PRODUCT INFORMATION & MANUAL

Nuclear Extraction Kit

NBP2-29447

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

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I. INTRODUCTION

The Nuclear Extraction Kit provides a simple and convenient method for the isolation of nuclear and cytoplasmic extracts from mammalian cells and tissue samples. This procedure is relevant to the monitoring of translocation of cell signaling molecules from cytoplasm to the nucleus. Examples include translocation of NF-κB molecules to the nucleus in TNFα treated cells, and translocation of mitogen-activated protein kinase to the nucleus in growth factor treated cells. The Nuclear Extraction Kit can be used in the preparation of purified proteins for use in Western blotting, Electrophoretic Mobility Assays (EMSA) and preparative purification of nuclear proteins.

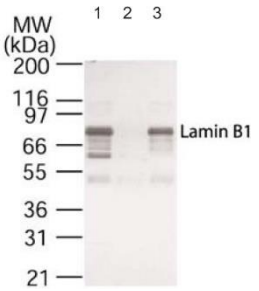

Figure 1. Western blot analysis of Lamin B1 using NB100-56403.
20 µg of HeLa cell proteins were separated on 4-20% SDS-PAGE.
Lane 1. Nuclear extract.
Lane 2. Cytoplasmic extract.
Lane 3. Total cell lysate.

II. KIT DESCRIPTION AND ADVANTAGES

The Nuclear Extraction Kit can be used to isolate cytosolic and nuclear fractions from the same cell or tissue preparation.

The reagents provided here are sufficient for 100 extractions using 100-mm tissue culture plates or 20 extractions using 1 gram of tissue.

III. KIT COMPONENTS AND STORAGE

Kit Components and storage for The Nuclear Extraction Kit

REAGENTS (4°C STORAGE)		
KC-401	10X Hypotonic Lysis Buffer	10 mL
KC-402	1X Nuclear Extraction Buffer	10 mL
KC-403	10% Detergent Solution	10 mL
KC-117	10X PBS	2 x 50 mL
REAGENTS (-20°C STORAGE–NON-FROST-FREE)		
KC-404	1 M DTT (for Nuclear Extraction from tissue)	100 µL
KC-405	10 mM DTT (for Nuclear Lysis Buffer)	500 µL
KC-406	100X Protease Inhibitor Cocktail (PIC)	100 µL
KC-407	100 mM PMSF	10 mL

Additional materials needed but not provided in the kit:

- Teflon homogenizer (tissues)
- Cell scraper (cells)
- High-speed cold centrifuge and compatible centrifuge tubes
- Microcentrifuge tubes
- Deionized Water
- Vortex

IV. BUFFER PREPARATION

1X Hypotonic Buffer:

Dilute 10X Hypotonic Buffer to 1X in deionized water. 1X Hypotonic Buffer can be stored at 4°C for 1 month.

Buffers / Components	60-mm plate ($4x10^6$ cells)	100-mm plate ($10x10^6$ cells)	150-mm plate ($20x10^6$ cells)
10X Hypotonic buffer	50 µL	100 µL	200 µL
Deionized water	450 µL	900 µL	1800 µL
Total Volume required	500 µL	1 mL	2 mL

1X PBS-PMSF:

Dilute 10X PBS in deionized water to make 1X PBS. Add 500 µL of 100 mM PMSF to 50 mL of 1X PBS. The 1X PBS-PMSF solution should be used within 24 h after dilution (diluted PMSF has a half-life less than 24 h).

Buffers / Components	60-mm plate ($4x10^6$ cells)	100-mm plate ($10x10^6$ cells)	150-mm plate ($20x10^6$ cells)
10X PBS	500 µL	1 mL	2 mL
Deionized water	4.45 mL	8.9 mL	17.8 mL
100 mM PMSF	50 µL	100 µL	200 µL
Total volume required	5 mL	10 mL	20 mL

Nuclear Lysis Buffer:

Add 0.5 mM DTT and 1X PIC to Nuclear Extraction Buffer, just prior to use.

Buffers / Components	60-mm plate ($4x10^6$ cells)	100-mm plate ($10x10^6$ cells)	150-mm plate ($20x10^6$ cells)
10 mM DTT	2.5 µL	5 µL	10 µL
Nuclear Extraction Buffer	47 µL	94 µL	188 µL
100X PIC	0.5 µL	1 µL	2 µL
Total volume required	50 µL	100 µL	200 µL

V. NUCLEAR EXTRACTION KIT PROCEDURE

A. Preparation of Nuclear Extract from Cells

i) Cell Culture

1. Grow cells to 70-80% confluency for adherent cells or about 1.5 x 10^6/mL for suspension cells.
2. If necessary, treat cells with desired experimental protocol.

ii) Cell Collection (following protocol is based on 10 x 10^6 HeLa cells grown on 100-mm tissue culture plate):

1. For adherent cells, wash cells with 5 mL of ice cold 1X PBS-PMSF. Aspirate buffer out and add 5 mL of ice cold 1X PBS-PMSF.
2. Dislodge the cells using a cell scraper and transfer into a 15-mL conical tube.
3. To pellet the cells, centrifuge for 5 min at 1000 rpm at 4 °C.
4. Aspirate and discard the supernatant. Keep the cell pellet on ice.

iii) Cytoplasmic Fraction Collection:

