CHEMICON®
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Nuclear Extraction Kit

100 Extractions

Cat. No. 2900

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Application

CHEMICON®'s Nuclear Extraction Kit (Catalog No. 2900) provides a simple and convenient method for the isolation of cytoplasmic and nuclear samples from mammalian cell culture or tissue samples. The Nuclear Extraction Kit can be used in the preparation of purified proteins for use in Western blotting, Electrophoretic Mobility Shift Assays (EMSA), and in CHEMICON®'s Transcription Factor Assay product line.

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Kit Components

1. Cytoplasmic Lysis Buffer, 10x: - (Part No. 90497) One vial containing 10mL of a concentrated lysis buffer. Dilute to a 1x in deionized water.
2. Nuclear Extraction Buffer: - (Part No. 90498) One vial containing 50mL of a 1x nuclear extraction buffer.
3. PBS Packets: - (Part No. 60093) Two pouches containing enough dry reagent to prepare 1 liter of a 1x PBS solution per pouch. Dilute packet in deionized water.
4. DTT, 1M: - (Part No. 90499) One vial containing 100µL of 1M Dithiothreitol. Prior to use, dilute to a final concentration of 0.5mM (1:2000) in 1x Cytoplasmic Lysis Buffer and 1x Nuclear Extraction Buffer.
5. Protease Inhibitor Cocktail: - (Part No. 90492) One vial containing 100µL of protease inhibitors in DMSO for use with mammalian cell and tissue extract buffers. A mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine and aspartic acid proteases and aminopeptidases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pepstatin A, E-64, bestatin, leupeptin, and aprotinin. Contains no metal chelators (e.g. EDTA, EGTA). Prior to use, dilute 1/1000 in 1x Cytoplasmic Lysis Buffer and 1x Nuclear Extraction Buffer.
6. Detergent, 10%: - (Part No. 90500) One vial containing 3mL of 10% IGEPAL CA-630 (detergent). Cytoplasmic Lysis Buffer and Nuclear Extraction Buffer already contain detergent, however, under certain conditions, more detergent may be required - refer to Extraction Procedure.

Materials Not Supplied

1. Tissue Culture Reagents
2. Cell Detachment Buffer (Trypsin) or Cell Scrapers
3. Syringes, 1mL – 27 Gauge Needle
4. 1.5 mL Microcentrifuge Tubes
5. 50 mL Conical Tubes
6. Deionized Water
7. Phosphatase Inhibitor (if needed)
8. Microcentrifuge, 4ºC
9. Table-top Centrifuge (capable of 16,000 x g), 4ºC
10. Rotator/Orbital Shaker, 4^{o}C

Storage

The Nuclear Extraction Kit is shipped and stored at -20ºC.

- Cytoplasmic Lysis Buffer (10x), Nuclear Extraction Buffer (1x), 10% Detergent, and the PBS Packets can be stored at 2-8ºC.
- DTT, 1M and Protease Inhibitor Cocktail must be stored at -20ºC. Avoid repeated freeze-thaw cycles.

Preparation of Reagents

***Note**: Chill all buffers on ice prior to use*.

- Rehydrate **PBS Packet** in 1 liter of deionized water.
- Dilute **10x Cytoplasmic Lysis Buffer** to a 1x solution with deionized water.
- Prior to use, add 0.5mM (final) **DTT** and 1/1000 dilution of **Protease Inhibitor Cocktail** to **1x Cytoplasmic Lysis Buffer** and/or **1x Nuclear Extraction Buffer**.

Extraction Procedure

A. Cell Culture

1. Grow cells to 70-90% confluency for adherent cells or about 1.5 x 10^6/mL for suspension cells.
2. If necessary, treat cells with desired method.

B. Cell Disruption

1. For adherent cells, wash the cells with 1x **PBS** solution, remove, and then add warmed trypsin cell detachment buffer to the culture flask(s). Let the trypsin sit for approximately 2-5 minutes (depending on cell type) and shake cells off.

