KAMIYA BIOMEDICAL COMPANY

Nuclear/Cytosol Fractionation Kit

For the separation of nuclear extract from the cytoplasmic fraction of mammalian cells

Cat. No. KT-388

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Nuclear/Cytosol Fractionation Kit is for the separation of nuclear extract from the cytoplasmic fraction of mammalian cells. For research use only, not for use in diagnostic procedures.

PRINCIPLE
This Nuclear/Cytosol Extraction Kit provides a complete system that enables the separation of nuclear extract from the cytoplasmic fraction of mammalian cells. The optimized reagents and procedures provided with the kit allow separation of nuclear and cytoplasmic fractions quickly with little or no cross-contaminations. The extracted nuclear and cytoplasmic protein fractions are functional and compatible with downstream assays such as transcriptional activity, RNA splicing, gel shift assay, reporter assays, enzyme activity assays, and Western blotting.

COMPONENTS

• Cytosol Extraction Buffer A (CEB-A)	20 mL	(WM cap)
• Cytosol Extraction Buffer B (CEB-B)	1.2 mL	(Green cap)
• Nuclear Extraction Buffer (NEB)	10 mL	(NM cap)
• DTT (1 M)	100 µL	(Blue cap)
• Protease Inhibitor Cocktail (lyophilized)	1 vial	(Red cap)

PROTOCOLS
A. General Consideration and Reagent Preparations:
- After opening the kit, you may store buffers at 4°C or –20°C. Store Protease Inhibitor Cocktail and DTT at –20°C.
- Add 250 µL DMSO to dissolve the 500X Protease Inhibitor Cocktail before use.
- 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 µL Protease Inhibitor Cocktail and 1 µL DTT to each of 1 mL of NEB and each of 1 mL of CEB-A, individually.
- Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures should be performed at 4°C.
- 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.

B. Nuclear/Cytosol Fractionation Protocol:
1. Collect cells by centrifugation at 600 x g for 5 minutes at 4°C.
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 seconds to fully resuspend the cell pellet. Incubate the tube on ice for 10 minutes.
4. Add 11 µL of ice-cold Cytosol Extraction Buffer-B to the tube. Vortex 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-chilled tube. Place the tube on ice.
7. Resuspend the pellet (contains nuclei) in 100 µL of ice-cold Nuclear Extraction Buffer Mix (prepared as in Section A).
8. Vortex on the highest setting for 15 seconds. Return the sample to ice.
9. Repeat step 8 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 -80°C 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.

STORAGE

Store at -20°C until the expiration date.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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