ab289882 – Nuclear/Cytosol Fractionation Kit

For isolation of nuclear/cytosol fractions without ultracentrifugation.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab289882

Storage and Stability

The entire kit can be stored at -20°C. After opening the kit, store all Buffers, Protease Inhibitor Cocktail I and DTT II at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
Extraction Buffer VI	20 mL	-20°C
Lysis Buffer X	1.2 mL	-20°C
Extraction Buffer V	10 mL	-20°C
DTT II	100 µl	-20°C
Protease Inhibitor Cocktail I	1 vial	-20°C

Reagent Preparation

- Always keep all buffers on ice during the experiment.
- All centrifugation procedures should be performed at 4°C.

Protease Inhibitor Cocktail I: Add 250 µL DMSO to dissolve the 500X Protease Inhibitor Cocktail I before use.

Extraction Buffer V: Before starting the procedure, prepare enough Extraction Buffer V and Extraction Buffer VI for your experiment: Add 2 µL Protease Inhibitor Cocktail I and 1 µL DTT II to each of 1 mL of Extraction Buffer V and each of 1 mL of Extraction Buffer VI, individually.

ΔNote: *If you plan on using the Histone Acetyltransferase Activity Assay Kit, ab65352, OMIT the DTT II as this will interfere with the Histone Acetyltransferase Activity Assay.*

Assay Protocol

– This 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 109 cells) by scaling up the volume.

1. Collect cells by centrifugation at 600 x g for 5 min at 4°C.
2. Add 0.2 mL Extraction Buffer VI Mix containing DTT II and Protease Inhibitors (prepared as in Reagent Preparation section). 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 Extraction Buffer VI.
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 Lysis X to the tube. Vortex 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 prechilled tube. Place the tube on ice.
7. Resuspend the pellet (contains nuclei) in 100 µL of ice-cold Extraction Buffer V (prepared as in Reagent Preparation section).
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.

ΔNote: 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 Extraction Buffer V (such as 20 µL) in Step 7.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)