allow for mild but efficient lysis with high protein yield. The performance is optimized and validated for several cell lines and tissues. There is no need for mechanical cell disruption. The single formulation reagents provide versatility, because protease or phosphatase inhibitors, reducing agents etc. may be added prior to cell lysis. The reagents are compatible with standard protein quantification assays like BCA Protein Assay.

- Contain ready-to-use optimized extraction buffers and protease inhibitor cocktail
- High protein yields from cells or tissues
- Gentle formulation preserves protein activity for downstream assays
- No need for mechanical disruption or ultracentrifugation
- Lysates are directly compatible with immunoprecipitation, SDS-PAGE, Western Blot, ELISA, EMSA and other functional assays

Low Cross Contamination

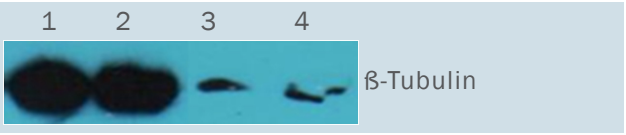

Fig. 1 Membrane proteins were isolated from HEK293 cells using the SERVA Mammalian Membrane Protein Extraction Kit and the Membrane Protein Extraction Kit of a competitor, following the respective protocols. To determine the percentage of cross contamination by cytoplasmic proteins in the membrane protein fraction, ß-Tubulin was detected in the cytoplasmic (lane 1,2) and membrane extract (lane 3,4) by Western blot.

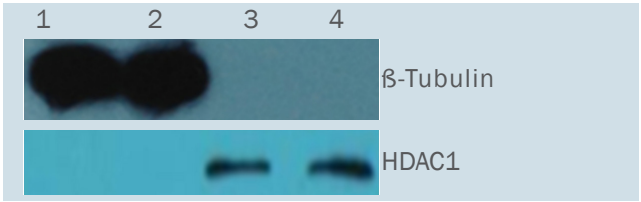

Fig. 2 Cytoplasmic and nuclear proteins were isolated from HEK293 cells using the SERVA Mammalian Membrane Protein Extraction Kit and the Membrane Protein Extraction Kit of a competitor, following the respective protocols. To analyze the efficiency of cellular fractionation, ß-Tubulin and HDAC1 were detected in the cytoplasmic (lane 1,2) and nuclear extract (lane 3,4) by Western blot.

High Protein Yield from Cells and Tissues

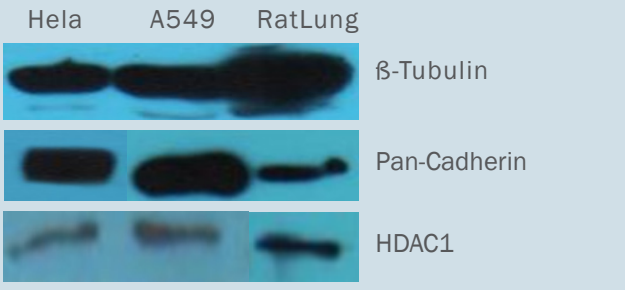

Fig. 3 Total proteins were isolated from Hela cells and A549 cells and rat lung tissue (1x 10^7 cells and 50 mg tissue) using the SERVA Mammalian Total Protein Extraction Kit. 20 µg of the extract were run on a 4-20 % SDS PAGE gel and analyzed by Western Blot using 1:200 dilutions of primary antibodies to various specific proteins.

Extracted Proteins Are Directly Compatible with Enzyme Activity Assays

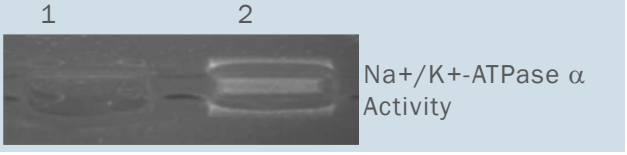

Fig. 4 Enzymatic activity assay of NA/K-ATPase α (lane 2, lane 1 = negative control) shows that proteins isolated with the Mammalian Protein Extraction Kit are fully functional and that activity is preserved for downstream assays.

Version 04/18

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Mammalian Protein Extraction Kits

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Mammalian Total Protein Extraction Kit

Workflow 55 min

Harvest cells
⇩
Wash
⇩
Add TPEB Buffer
(lysis of cells and solubilizing of total proteins)
⇩
Vortex, incubation on ice for 30 min
⇩
Centrifuge for 10 min,
collect supernatant

Extract total native proteins (cytoplasmic, membrane and nuclear proteins) from mammalian cells and tissues.

Mammalian Membrane Protein Extraction Kit

Workflow 75 min

Harvest cells
⇩
Wash
⇩
Add MPEB I Buffer
(permeabilization of cells and release of soluble cytoplasmic proteins)
⇩
Vortex, incubation on ice for 10 min
⇩
Centrifuge for 15 min, cytoplasmic proteins in supernatant
⇩
Resuspend pellet in MPEB II Buffer
⇩
Vortex, Incubation on ice, 30 min
⇩
Add MPEB III Buffer, vortex
(solubilisation of membrane proteins)
⇩
Centrifuge for 15 min, membrane proteins in supernatant

Extract native membrane and cytoplasmic proteins from mammalian cells and tissues. Efficiency is up to 90 % for membrane proteins with at least 1 – 2 transmembrane domains. Cross-contamination of cytoplasmic proteins is usually less than 10 %.

Mammalian Nuclear and Cytoplasmic Protein Extraction Kit

Workflow 80 min

Harvest cells
⇩
Wash
⇩
Add CPEB I Buffer
(cell membrane swelling)
⇩
Vortex, incubation on ice for 10 min
Add CPEB II Buffer
(release of cytoplasmic proteins)
⇩
Centrifuge for 15 min, cytoplasmic proteins in supernatant
⇩
Resuspend pellet in CPEB I Buffer, vortex, centrifuge for 5 min
⇩
Resuspend pellet in NPEB Buffer
(lysis of nuclei and solubilisaion of nuclear proteins)
⇩
Vortex, incubation on ice for 30 min
⇩
Centrifuge for 10 min, nuclear proteins in supernatant

Extract native nuclear and cytoplasmic proteins from mammalian cells and tissues. Cross-contamination of cytoplasmic proteins is usually less than 10 %.

Ordering Information

Product	Size	Cat. no.
Mammalian Total Protein Extraction Kit	100 Samples	39241.01
Mammalian Nuclear and Cytoplasma Protein Extraction Kit	50 Samples	39243.01
Mammalian Membran Protein Extraction Kit	50 Samples	39242.01

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