Nc-nucleus/plasma protein extraction kit

Project number: N666081

Storage conditions: -20° C.

Products

individual parts making up a compound	50T
Nc-Buffer A	50ml
Nc-Buffer B	3ml
Nc-Buffer C	25ml
Protease Inhibitor Cocktail	750 μl

Products

The Nc-Nucleus/Plasma Protein Extraction Kit is a simple and rapid method for extracting nucleus and plasma proteins from mammalian cells and tissues, and the extracted proteins remain biologically active. The kit first cleaves the cell membrane and releases plasma proteins using the plasma protein extraction reagent, and then centrifuges the nucleus to obtain a nucleus precipitate. Finally, the nuclear proteins are extracted by the nuclear protein extraction reagent. The extracted nuclear and plasma proteins are of high purity, effectively avoiding cross-contamination of nuclear and plasma proteins, and can be used for subsequent operations such as Western, Gel Shift, reporter gene detection and enzyme activity determination.

caveat

1. If phosphorylated proteins are to be extracted, add a phosphatase inhibitor to the extraction reagent.
2. All sample handling should be done on ice.
3. The amount of reagents can be adjusted according to the specific experimental situation to ensure that the ratio of each reagent used is Nc-Buffer A:Nc-Buffer B:Nc-Buffer C = 100:5.5:50.
4. Higher speeds can be used for centrifugation.

procedure

I Extraction of cytoplasmic and cytosolic proteins from cells

1. Please remove the extraction reagents Nc-Buffer A and Nc-Buffer C for pre-cooling before protein extraction.
2. Collect the cells and count them. Centrifuge to remove supernatant.

3. 1×107 cells were added with 1 ml of Nc-Buffer A (added to Protease Inhibitor Cocktail at a ratio of 1:99 within 2-3 minutes prior to protein pumping), vortexed for 5 seconds to mix well, and incubated on ice for 20 minutes.
Note: The characteristics of various cells are different, and the amount of Nc-Buffer A needs to be adjusted according to the characteristics of different cells. If the protein concentration is small, reduce the amount of Nc-Buffer A and subsequent Nc-Buffer B and Nc-Buffer C proportionally.
4. Add 55 μl of Nc-Buffer B, vortex for 5 seconds to mix thoroughly, and incubate on ice for 1 minute.
5. Centrifuge at 12,000 rpm (~13,400 x g) for 15 minutes at 4° C, collect the supernatant (as clean as possible) into a new centrifuge tube and store at -20° C (this extract is cytoplasmic protein).
6. Add 500 μl of Nc-Buffer C (add Protease Inhibitor Cocktail at a ratio of 1:99 before use) to the precipitate obtained in the previous step, vortex for 5 seconds to mix thoroughly, resuspend the precipitate and incubate on ice for 40 minutes, vortexing and mixing at 10-minute intervals for about 15-30 seconds each time.
7. Centrifuge at 12,000 rpm for 15 minutes at 4° C, collect the supernatant (as clean as possible) into a new centrifuge tube and store at -20° C (this extract is for cytosolic proteins).
II Extraction of cytoplasmic and cytosolic proteins from tissues
1. Sampling and preservation of tissues.
2. Remove the extraction reagents Nc-Buffer A and Nc-Buffer C for pre-cooling before protein extraction.
3. Weigh the tissue and add 1 ml of Nc-Buffer A per 100 mg of tissue (add Protease Inhibitor Cocktail 2-3 minutes before protein extraction at a ratio of 1:99), homogenize well on ice with a homogenizer, and incubate on ice for 20 minutes.
Note: The characteristics of various tissues are different, and the amount of Nc-Buffer A needs to be adjusted according to different tissues. If the protein concentration is small, reduce the amount of Nc-Buffer A and subsequent Nc-Buffer B and Nc-Buffer C proportionally.
4. Add 55 μl of Nc-Buffer B, vortex for 5 seconds to mix thoroughly, and place on ice for 1 minute of incubation.
5. Centrifuge at 12,000 rpm for 15 minutes at 4° C, collect the supernatant (as clean as possible) into a new centrifuge tube and store at -20° C (this extract is cytoplasmic protein).
6. Add 500 μl of Nc-Buffer C (add Protease Inhibitor Cocktail at a ratio of 1:99 before use) to the precipitate obtained in the previous step, vortex for 5 seconds to mix thoroughly, resuspend the precipitate and incubate on ice for 40 minutes, vortexing and mixing at 10-minute intervals at , each time for about 15-30 seconds.
7. Centrifuge at 12,000 rpm for 15 minutes at 4° C, collect the supernatant (as clean as possible) into a new centrifuge tube and store at -20° C (this extract is cytosolic protein).