Nucleic and Cytoplasmic fluid extraction isolation kit

Product description

The study of the components of the nucleus and cytoplasm has always been an important direction of cell research, and the components in the cell, especially after the extraction and isolation of the nucleus, can not only realize the research of intracellular protein localization, but also realize the research on the transcriptional regulation mechanism of intranuclear proteins.

This kit provides a simple and convenient method for extracting cytoplasmic and intranuclear proteins from cultured cells and fresh tissue homogenizers, which can be used for subsequent experiments such as Western blot.

Composition and storage conditions

Size / Components	50rxns	100rxns	200rxns
Buffer A	25 mL	50 mL	100 mL
Buffer B	0.5 mL	1 mL	2 mL
Buffer C	2.5 mL	5 mL	10 mL
Buffer D	0.3 mL	0.6 mL	1.2 mL
Cell wash Buffer	250 mL	500 mL	1 L
Protease Inhibitor Cocktail (EDTA-Free, 100X in DMSO) (K1010)	0.3 mL	0.6 mL	1.2 mL

Buffer A/C and Cell wash Buffer: Store at 4 °C; Others: Store at -20°C.

Experimental manipulation

Cultured cell in vitro: all experiments require been operated on ice.

I. Preparation before use

1. Buffer A working solution: firstly, add Buffer D to Buffer A at the ratio of Buffer D: Buffer A=1:100; then add Protease Inhibitor Cocktail into the liquid in the previous step at the ratio of Protease Inhibitor Cocktail: Buffer A=1:100.
2. Buffer C working solution: firstly, add Buffer D into Buffer C at the ratio of Buffer D: Buffer

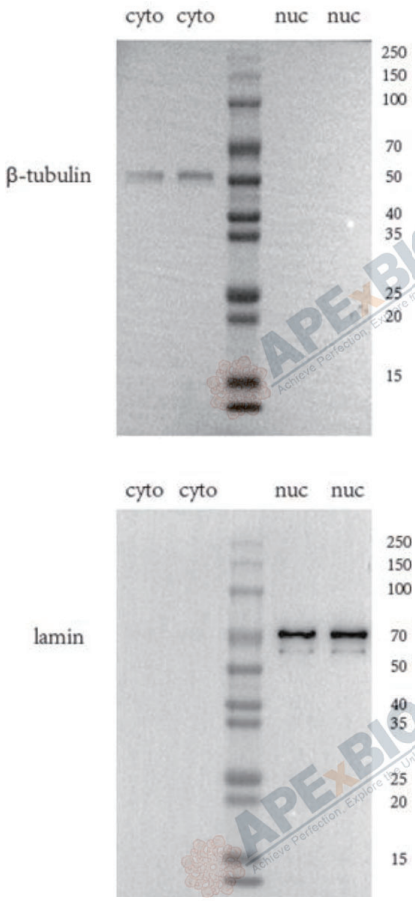
C=1:100; then add Protease Inhibitor Cocktail into the liquid in the previous step at the ratio of Protease Inhibitor Cocktail: Buffer C=1:100.

3. Cell wash Buffer is pre-cooled in advance.
4. Pre-cool the centrifuge at 4 degrees.

II. **Experimental steps**

1. The collected Cultured cells (1*10^6 cells) were washed with pre-cooled Cell wash Buffer and collected into 1.5 mL EP tubes, centrifuged at 500 g for 5 min and the supernatant was discarded.
2. Cells were washed again with pre-cooled Cell wash Buffer and centrifuged at 500 g for 5 min, discarding the supernatant.
3. 100 μL of Buffer A working solution was used to resuspend the centrifuged cells and incubated on ice for 10 min.
4. Rapidly add 5 μL of Buffer B to each EP tube and vortex 4 times for 10 s each time (*Note:* Fast).
5. Centrifuge the liquid from the previous step (Step 4) for 5 min at 5,000 rpm to obtain the supernatant, containing cytoplasmic proteins, which can be pipetted to a new 1.5 mL EP tube for subsequent use. (*Note:* The precipitate is cellular debris as well as components such as nuclei, so when aspirating cytoplasmic proteins, do not aspirate the substrate precipitate which may lead to contamination of subsequent nuclear protein extracts by cytoplasmic proteins).
6. After aspirating all the liquid from the EP tubes in the previous step (Step 5), add 100 μL of Buffer A working solution to each EP tube and resuspend the precipitate.
7. Centrifuge the resuspended liquid at 5,000 rpm for 5 min and discard the supernatant.
8. Repeat Steps 6 and 7 to completely remove cytoplasmic components (*Note:* optional step).
9. Slowly add 20-30 μL of Buffer C working solution into the sediment after centrifugation in the previous step (Step 7 or Step 8), while blowing evenly with a lance tip to obtain the final mucilage. (*Note:* The experimental operation time limit for this step is about 1 min)
10. Place the product obtained in the previous step (Step 9) on ice for 30 min, and vortex and oscillate for 10 s every 5 min.
11. Centrifuge the vortexed liquid at 16,000 g for 10 min and remove the supernatant, which is the nucleus solution.
12. The nucleus solution (step 11) and cytoplasmic solution (step 5) can be stored at -80°C for subsequent use.

Experimental validation plot



Western Blot analysis of cytoplasmic protein β-tubulin and intracellular protein Lamin A of 293T cells isolated by the kit listed above.

Instructions for use

1. Tissue sample processing: In an ice bath at 0°C, liver tissue can be ground directly to obtain individual cells and subsequently followed according to the instructions above, while other tissues are recommended to be treated with collagenase to obtain individual cells and subsequently followed according to the instructions.
2. The protein sample obtained by lysis cannot be determined by Bradford method due to interference with a higher concentration of detergent, and the protein concentration can be determined using the BCA protein quantification kit.
3. For tissue samples, this kit is suitable for fresh tissue and is less effective for frozen tissue extraction.
4. This product is for scientific use only.