Nuclear and Cytoplasmic Protein Extraction Kit

I. Kit Content, Storage, and Stability

Content	Storage	(PP2201)
Cytoplasmic Protein Extraction Reagent A (CER A)	4 °C	26 ml
Nuclear Protein Extraction Reagent B (NER B)	4 °C	5 ml

All reagents are stable for 12 months if stored properly.

II. Principle

Nuclear and Cytoplasmic Protein Extraction Kit provides a simple and convenient method for extracting nuclear and cytoplasmic protein from cultured cells. The user can complete the separation of nuclear and cytoplasmic proteins in 90 minutes. High quality of protein is extracted and can be directly use for following reactions, such as Western blot, EMSA, foot printing，reporter gene detection, and measurement of enzyme activity. The Kit breaks cell membrane and releases proteins because of cell burst under hypotonic condition caused by Cytoplasmic Protein Extraction Reagent A; then centrifuge for collection of the nucleoli. At last, extract the nuclear proteins by Nuclear Protein Extraction Reagent B. The kit can be used for 100 samples (40 mg/sample, or 2 $\times 10^6$ Hela cells). You can adjust the volume of reagent accordingly for different sample volume.

III. Notes

1. PMSF (phenylmethylsulfonyl fluoride) should be prepared by user. PMSF has to be added into reagents 2-3 minutes before adding the reagents into samples to avoid that the PMSF gets inactive.
2. All steps for extraction should be performed on ice or at 4℃.
3. The Kit is designed for cultured cells and fresh tissue samples; it will not work efficiently for frozen tissue samples.

IV. Procedure

1. Prepare solutions: thaw two reagents in the kit, put them on ice once completely dissolved, and mix thoroughly. Take the proper volume of CER A and NER B and add PMSF (2-3 minutes before use) with 1 mM final concentration. If the target protein is rich in cysteine, please add DTT into CER A and NER B with 0.5 mM final concentration.
2.

2-1 : For adherent cells: wash with PBS and scrape cells, or treat cells by EDTA (Do not digest with pancreatin to avoid degradation of proteins). Then centrifuge at 1000 rpm for 2-3 minutes, discard supernatant, and keep cell pellets for use.

2-2 : For suspending cells: centrifuge at 1000 rpm for 2-3 minutes, discard supernatant, and keep the pellets for use.

2-3 : For fresh tissues (take the step A or B):

A: Cut the tissue into fine pieces and homogenize the pieces in PBS. Then centrifuge at 1000 rpm for 2-3 minutes, discard supernatant, estimate the volume of cell block, and proceed Step 4.

B: Weight the tissue, and cut the tissue into fine pieces. Add 500 μl CER A into

50 mg sample and transfer into a new tube after homogenization. Incubate for 10 min on ice. Proceed Step 7.

3 Add 200 μl CER A (containing PMSF) into 20 μl cell pellets (the volume of 2×10^6 Hela cells is about 20 μl or 40 mg).

4 Vortex for 5 seconds to suspend cells (If not complete, prolong the time of vortex).

5 Centrifuge at 1000 rpm for 1 minute and discard the supernatant.

6 Add 60 μl CER A (about 3 times of the volume of cell pellet), vortex for 5 seconds at high-speed, and incubate for 10 minutes on ice.

7 Vortex for 5 seconds at high-speed; and centrifuge at 11,000-12000 rpm at 4℃ for 10 minutes.

8 Immediately take the supernatant (**cytoplasmic proteins**) into a new tube. Then store at -70℃ or use directly. (Do not touch the precipitate, leave very small volume of supernatant to avoid touching.

9 Add 50 μl PBS to the precipitate, centrifuge, and remove the PBS. Add 50 μl NER B (containing PMSF).

10 Vortex for 30 seconds to suspend pallets; put back on the ice and vortex for 30 seconds every 5 minutes in following 40 minutes.

11 Centrifuge 11,000-12,000 rpm at 4℃ for 10 minutes.

12 Immediately take the supernatant (**nuclear proteins**) into a new pre-cooling tube. Then store at -70℃ or use directly.

V. Troubleshooting

Problem	Possible Reason	Advices
Low concentration	The ratio of reagents and cells is not appropriate.	20 μl (40 mg) cell block/200 μl CER A
Inactive or low activity	Operation is not on ice	Always operate on ice
	Proteinase activity is high	In addition to PMSF, also add other protease inhibitors to prevent degradation of proteins.
Low yield of nuclear protein	Incomplete lysis of cell block	Vortex more vigorously.
	Incomplete lysis of Nucleoli	Prolong the incubation time after adding CER A
There is a bad mixture of cytoplasmic and nucleic proteins	Incomplete lysis of cell	Increase the time of vortex and incubation on ice
	cell lyses time is too long	Decrease the time of vortex and incubation on ice
	Over or inadequate homogenization	Optimize the time and condition of homogenization