   Alternately, a cell scraper may be used instead of trypsin. Collect cells and transfer to a clean centrifuge tube. Rinse the culture flask with two volumes of ice cold **PBS** and add to centrifuge tube. Centrifuge the sample at 250 x g for 5 minutes at 4°C. Discard the supernatant and resuspend the cell pellet in 40 mL of ice-cold **PBS** to wash. Centrifuge the suspension at 250 x g as before. Repeat. Pour off supernatant.

   Note: *All work done after cell trypsinization/detachment needs to be performed on ice and/or with chilled buffers. It is imperative that the cell pellets and suspension remain as cold as possible without freezing during the extraction process.*

2. Estimate the approximate volume of the cell pellet. This value will be needed for determining the amount of buffer volume necessary for nuclear extraction. (Two T175 tissue culture flasks of confluent HeLa cells will generate a cell pellet of approximately 100µL.)
3. Add 5 cell pellet volumes of ice cold 1x **Cytoplasmic Lysis Buffer** containing 0.5mM DTT and 1/1000 dilution or inhibitor Cocktail.
4. Resuspend the cell pellet by gently inverting the tube. Avoid foam production. ***Do not vortex!***
5. Incubate the cell suspension on ice for 15 minutes.
6. Centrifuge the cell suspension at 250 x g for 5 minutes at 4°C. Discard supernatant and resuspend the cell pellet in two volumes of ice cold 1x **Cytoplasmic Lysis Buffer**.

C. Cell Lysis

1. Using a syringe with a small gauge needle (27 gauge), draw the cell suspension prepared in Section B from the sample tube into the syringe and then eject the contents back into the sample tube. Repeat approximately 5 times (drawing and ejecting). If the cells "clump" and you are not able to draw them into the syringe, more **Detergent**, **10%** may be added.

   Note: *Lysis and extraction buffers already contain detergent. However, if cell clumping occurs during the lysis procedure it may be necessary to add additional detergent to the Cytoplasmic Lysis Buffer and Nuclear Extraction Buffer.*

2. Centrifuge the disrupted cell suspension at 8,000 x g for 20 minutes at 4°C.
3. The supernatant contains the cytosolic portion of the cell lysate. Transfer the supernatant to a fresh tube. To keep the cytosolic fraction, aliquot, snap-freeze and store at -80°C. Avoid repeated freeze-thaw cycles.
4. The remaining pellet contains the nuclear portion of the cell lysate.

D. Nuclear Extraction

1. Resuspend the nuclear pellet in 2/3 of the original cell pellet volume (determined in step B.2) of ice cold **Nuclear Extraction Buffer** containing 0.5mM DTT and 1/1000 Protease Inhibitor Cocktail.
2. Using a fresh syringe, with a 27-gauge needle, repeat Step C.1. to disrupt the nuclei, add more **Detergent, 10%** if necessary.

   Note*: The nuclear extract sample can be stored at –80°C at this point if needed.*

3. Use a rotator or orbital shaker (low speed) to gently agitate the nuclear suspension at 4°C for 30-60 minutes.
4. Centrifuge the nuclear suspension at 16,000 x g for 5 minutes at 4°C.
5. Transfer the supernatant to a fresh tube. This fraction is the nuclear extract.
6. Determine protein concentration.

   Note*: If additional detergent was added during cell lysis (step C.1 or D.2) it may interfere with certain methods of protein concentration determination. Extract may need to be diluted 10-fold or more depending upon final detergent concentration.*

7. Snap-freeze the nuclear extract in aliquots and store at –80°C. Avoid repeated freezing and thawing of nuclear extract.

Warranty

These products are warranted to perform as described in their labeling and in CHEMICON® literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND CHEMICON® DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CHEMICON® *'s* sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CHEMICON®, to repair or replace the products. In no event shall CHEMICON® be liable for any proximate, incidental or consequential damages in connection with the products.

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December 2003
Revision A: 41599