Product Information

Nuclear/Cytosol Fractionation Kit

I. Kit Contents:

Components	K2111-25 25 assays	K2111-100 100 assays	Cap Color	Part Number
Cytosol Extraction Buffer A (CEB-A)	5 ml	20 ml	WM	K2111-C-1
Cytosol Extraction Buffer B (CEB-B)	300 µl	1.2 ml	Green	K2111-C-2
Nuclear Extraction Buffer A (NEB)	2.5 ml	10 ml	NM	K2111-C-3
DTT (1 M)	100 µl	100 µl	Blue	K2111-C-4
Protease Inhibitor Cocktail	1 vial	1 vial	Red	K2111-C-5

II. Introduction:

Nuclear/Cytosol Extraction Kit enable a fast and convenient separation of nuclear extract from the cytoplasmic fraction of mammalian cells with little or no cross-contaminations. The extracted nuclear and cytoplasmic protein fractions can be analyzed by western blotting, enzyme activity assays and reporter assays etc.

III. General Consideration and Reagent Preparation:

1. After opening the kit, you may store buffers at +4℃ or -20℃. Store Protease Inhibitor Cocktail and DTT at -20℃.
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. y.

Note: If you plan on using the HAT Activity Assay Kit, 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℃.
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 minutes at 4℃.
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 second 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. Vertex 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 IIIA).
8. Vortex on the highest setting for 15 seconds. Return the sample to ice.
9. Repeat Step 8 for 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℃ 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.

Frequently Asked Questions:

1. Can we use frozen cells?

No,freezing will break up the nucleus.

2. What kind of protein yield you can get using the kit?

Around 50 - 100 µg nuclear protein extract.

3. Where are the membrane, ER, mitochondrial, etc. proteins?

All are in cytosol.

4. What should I do if my sample is too dilute?

Spin-filter at 3500 RPM. This will get rid of water and the salts it contains and also the glycerol which is part of nuclear fraction.

5. What should I do if the cells clumped when I vortexed them in step 8?

This happens often. That is why step 9 says “Repeat step 8 every 10 minutes”. What also helps is if you break the clump by pipetting up and down several times.

6. What Can I do with contaminations between the two fractions?

There are 3 things the customer can do to reduce contamination:

1) Make sure all the cells are suspended well in the CEB-A mix before adding CEB-B by combination of pipette and vortex, so that all cells are lysed by CEB-B. Some cells tend to associate together, over-trypsinization also can make the cells associate together.

2) Completely remove the cytosol fraction by a quick spin to remove any leftover cytosol fractions in the nuclei.

3) If contamination is still a issue, wash the nuclei with PBS once or twice before extracting the nuclei.

7 .Can we obtain the native chromatin through this kit or only the total protein?

You can isolate only the protein. At step 7, you obtained the whole nuclei (including chromatin and all nuclear proteins). However, at the final step, you obtained the total soluble nuclear protein extracts. The chromatin bound insoluble proteins have been precipitated at step 10.

8. How does the nuclear/cytosol seperation occur?

The CEB-A helps in suspending the cells. CEB-B is detergent which can lyse the cell membrane, but not the nuclear. NEB extracts the nuclear fraction.

9. Why do I have some ppt. in my reaction?

This precipitate is due to a small amount of protease inhibitor precipitating when diluted in buffer. Either spin down to remove this precipitate, or do not remove the precipitate and continue the experiment as is. It will cause no adverse effect.

10. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

11. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

12. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

13. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the data sheet for storage information and shelf life of each of the components.

14. Why are my standard curve values lower than those shown on the data sheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

15. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

16. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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