1. Resuspend cell pellet in 1 mL of ice cold 1X Hypotonic Buffer by pipetting up and down several times and transfer to a pre-chilled microcentrifuge tube.
2. Incubate the cells on ice for 15 min.
3. Add 50 µL of the 10% Detergent Solution a nd vortex vigorously for 10 sec (Whole Cell Lysate).
4. Centrifuge the tubes for 5 – 10 min at 14,000 – 16,000 rpm in a cold microcentrifugeS.
5. Carefully remove the supernatant (Cytoplasmic Fraction) into a pre-chilled microcentrifuge tube and store at 4 °C. The pellet is the nuclear fraction.

iv) Nuclear Fraction Collection:

1. Resuspend nuclear pellet in 100 µL Nuclear Lysis Buffer by pipetting up and down. Vortex vigorously and incubate suspension at 4 °C, for 30 min on a rocking platform.
2. Vortex suspension for 30 sec. Centrifuge the suspension at 14,000 rpm for 10 min at 4 °C in a microcentrifuge.
3. Transfer the supernatant (Nuclear Fraction) into a pre-chilled micro-centrifuge tube. Store the nuclear fraction at -80 °C until further use. Avoid multiple freeze/thaw cycles.
4. Determine the protein concentration in the nuclear extract using a detergent compatible assay technique (e.g.: Bio-Rad DC Protein Assay Method). We recommend using the Nuclear Lysis Buffer as the blank and performing a 1:50 and 1:100 dilution of your sample.

B. Preparation of Nuclear Extract from Tissues

i) Cytoplasmic Fraction Collection

(Tissue Homogenization based on 1 gram Mouse Spleen).

1. Weigh tissue and cut into small pieces using clean razor blade and wash in 5 mL of cold 1X PBS-PMSF. Collect cut pieces in a clean homogenizer.
2. Add 5 mL of ice cold 1X Hypotonic Buffer supplemented with 1 mM DTT and 1% Detergent Solution. For example, add 5 µL of 1M DTT and 500 µl of 10% Detergent Solution to 4.495 mL of ice cold 1X Hypotonic Buffer per gram of tissue and homogenize. Incubate on ice for 15 to 30 min. (Whole Cell Lysate).
3. Centrifuge for 10 min at 10,000 rpm at 4 ° C. Transfer the supernatant (Cytoplasmic Fraction) into a 15-mL tube and store at 4°C. The pellet is the nuclear fraction.

ii) Nuclear Fraction Collection

1. Resuspend nuclear pellet in 500 µL Nuclear Lysis Buffer by pipetting up and down. Vortex vigorously and incubate suspension at 4 ° C, for 30 min on a rocking platform.
2. Vortex suspension for 30 sec. Centrifuge the suspension at 14,000 rpm for 10 min at 4 ° C in a microcentrifuge.
3. Transfer the supernatant (Nuclear Fraction) into a pre-chilled microcentrifuge tube. Store the nuclear fraction at -80°C until further use. Avoid multiple freeze/thaw cycles.
4. Determine the protein concentration in the nuclear extract using a detergent compatible assay technique (e.g.: Bio-Rad DC Protein Assay Method). We recommend using the Nuclear Lysis Buffer as the blank and performing a 1:50 and 1:10 dilution of your sample.

VI. TROUBLE SHOOTING

Problem	Probable Cause	Suggestion
Low protein concentration on cytosolic fraction.	Cell density is too high for effective lysis of cell membrane.	Larger volume of lysis buffer should be used
Low or no protein yield in cytoplasmic fraction or nuclear fraction.	Cell type is not compatible with this extraction procedure.	Hypotonic and nuclear extraction buffers should be optimized for this type of cells.

VII. PRODUCT CITATIONS

1. Molecular cloning and characterization of human Castor, a novel human gene upregulated during cell differentiation. Liu Z, X Yang, F Tan, K Cullion and CJ Thiele. Biochemical and Biophysical Research Communications, 344:834-844 (2006). Nuclear and cytoplasmic proteins were extracted from Sk-N-AS human neuroblastoma cells [see Materials and Methods (Western blot analysis) and Fig. 7A]
2. Involvement of AMP-activated protein kinase in beneficial effects of betaine on high-sucrose diet-induced hepatic steatosis. Song Z, I Deaciu, A Zhou, M Song, T Chen, D Hill, and CJ McClain. Am J Physiol Gastrointest Liver Physiol, In press (Aug 16, 2007). doi:10.1152/ajpgi.00133.2007. Nuclear proteins were isolated from fresh mouse liver tissue [see Materials and Methods (Preparation of Nuclear Extracts) and Fig. 5]
3. Adenovirus-mediated expression of a dominant negative Ku70 fragment radiosensitizes human tumor cells under aerobic and hypoxic conditions. He F, L Li, D Kim, B Wen, X Deng, PH Gutin, CC Ling and GC Li. Cancer Res 67:634-642: Nuclear fractions isolated from human U-87 glioma and HCT-8 colon carcinoma cells used for WB with phosphospecific antibodies (Fig. 5)
4. Impact of progesterone on cytokine-stimulated nuclear factor kappa B signaling in HeLa cells. Vidaeff AC, SM Ramin, LC Gilstrap III, KD Bishop, and JL Alcorn. J Maternal-Fetal & Neonatal Medicine doi:10.1080/14767050601128019 (2007). Nuclear and cytosolic fractions isolated from HeLa cells used for WB with IkBa (Fig. 2) and p65 (Fig. 3) antibodies.
5. MicroRNA Let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. Sampson VB, NH Rong, J Han, Q Yang, V Aris, P Soteropoulos, NJ Petrelli, SP Dunn and LJ Krueger. Cancer Res 67:9762-9770. Nuclear and cytosolic fractions isolated from Namalwa Burkitt lymphoma cells used for IP with MYC antibodies and WB with MYC and MAX antibodies (Fig. 2).