Nuclear and Cytoplasmic Protein Extraction Kit

Cat #: orb1566784 (manual)

Size: 50 tests

Product Composition

Reagent name	Specifications
Cytoplasmic Extraction Reagent A (CER A)	15 mL
Cytoplasmic Extraction Reagent B (CER B)	0.5 mL
Nuclear Extraction Reagent (NER)	2.5 mL
Manual	1 copy

Product Introduction

Nuclear and Cytoplasmic Protein Extraction Kit provides a simple and convenient method for extracting nuclear and cytoplasmic proteins from cultured cells or fresh tissues. The separation of nuclear and cytoplasmic proteins from cultured cells can be completed in about 90 minutes. The extracted proteins are non-denatured and active, and can be used for subsequent operations such as Western, EMSA, foot printing, reporter gene detection, and enzyme activity determination.

The extraction principle of this kit is to use CER A and CER B to fully swell the cells under low osmotic pressure conditions, destroy the cell membrane, release cytoplasmic proteins, and then obtain the cell nucleus precipitate by centrifugation. Finally, the nuclear protein is extracted by high-salt NER.

For cell samples, this kit can extract 50 samples, with a quantity of 2×10^6 Hela cells (about 40mg); for tissue samples, if the weight of each sample does not exceed 30mg, this kit can extract 50 samples, and if each sample is about 30-60mg, this kit can extract about 37 samples. The extraction scale can be scaled up or down as needed.

Operation Procedure

Prepare the solution: dissolve the three reagents in the kit at room temperature, place on ice immediately after dissolution, and mix well. Take appropriate amounts of CER A and NER for use, and add PMSF to a final concentration of 1mM within a few minutes before adding. If the target protein is rich in cysteine, add DTT to CER A and NER to a final concentration of 0.5mM.

1. **For adherent cells:** wash once with PBS, scrape the cells with a cell scraper, or treat the cells with EDTA solution so that the cells are no longer tightly attached to the wall, and blow the cells with a pipette (try to avoid digesting the cells with trypsin to prevent trypsin from degrading the target protein to be extracted). Centrifuge at 500g for 2-3 minutes, discard the supernatant as much as possible, and keep the cell pellet for use. Go to step 4.

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2. **For suspended cells**: wash once with PBS, centrifuge at 500g for 2-3 minutes, discard the supernatant as much as possible, and keep the cell pellet for use. Go to step 4.

3. **For fresh tissue:**

(Method 1) Cut the tissue into very small pieces as possible. Homogenize in PBS to make a cell suspension, centrifuge at 500g for 2-3 minutes, discard the supernatant, estimate the volume of the cell pellet, and proceed to step 4.

(Method 2) After weighing the tissue, cut the tissue into very small pieces as possible, add 500µl of CER A for every 50mg of tissue, transfer the homogenate to a plastic centrifuge tube after homogenization, and proceed to step 5. (In step 7, add 10µl of CER B for every 200µl of CER A.)

4. Add 200µl of CER A supplemented with PMSF for every 20µl of cell pellet. (For 2×10^6 Hela cells, the volume of the cell pellet is approximately 20µl or 40mg.)

5. Vortex at the highest speed for 5 seconds to completely suspend and disperse the cell pellet. (If the cell pellet is not completely suspended and dispersed, the vortex time can be appropriately extended.)

6. Ice bath for 10-15 minutes.

7. Add 10µl of CER B. Vortex at the highest speed for 5 seconds, and ice bath for 1 minute.

8. Vortex at the highest speed for 5 seconds, and centrifuge at 14,000-16,000g at 4℃ for 5 minutes.

9. Immediately aspirate the supernatant into a pre-cooled centrifuge tube, which is the extracted cytoplasmic protein. It can be used immediately or frozen at -70℃. (Do not touch the pellet. A very small volume of supernatant can be retained above the pellet to avoid touching the pellet.)

10. For the pellet, completely aspirate the remaining supernatant and add 50µl of NER with PMSF added. (Not aspirating the supernatant will contaminate the cytoplasmic protein.)

11. Vortex at the highest speed for 15-30 seconds to completely suspend and disperse the cell pellet. Then put it back in the ice bath, and vortex vigorously for 15-30 seconds every 1-2 minutes for a total of 30 minutes.

12. Centrifuge at 14,000-16,000g at 4℃ for 10 minutes.

13. Immediately aspirate the supernatant into a pre-cooled plastic tube, which is the extracted nuclear protein. It can be used immediately or frozen at -70℃.

Storage conditions

Store at -20℃, valid for one year.

Precautions

1. You need to prepare PMSF by yourself. PMSF must be added within 2-3 minutes before the extraction reagent is added to the sample, to prevent PMSF from quickly becoming ineffective in the aqueous solution.

2. All steps of protein extraction must be performed on ice or at 4℃. All reagents used need to be pre-cooled or thawed immediately to ensure a low temperature environment during the operation.

3. For tissue samples, this kit is only suitable for fresh tissues, and the extraction effect on frozen tissues is poor. The number of tissue samples that can be extracted is usually less than 50.

4. The nuclear protein and cytoplasmic protein extracted using this kit can be directly measured by BCA protein quantification kit. However, it is not suitable for measuring protein concentration using Bradford method.

5. For your safety and health, please wear a lab coat and disposable gloves during operation.

Troubleshooting

Problems	Possible Causes	Solutions
Low production of cytoplasmic proteins	Cells are not completely lysed.	Increase the dosage of CER B.
	Cell clumps are not completely dispersed.	Vortex violently and completely
Low production of nuclear protein	Cell clumps are not completely dispersed.	Vortex violently and completely
	Incomplete nuclear separation	After adding CER B, increase the centrifugation time
Low protein concentration	The volume ratio of extraction reagents to cell precipitates is not suitable	Add 200 μ l CER A according to the ratio of 20 μ l cell pellet (about 40mg)
Low or no protein activity	The sample was not subjected to low-temperature operation	Always centrifuge at low temperature, keep the sample on ice and pre cool the reagent
	High protease activity	In addition to PMSF, multiple protease inhibitors can be added to jointly inhibit protease activity
There is severe mixing between nuclear proteins and cytoplasmic proteins	Incomplete clearance of cytoplasmic extracts (cytoplasmic proteins)	Before extracting the nucleus, carefully remove all the supernatant extracted from the cytoplasm
	Incomplete cell lysis	Increase Vortex time and ice bath time
	Excessive cell lysis	Reduce Vortex time and ice bath time
	Excessive, insufficient, or uneven homogenization	Optimize homogenization time and conditions
Simultaneous low or no cytoplasmic/nuclear protein production	It may be related to the type of cells	This cell line may not be suitable for this method
During the cracking process, viscous liquid was found or nuclear cracking was observed under a microscope	Excessive lysis, complete nuclear lysis, and release of DNA	Reduce the proportion of CER B usage or do not add it; Reduce Vortex time and ice bath time

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