Nuclear/Cytosol Isolation Kit

Cat. No. NCI-013K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This Nuclear/Cytosol Extraction Kit provides a complete system that enables the separation of nuclear extract from the cytoplasmic fraction of mammalian cells and tissues The new kit provides a system that maintains the nuclear and cytoplasmic compartments separate and intact. Transcriptional activity/reporter assays/enzyme activity assays/Western blotting and more downstream assays.
Storage	Store kit at -20centigrade. Briefly spin small vials prior to opening.
Kit Components	• Cytosol Extraction Buffer A (CEB-A) • Cytosol Extraction Buffer B (CEB-B) • Nuclear Extraction Buffer (NEB) • DTT (1 M) • Protease Inhibitor Cocktail (lyophilized)
Target Species	Mammalian
Features & Benefits	• Simple procedure; takes less than 2-3 hours • Fast and convenient • The optimized reagents and procedures provided with the kit allow separation of nuclear and cytoplasmic extracts from cells relatively quick with little or no cross-contaminations
Preparation	1. After opening the kit, you may store buffers at +4centigrade or –20centigrade. Store Protease Inhibitor Cocktail and DTT at –20centigrade.

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	2. Add 250 µDMSO to dissolve the 500X Protease Inhibitor Cocktail before use. 3. Before starting the procedure, prepare enough Nuclear Extraction Buffer Mix (NEB Mix) and Cytosol Extraction Buffer A Mix (CEB-A Mix) for your experiment: Add 2 µ Protease Inhibitor Cocktail and 1 µDTT to each of 1 ml of NEB and each of 1 ml of CEB-A, individually. 4. Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures should be performed at 4centigrade. 5. The following protocol is described for fractionation of up to 2 x 10^6 cells. The procedure is also applicable for large-scale preparations (e.g., up to 10^9 cells) by scaling up the volume.
Separation Protocol	1. Collect cells by centrifugation at 600 x g for 5 minutes at 4centigrade. 2. Add 0.2 ml CEB-A Mix containing DTT and Protease Inhibitors (prepared as in Section A). If using tissue samples, cut the tissue (100-200 mg) into small pieces, add ice cold PBS (1-2 ml), and homogenize in a tissue homogenizer. Pellet the cells by centrifugation at 500 x g for 2-3 minutes and remove the supernatant. Add 0.2 ml of the CEB-A mix. 3. Vortex vigorously on the highest setting for 15 second to fully resuspend the cell pellet.Incubate the tube on ice for 10 minutes. 4. Add 11 µof ice-cold Cytosol Extraction Buffer-B to the tube. Vertex 5 seconds on the highest setting. Incubate on ice for 1 minute. 5. Vortex 5 seconds on the highest setting. Centrifuge the tube for 5 minutes at maximal speed in a microcentrifuge (16,000 x g). 6. Immediately transfer the supernatant (Cytoplasmic extract) fraction to a clean pre-

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chilled tube. Place the tube on ice.

7. Resuspend the pellet (contains nuclei) in 100 µl of ice-cold Nuclear Extraction Buffer Mix.

8. Vortex on the highest setting for 15 seconds. Return the sample to ice.

9. Repeat Step 8 for every 10 minutes for a total 40 minutes.

10. Centrifuge the tube at full speed (16,000 x g) in a microcentrifuge for 10 minutes.

11. Immediately transfer the supernatant (Nuclear extract) to a clean pre-chilled tube. Place on ice. Store extract at –80centigrade for future use.

Note: Nuclear extract prepared using the above procedure contains proteins in a concentration ~1 mg/ml. If higher concentration is desired, the nuclei can be resuspended in less volume of NEB-Mix (such as 20 µl) in Step 7.

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