Nuclear & Cytoplasmic Protein Extraction Kit

Cat. No. NCE-317K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit is a complete system for preparing nuclear and cytoplasmic protein extracts from mammalian tissue or cultured cells.
Applications	This kit was used to study the impact of salt on cardiac differential gene expression and coronary lesion in normotensive mineralocorticoid-treated mice. It was also used to test the therapeutic potential of andrographolide for treating endometriosis
Usage	1 Kit sufficient for 10 extractions (1 ml packed cell volume) or for 100 extractions (100 µl packed cell volume).
Storage	This kit is shipped on dry ice and storage at -20 centigrade is recommended. The DTT solution and the protease inhibitor cocktail must be stored at -20 centigrade and should be added to buffers immediately before use. All the buffer solutions may be stored at 2-8 centigrade for several weeks. The 10% IGEPAL CA-630 Solution may be stored for long term at 2-8 centigrade.
Kit Components	· 10X Lysis Buffer, hypotonic (100 mM HEPES, pH 7.9, with 15 mM MgCl2 and 100 m M KCl) 7 ml · 5X Lysis Buffer, isotonic (50 mM Tris HCl, pH 7.5, with 10 mM MgCl2, 15 mM CaCl 2, and 1.5 M Sucrose) 14 ml · Extraction Buffer (20 mM HEPES, pH 7.9, with 1.5 mM MgCl2, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) Glycerol) 10 ml · 3X Dilution and Equilibration Buffer (60 mM HEPES, pH 7.9, with 4.5 mM MgCl2, 0.6 mM EDTA, 30 mM KCl, and 75% (v/v) Glycerol) 90 ml

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	· Dithiothreitol (DTT) (1 M DTT in deionized water) 0.4 ml · Protease Inhibitor Cocktail (contains 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEB SF), Pepstatin A, Bestatin, Leupeptin, Aprotinin, and trans-EpoxysuccinylL-leucyl-amido(4-guanidino)-butane (E-64)) 1 ml · IGEPAL CA-630 10% Solution (10% IGEPAL CA-630 in deionized water) 4 ml
Materials Required but Not Supplied	· Centrifuge tubes · Centrifuge (Eppendorf with A-4-62 rotor or equivalent) (optional) · Microcentrifuge (Eppendorf 5417R or equivalent) · Glass tissue homogenizer (grind tube and type B pestle) (optional) · Microscope · Syringes, 1 ml capacity · Precision Glide Needles, SS, 27 gauge · Microscope slides · Micro cover glass · Cells scrapers · Dulbecco's phosphate-buffered saline (PBS) · Trypan Blue solution
Separation Protocol	Perform all steps at 2-8 centigrade. Use precooled buffers and equipment. Ensure all the solutions are defrosted and homogeneous. All centrifugations are done at 4 centigrade with precooled rotors. The final concentration of DTT in the solutions should be 1 mM. The protease inhibitor cocktail should be diluted 100-fold in the final solutions. A. Nuclear Protein Extraction from 100 ml of packed cell volume using a detergent (IGEPAL CA-630) Calculate accordingly for different packed cell volumes. 1. Dilute the 1 M DTT solution with deionized, sterile water to a concentration of 0.1 M. For small-scale preparations (below 100 ml total) the 1 M DTT stock solution should be diluted to 0.01 M. 2. Prepare 1X Lysis Buffer, hypotonic, from the 10X Lysis Buffer, hypotonic, by

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diluting 10-fold with sterile, deionized water. For fragile cells use 1X Lysis Buffer, isotonic, prepared from the 5X Lysis Buffer, isotonic, to replace the 1X Lysis Buffer, hypotonic. To 500 ml of 1X Lysis Buffer (either hypotonic or isotonic), add 5 ml of the prepared 0.1 M DTT solution and 5 ml of the protease inhibitor cocktail.

3. Collect cells

a. Adherent cells from 70-90% confluent monolayer culture.

· Remove the growth medium from the cells.

· Rinse the cells twice with PBS, being careful not to dislodge any of the cells.

· Discard the PBS. · Scrape the cells using fresh PBS into an appropriate conical centrifuge tube.

· Centrifuge for 5 minutes at 450 x g. · Decant and discard the supernatant.

b. Cells in suspension

· Collect the cells into an appropriate conical centrifuge tube.

· Centrifuge for 5 minutes at 450 x g.

· Decant and discard the supernatant.

· Wash cells twice by resuspending the cell pellets in PBS and centrifuge for 5 minutes at 450 x g.

· Decant and discard the supernatant.

4. Estimate the packed cell volume (PCV).

5. Add 500 ml (5X PCV) of 1X Lysis Buffer (including DTT and protease inhibitors) to 100 ml of PCV. Resuspend the cell pellet gently. Avoid foam formation. If working with small volumes, the suspended cells may be transferred to a microcentrifuge tube.

