Nuclear/Cytosol Fractionation Kit

(Catalog #K266-25, -100; Store kit at -20°C)

I. Introduction:

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.

II. Kit Contents:

Components	K266-25 25 Assays	K266-100 100 Assays	Cap Code	Part Number
Cytosol Extraction Buffer A (CEB-A)	5 ml	20 ml	NM/WM	K266-xx(x)-1
Cytosol Extraction Buffer B (CEB-B)	300 µl	1.2 ml	Green	K266-xx(x)-2
Nuclear Extraction Buffer A (NEB)	2.5 ml	10 ml	Amber NM	K266-xx(x)-3
DTT (1 M)	100 µl	100 µl	Blue	K266-xx(x)-4
Protease Inhibitor Cocktail	1 vial	1 vial	Red	K266-xx(x)-5

III. General Consideration and Reagent Preparation:

1. After opening the kit, you may store the buffers at +4°C or -20°C. Store Protease Inhibitor Cocktail and DTT at -20°C.
2. Add 250 µl DMSO 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 µl Protease Inhibitor Cocktail and 1 µl DTT to each of 1 ml of NEB and each of 1 ml of CEB-A, individually. ***[Note: If you plan on using the HAT Activity Assay Kit, K332-100, OMIT the DTT-this will interfere with the HAT Activity Assay]***
4. Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures should be performed at 4°C.
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

IV. Nuclear/Cytosol Fractionation Protocol:

1. Collect cells by centrifugation at 600 x g for 5 min 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 min and remove the supernatant. Add 0.2 ml of the CEB-A mix.
3. Vortex vigorously on the highest setting for 15 sec to fully resuspend the cell pellet .Incubate the tube on ice for 10 min.
4. Add 11 µl of ice-cold Cytosol Extraction Buffer-B to the tube. Vertex 5 sec on the highest setting. Incubate on ice for 1 min.
5. Vortex 5 sec on the highest setting. Centrifuge the tube for 5 min 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 IIIA).
8. Vortex on the highest setting for 15 sec. Return the sample to ice.
9. Repeat Step 8 for every 10 min for a total 40 min.
10. Centrifuge the tube at full speed (16,000 x g) in a microcentrifuge for 10 min.
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.

V. Published References Citing the Product:

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