

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

III. Nuclear/Cytosol Fractionation Protocol:

A. General Consideration and Reagent Preparation:

- After opening the kit, you may store buffers at $+4^{\circ}\text{C}$ 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 2×10^6 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:

- Collect cells by centrifugation at 600 x g for 5 minutes at 4°C .
- 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.

- Vortex vigorously on the highest setting for 15 second to fully resuspend the cell pellet. Incubate the tube on ice for 10 minutes.
- 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.
- Vortex 5 seconds on the highest setting. Centrifuge the tube for 5 minutes at maximal speed in a microcentrifuge (16,000 x g).
- Immediately transfer the supernatant (Cytoplasmic extract) fraction to a clean pre-chilled tube. Place the tube on ice.
- Resuspend the pellet (contains nuclei) in 100 μl of ice-cold Nuclear Extraction Buffer Mix (prepared as in Section IIIA).
- Vortex on the highest setting for 15 seconds. Return the sample to ice.
- Repeat Step 8 for every 10 minutes for a total 40 minutes.
- Centrifuge the tube at full speed (16,000 x g) in a microcentrifuge for 10 minutes.
- 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.

IV. Published References Citing the Product:

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