6. Incubate the packed cells in the selected lysis buffer on ice for 15 minutes, allowing cells to swell. Take several microliters of the cells in the lysis buffer and view them under the microscope. If massive cell lysis is detected under the microscope or a gelatinous mass is observed, the cells may be fragile. In this case, use the Lysis Buffer, isotonic, for cell lysis and consider eliminating the incubation step.

7. To the swollen cells in lysis buffer, add the 10% IGEPAL CA-630 solution to a final concentration of 0.6% (6 ml per 100 ml of mixture). Vortex vigorously for 10 seconds.

8. Centrifuge immediately for 30 seconds at 10,000-11,000 x g.
In order to assess the degree of lysis, before centrifugation, take a sample of the suspended cells and view the nuclei under the microscope. Lysis can be observed by the addition of the Trypan Blue solution to an aliquot of cells. The dye is excluded from the intact cells, but stains the nuclei of lysed cells. If lysis of nuclei is observed under the microscope or if a gelatinous mass is observed, lyse the cells with a lower final concentration of IGEPAL CA-630.
· If cells are not lysed, increase the final percentage of IGEPAL CA-630 in the resuspended cells (step 7).
· For fragile cells use lower concentrations of IGEPAL CA-630. Avoid vortexing the cells and centrifuge at a slower speed.
9. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
10. Add 1 ml of the prepared 0.1 M DTT solution and 1 ml of the protease inhibitor cocktail to 98 ml of the Extraction Buffer. If it is necessary to extract the proteins of interest at a lower salt concentration, dilute the Extraction Buffer with 1X Dilution and Equilibration Buffer. Note: The salt concentration in the Extraction Buffer is 0.42 M, a commonly used extraction condition. In rare cases a lower or a higher salt concentration may be needed for a better extraction of a particular protein. In that case, dilute the Extraction Buffer with the 1X Dilution and Equilibration Buffer or add NaCl to the Extraction Buffer to reach the desired salt concentration.
11. Resuspend the crude nuclei pellet in ~70 ml (2/3X PCV) of Extraction Buffer containing the DTT and protease inhibitor cocktail.
12. Mount the tube on a vortex mixer and agitate at medium to high speed for 15-30 minutes. Avoid foam formation.
13. Centrifuge for 5 minutes at 20,000-21,000 x g.
14. Transfer the supernatant to a clean, chilled tube
15. Snap-freeze the supernatant in aliquots with liquid nitrogen and store at -70 centigrade. B. Nuclear Protein Extraction from 200 ml of packed cell volume without the use of a detergent Calculate accordingly for different packed cell volumes. Detergents can interfere with the activity or binding of the extracted proteins.

Therefore, a procedure for nuclear protein extraction without the use of a detergent is included. This protocol describes the preparation of crude nuclear extracts using a syringe or a glass tissue homogenizer. Note: The procedure requires at least 100 ml of PCV. Use of a syringe is recommended for small-scale preparations (0.1-1 ml). Passage of more than one millliliter through a syringe may cause difficulties due to the needle gauge size.

1. Dilute the 1 M DTT solution to 0.1 M with deionized, sterile water.
2. Prepare 1X Lysis Buffer, hypotonic. For protein extraction from fragile cells, prepare 1X Lysis Buffer, isotonic, to replace the 1X Lysis Buffer, hypotonic. To 1,400 ml of 1X Lysis Buffer (hypotonic or isotonic), add 14 ml of the prepared 0.1 M DTT solution and 14 ml of the protease inhibitor cocktail.
3. Collect cells

a. Adherent cells from 70-90% confluent monolayer culture.

· Remove the growth medium from the cells.
· Rinse the cells twice with PBS, being careful not to dislodge any of the cells.
· Discard the PBS.
· Scrape the cells using fresh PBS into an appropriate conical centrifuge tube.
· Centrifuge for 5 minutes at 450 x g. · Decant and discard the supernatant.

b. Cells in suspension

· Collect the cells into an appropriate centrifuge conical tube.
· Centrifuge for 5 minutes at 450 x g.
· Decant and discard the supernatant.
· Wash cells twice by resuspending the cell pellets in PBS and centrifuge for 5 minutes at 450 x g.
· Decant and discard supernatant.

4. Estimate the packed cell volume (PCV).
5. Add 1 ml (5X PCV) of 1X Lysis Buffer (including DTT and protease inhibitors) to 200 ml of PCV. Resuspend the cell pellet gently. Avoid foam formation. If working with small volumes, the suspended cells may be transferred to a microcentrifuge tube. Incubate the packed cells in lysis buffer for 15 minutes, allowing cells to swell.

