PI-0107-25, -100

Nuclear/Cytosol Fractionation Kit

(25 or 100 preps, Store kit at 4°C)

Introduction:

Isolation of nuclear material, free of cytoplasmic contaminants and/or cytoplasmic proteins, free of nuclear components is needed for a range of analytical purposes. AkrivisBio's Nuclear/Cytosol Fractionation Kit provides an efficient system which enables the separation of nuclear material from the cytoplasmic fraction of mammalian cells with little to no cross-contamination. Our optimized reagents and protocol require only a tabletop centrifuge, sample tubes and pipettors. A complete isolation procedure can be completed in as little as 2 hours. The extracted nuclear and cytoplasmic protein fractions are functional and compatible with a variety of downstream applications such as measurement of transcriptional activity, RNA splicing, gel shift assay, reporter assay, enzyme activity assay, or blotting.

Kit Components:	**25 Preps**	**100 Preps**		
Cytosol Extraction Buffer (CEB)	5 ml	20 ml	NM/WM	PI-0107A
Detergent Solution	1.2 ml	1.2 ml	Green	PI-0107B
Nuclear Extraction Buffer (NEB)	2.5 ml	10 ml	Amber NM	PI-0107D
DTT	100 µl	100 µl	Blue	PI-0107D
Protease Inhibitor Cocktail	lyoph	lyoph	Red	PI-0107E

User Supplied Materials:

DMSO
PBS

Storage and Handling:

Store unopened kit at 4°C. Centrifuge all vials briefly before opening to recover any material which might be lodged around the cap. Bring all components to room temperature before using.

Protease Inhibitor Cocktail: Add 250 µl of anhydrous DMSO and dissolve to make a 500X solution. Store at -20°C after reconstitution.
DTT: Ready to use as supplied. Store at 4°C.
Cytosol Extraction Buffer, Nuclear Extraction Buffer: Prepare 1 ml of each buffer for each 10 x 10^6 cells to be fractionated. For each 1 ml of buffer, add 2 µl of 500X Protease Inhibitor Cocktail and 2 µl of DTT solution and vortex.

Note: Some down-stream applications are affected by the presence of DTT such as some enzyme activity assays, native gel electrophoresis, redox sensitive analyses, etc. If you intend to use the isolated preparations for one of these, the DTT may be omitted.

Note: Place buffers and detergent solution on ice for at least 15 minutes before starting the isolation protocol. Keep everything ice-cold at all times to optimize enzyme stability in the final preparations.

The following protocol is designed for 2 x 10^6 cells. It can be scaled up as needed by increasing the buffer volumes but maintaining ice-cold temperatures at all times can be challenging for larger volumes.

Fractionation Protocol:

1. Mince 100-300 mg tissue into small (~1-2 mm) pieces with scissors or razor blade in 1-2 ml of ice-cold PBS and work as rapidly as possible.
2. Transfer to a small homogenizer on ice and homogenize with 3-5 passes with a loose pestle.
3. Pellet cells (cultured cells enter the protocol here) by centrifugation at 600 x g (~ 2700 rpm on a tabletop centrifuge) for 5 min at 4°C. Remove supernatant without disturbing the pellet.
4. Add 0.2 ml prepared Cytosol Extraction Buffer Mix containing DTT and Protease Inhibitor Cocktail.
5. Vortex vigorously on the highest setting for 15 sec to fully resuspend the cell pellet and place on ice for 10 minutes.
6. Add 11 µl of ice-cold Detergent Solution to the tube. Mix by vortexing 5 sec on the highest setting then place on ice for 1 min.
7. Vortex 5 sec on the highest setting. Centrifuge the lysed cells for 5 min at maximum speed (16,000 x g) in a refrigerated tabletop centrifuge.
8. Quickly transfer the supernatant (Cytoplasmic Fraction) to a labeled, fresh pre-chilled tube, take 1-5 µl for a protein determination and store the rest at -80°C.
9. Resuspend the pellet (Nuclear Fraction) in 100 µl of ice-cold Nuclear Extraction Buffer Mix containing DTT and Protease Inhibitor Cocktail.
10. Vortex vigorously on the highest setting for 15 seconds then return the Nuclear Fraction to the ice.
11. Repeat Step 10 3 more times, every 10 min for a total of 40 min.
12. Centrifuge Nuclear Fraction in a refrigerated tabletop centrifuge at maximum speed (16,000 x g) for 10 minutes.
13. Transfer the supernatant (Nuclear Fraction) to a labeled, fresh pre-chilled tube.
14. Take 1-5 µl for protein determination and store the rest at -80°C.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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