

Instruction Manual

Catalog Number	PK-CA577-K266-25		
Description	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.		
Quantity	25 assays		
Kit Components		Quantity	Cap code
		(25 assays)	(100 assays)
	Cytosol Extraction Buffer A (CEB-A)	5 ml	20 ml
	Cytosol Extraction Buffer B (CEB-B)	300 μ l	1.2 ml
	Nuclear Extraction Buffer (NEB)	2.5 ml	10 ml
	DTT (1 M)	100 μ l	100 μ l
	Protease Inhibitor Cocktail (lyophilized)	1 vial	1 vial
			WM Green NM Blue Red
General Consideration & Reagent Preparation	<ol style="list-style-type: none"> 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 1 ml of NEB and each 1 ml of CEB-A, individually. Note: If you plan on using the HAT Activity Assay Kit (PK-CA577-K332) omit the DTT as this will interfere with the HAT Activity Assay! Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures should be performed at 4°C. 		
Applications / Assay Protocol	<p>The following protocol is described for fractionation of up to 2 x 10⁶ cells. The procedure is also applicable for large-scale preparations (e.g., up to 10⁵ cells) by scaling up the volume.</p> <p>Nuclear/Cytosol Fractionation Protocol:</p> <ol style="list-style-type: none"> 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). <p>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 (e.g. PK-CA577-1998). 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.</p> <ol style="list-style-type: none"> Vortex vigorously on the highest setting for 15 seconds 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 (see <i>Reagent Preparation</i> above). Vortex on the highest setting for 15 seconds. Return the sample to ice. Repeat Step 8 every 10 minutes for a total of 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.
Storage & Stability	Store kit at -20°C upon arrival. Store individual reagents as indicated on the respective labels.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.

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