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6. Centrifuge the suspended cells for 5 minutes at 420 x g. Decant supernatant and resuspend the pellet of packed cells in 400 ml (2X PCV) of the 1X Lysis Buffer.
7. Cell disruption a. Using a glass tissue homogenizer, transfer the cells into a glass tissue grind tube. Grind on ice slowly with five up-and-down strokes using a type B pestle. Avoid foam formation. OR b. Using a syringe with a narrow-gauge (No. 27) hypodermic needle, fill the syringe with 1X Lysis Buffer. The syringe plunger is used to displace the buffer as fully as possible. This removes all the air from the syringe and prevents excess air being pumped into the cell suspension during lysis. Draw the cell suspension slowly into the syringe and then eject with a single rapid stroke. Repeat five times.
Note: The number of strokes needed (using the tissue homogenizer or the syringe) varies between cell lines. Start with 5 strokes and then check lysis under the microscope. Lysis should be 80-90%. If the lysis is not sufficient, perform several more strokes until lysis is complete. Lysis can be observed by the addition of the Trypan Blue solution to an aliquot of cells. The dye is excluded from the intact cells, but stains the nuclei of lysed cells. If nuclear lysis or clumps of nuclei are visualized, or if a gelatinous mass is observed, the cell disruption was too vigorous or too many strokes were performed.
8. Centrifuge the disrupted cells in suspension for 20 minutes at 10,000–11,000 x g.
9. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
10. Add 1.5 ml of the prepared 0.1 M DTT solution and 1.5 ml of the protease inhibitor cocktail to 147 ml of the 1X Extraction Buffer. If the proteins of interest are extracted at a lower salt concentration, dilute the Extraction Buffer with the 1X Dilution and Equilibration Buffer. Note: The salt concentration in the Extraction Buffer is 0.42 M, a commonly used extraction condition. In rare cases a lower or a higher salt concentration may be needed for a better extraction of a particular protein. In that case, dilute the Extraction Buffer with the 1X Dilution and Equilibration Buffer or add NaCl to the Extraction Buffer to reach the desired salt concentration.
11. Resuspend the crude nuclei pellet in ~140 ml (2/3X PCV) of Extraction Buffer containing the DTT and protease inhibitors. If the procedure is being performed with a

tissue homogenizer, it is recommended to give 10 more strokes at this point.
12. Shake gently for 30 minutes.
13. Centrifuge for 5 minutes at 20,000-21,000 x g.
14. Transfer the supernatant to a clean, chilled tube.
15. Snap-freeze the supernatant in aliquots with liquid nitrogen and store at -70 centigrade. C. Nuclear protein extraction from 100 mg of tissue Calculate accordingly for different tissue weight.
1. Dilute the 1 M DTT solution to 0.1 M with deionized, sterile water.
2. Prepare 1X Lysis Buffer
· For tissues tested by Sigma the hypotonic buffer works better than the isotonic. Therefore, it is recommended to prepare 1X Lysis Buffer, hypotonic. If the tissue is found to be too fragile, one can use the 1X Lysis Buffer, isotonic.
· Add 10 ml of the prepared 0.1 M DTT solution and 10 ml of the protease inhibitor cocktail to 1000 ml of 1X Lysis Buffer.
3. Rinse the tissue twice with PBS buffer. Discard the PBS.
4. Resuspend the tissue gently in 1,000 ml (5X PCV) of the 1X Lysis Buffer (containing DTT and protease inhibitors).
5. Homogenize the tissue until more than 90% of the cells are broken and nuclei are visualized under the microscope.
6. Centrifuge the disrupted cells in suspension for 20 minutes at 10,000–11,000 x g.
7. Transfer the supernatant to a fresh tube. This fraction is the cytoplasmic fraction.
8. Add 1.5 ml of the prepared 0.1 M DTT solution and 1.5 ml of the protease inhibitor cocktail to 147 ml of the Extraction Buffer. Note: If one wishes to extract nuclei proteins with a lower salt concentration, the Extraction Buffer may be diluted with 1X Dilution and Equilibration buffer.
9. Resuspend the crude nuclei pellet in ~140 ml (2/3X PCV) of Extraction Buffer containing DTT and protease inhibitor. At this stage a short homogenization can be performed to facilitate nuclear extraction.
10. Shake gently for 30 minutes.
11. Centrifuge for 5 minutes at 20,000 – 21,000 x g.

12. Transfer the supernatant to a clean, chilled tube.
13. Snap-Freeze the supernatant in aliquots with liquid nitrogen and store at -70 centigrade.

D. Salt Removal The nuclear proteins extracted according to the protocol are suspended in Extraction Buffer, a high salt buffer. Usually the proteins extracted are highly concentrated and can be diluted with a low salt buffer (1X Dilution and Equilibration Buffer). Since small amounts of the concentrated extract in a high salt buffer are sufficient for analysis by EMSA, footprinting, and similar assays, the salt dilution occurs naturally in the reaction tube itself. If salts interfere with further experiments, removal of salts may be performed rapidly using desalting gel-filtration columns (see Reagents and Equipment Recommended for Salt Removal). The desalting columns require equilibration with 1X Dilution and Equilibration Buffer supplemented with DTT. The protease inhibitor cocktail should be added to the eluted protein fraction. The salts may also be removed by dialysis of the nuclear extracts against a dialysis buffer that is similar to the 1X Dilution and Equilibration Buffer, containing a final concentration of 1 mM DTT and protease inhibitor cocktail or 0.5 mM AEBSF.

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