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Product Datasheet
Nuclear and Cytoplasm Extraction kit

Introduction

The Nuclear & Cytoplasmic Extraction Kit is used for the separation of cytoplasmic and nuclear fractions from cultured mammalian cells for expression of proteins and transport studies as well as proteomic analysis. This kit is based on organic buffers and contains a proprietary combination of different reagents. The kit is supplied with enough reagents to purify 100 cell pellet fractions where a pellet fraction is a 20 µl wet cell volume of 2 x 106 cells.

Reagent Supplied

Hypotonic Buffer (HB)	25 ml
Cell Lysis Reagent (CLR)	1.5 ml
Nuclear Extraction Buffer (NEB)	15 ml

Storage Conditions

The kit is shipped at 4oC. Store the kit components at -20°C upon arrival. The kit is stable for one year when stored unopened. Use aseptic techniques when handling the reagent solutions.

Use Limitations

For research use only. Not for diagnostic or therapeutic use.

Items Required

a) Centrifuge and Centrifuge Tubes.
b) Phosphate Buffer Solution (PBS)
c) Protease Inhibitor Cocktail

Before Use

a) All buffer should be kept ice cold.
b) All Centrifugation steps should be performed at 4°C
c) Add appropriate Protease inhibitor cocktail to HB and NEB just before use

Procedure

Cultured Cells Preparation:. This procedure is validated for HEK293, HeLa, JURKAT and U937 Cell lines.
This procedure is for processing fresh samples using 2 -10 x 106 mammalian cells. It can be scaled up and down according to the Table 1.

1. Cell Harvest:
   i) Adherent Cells: Harvest adherent cells with trypsin-EDTA and centrifuge at 310 x g for 3 minutes.
   ii) Suspension Cells: Harvest cells by centrifuging at 310 x g for 3 minutes.
2. Carefully remove all the supernatant with a pipette leaving cell pellet as dry as possible.
3. Wash cells twice by suspending the cell pellet with 1ml ice cold PBS and centrifuge at 310 x g for 3 minutes.
4. Carefully remove all the supernatant with pipette leaving cell pellet as dry as possible .
5. Re-suspend cells in ice cold 1× HB by pipetting several times. Incubate tube on ice for 15 minute for Hek293 cells and 30 minutes for HeLa cells and other tested cell lines (need to optimize this step for other cell lines)
6. Add ice cold CLR to the tube.
7. Vortex the tube for 10 second on the highest setting.
8. Centrifuge the tube for 10 minutes at maximum speed (12000 x g) however for lymphoblast or white blood cells derived cell lines like Jurkat, K562 cells etc; it is recommended to use 3000 RPM for 5 min.
9. Immediately transfer the supernatant (cytoplasmic extract) to a clean pre-chilled tube. Place the tube on ice until use or store at -80°C / liquid nitrogen. Wash the pellet twice with 250 µl HB and remove the supernatant. Care should be taken not to lose the pellet.
10. Resuspend the insoluble pellet fraction which contains nuclei in ice cold NEB. Vigorously vortex for 15 seconds. Then, incubate for 30 minutes with vortexing at 10 minutes intervals.
11. Centrifuge tube for 30 minutes at maximum speed (>12000 x g) at 4°C.
12. Immediately transfer the supernatant fraction to a clean pre-chilled tube.
13. Store extracts at -80°C or liquid nitrogen until use.

Table 1

Volume of reagents for wet cell pellet volumes

Packed cell volume (µl)	HB (µl)	CLR (µl)	NEB (µl)
20	200	11	100
50	500	27.5	250
100	1000	55	500

*For HeLa cells, 2 × 106 cells is equivalent to 20 µL packed cell volume.

Troubleshooting

Problem	**Probable Cause**	**Suggestions**
Low cytoplasmic protein yield	Cells were not lysed	Increase amount of HB
	Cell pellet was not dispersed	Vortex thoroughly
Poor separation of nuclear and cytosolic proteins	Poor cell lysis	Ensure all residual PBS is removed prior to addition of HB Extend vortex time and use maximum vortex speed to ensure cells fully dispersed. Increase incubation times
	Cytoplasmic fraction not completely removed	Ensure all supernatant is removed from nuclear pellet prior to its lysis Briefly centrifuge nuclear pellet after supernatant is removed to collect any excess supernatant Wash nuclear pellet with HB
Low nuclear protein yield	Cell pellet was not dispersed	Vortex thoroughly
	Incomplete nuclei isolation	Increase time of centrifugation following addition of NEB
No or low protein activity detected	Samples were not kept cold	Centrifuge at 4°C and keep samples on ice between vortexing steps
	Presence of proteases	Use a protease inhibitor cocktail

Sample results

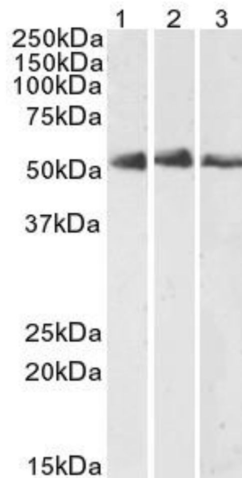

Fig: 1 Comparison of Different Nuclear lysates of cell lines; 1. HEK293 2. Hela 3. U937. Prepared using Nuclear and cytoplasm extraction Kit (#SB-NEK-100). Tested with Primary antibody goat anti-CD2BP2 at 0.1ug/ml

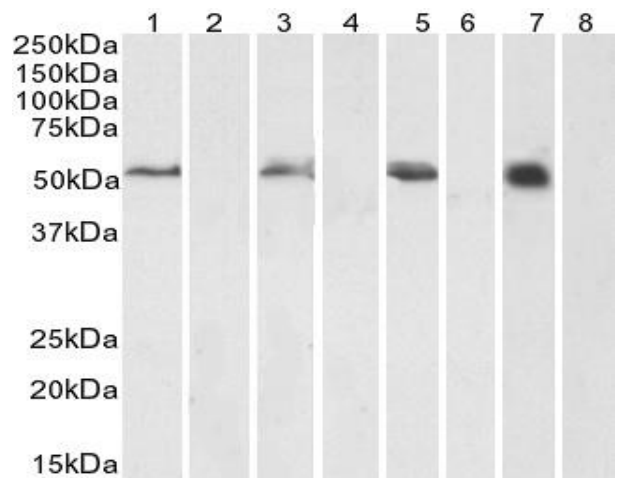

Fig: 2 Antibodies Tested on Nuclear and Cytosolic Lysates prepared using Nuclear and Cytoplasm Extraction Kit (#SB-NEK-100)
1-Nuclear Lysate of HeLa, 2- Cytosolic Lysate of Hela tested on Primary Antibody goat-anti CD2BP2 at 0.1ug/ml.
3-Nuclear Lysate of K56, 4- Cytosolic Lysate of K56 tested on Primary Antibody goat- PLCD3 at 0.1ug/ml.
5-Nuclear Lysate of U251, 6- Cytosolic Lysate of JURKAT tested on Primary Antibody goat-anti PLCD3 at 0.1ug/ml.
7-Nuclear Lysate of JURKAT, 8- Cytosolic Lysate of JURKAT tested on Primary Antibody goat-anti PLCD3 at 0.1ug/ml.