

Product Data Sheet

Product Name: Nuclear and Cytoplasmic Protein Extraction Kit

Cat. No.: GK10028

Components

Components	50 tests
Cytoplasmic protein extraction reagent A	15ml
Cytoplasmic protein extraction reagent B	0.5ml
Nuclear protein extraction reagent C	2.5ml
PMSF(100mM)	0.2ml

Features

Applications	A simple and convenient method for extracting nuclear and cytoplasmic proteins. 90 minutes to complete the separation of nuclear and cytoplasmic proteins. Extracted protein can be used for Western, EMSA and other subsequent operations.
Shipping	Ship with blue ice.
Storage	Store at -20°C for up to 1 year.
Usage	For Research Use Only! Not For Use in Humans.

Protocol

1. Prepare:

Keep the four reagents at room temperature to dissolution, mix and place on ice immediately after dissolution. Take an appropriate amount of cytoplasmic protein extraction reagent A for use, add PMSF just before use, and make the final concentration of PMSF 1mM. Take an appropriate amount of nuclear protein extraction reagent C for use, add PMSF just before use, and make the final concentration of PMSF 1mM.

2. Adherent cells:

Wash the cells with PBS, collect the cells with a cell scraper, or treat the cells with EDTA solution to loosen the cells, and blow the cells off with a pipette. Centrifuge to collect cells and discard the supernatant.

Avoid using trypsin to digest cells, to prevent degradation of the target protein that needs to be extracted.

3. Suspension cells:

Wash the cells with PBS, centrifuge to collect cells, and discard the supernatant.

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4. Add 200 μ L of cytoplasmic protein extraction reagent A containing PMSF to every 20 μ L of cell pellet. (For 2*10⁶ HeLa cells, the volume of the cell pellet is approximately 20 μ L or 40mg.)
5. Vortex vigorously for 5-10 seconds, avoid cell clumps.
6. Incubate on ice for 10-15 minutes.
7. Add cytoplasmic protein extraction reagent B 10 μ L. Vortex vigorously for 5 seconds, then incubate on ice for 1 minute.
8. Vortex vigorously for 5 seconds, centrifuge at 4°C 12,000g for 5 minutes.
9. Immediately transfer the supernatant to the new pre-cooled EP tube to obtain cytoplasmic protein. If not used immediately, it can be frozen at -70°C.
(Do not touch the pellet, you can keep a small volume of supernatant above the pellet to avoid touching it. The concentration of cytoplasmic protein in the supernatant after lysis with 200 μ L cytoplasmic protein extraction reagent A per 2*10⁶ cells was about 2-5mg/ml, varying by cell type.)
10. Aspirate all remaining supernatant from the tube containing the nuclear pellet, and add 50 μ L of nuclear protein extraction reagent C containing PMSF. (Failure to completely remove the supernatant can lead to contamination with cytoplasmic proteins.)
11. Vortex vigorously for 15-30 seconds to mix the pellet thoroughly. Then place back on ice, and Vortex vigorously every 1-2 minutes for a total of 30 minutes.
12. Centrifuge 12,000g at 4 ° C for 10 min.
13. Immediately transfer the supernatant to the new pre-cooled EP tube to obtain the nuclear protein. It can be used immediately or stored at -70°C. The supernatant obtained after lysis with 50 μ L nuclear protein extraction reagent C for every 2*10⁶ cells has a nuclear protein concentration of about 1.2-3.0mg/ml, which varying by cell type.
14. For fresh tissue:
 - a. Cut the tissue sample into as small pieces as possible. Mix the appropriate amount of cytoplasmic protein extraction reagent A and B in a ratio of 20:1 (for example, add 10 μ L cytoplasmic protein extraction reagent B to 200 μ L cytoplasmic protein extraction reagent A), then add PMSF until the final concentration was 1mM. Mix tissue and tissue homogenate at a ratio of 200 μ L tissue

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homogenate per 60mg tissue (for tissue samples not exceeding 30mg, only 100µL tissue homogenate should be added) and fully homogenate in a glass homogenizer. Homogenate should be carried out in ice bath or 4°C.

b. After homogenizing, transfer the homogenate to the EP tube and leave it in the ice bath for 15 minutes.

c. Centrifuge at 4°C 1,500g for 5 min. Transfer the supernatant to a new c pre-cooled EP tube to obtain a portion of the cytoplasmic protein. (Do not touch the pellet).

d. The remaining pellet contains many unbroken cells. Next, follow step 4 of this protocol to start the operation, that is, treat the precipitation as a cell precipitation operation that has been centrifuged and collected, and follow steps 4 to 13 to extract cytoplasmic proteins and nuclear proteins.

Note: For tissue samples starting with 30-60mg, directly follow steps 4-13; for samples not exceeding 30mg, use half of the volume of solutions used in steps 4-13.

The extracted cytoplasmic proteins can be combined with the extracted cytoplasmic proteins in step 14c. The protein concentration in the solution from fresh liver tissue lysis is approximately 3-10mg/ml for both cytoplasmic and nuclear proteins, which varies with different tissues samples.

15. Precautions :

a. PMSF should be added into protein extraction reagents 2-3 minutes before use to minimize its invalidation in solution.

b. All steps of protein extraction should be performed on ice or at 4°C.

c. This product is designed for cell samples and fresh tissue samples. It does not work efficiently for frozen tissues.

d. The isolated proteins using this kit could be quantified with the BCA Protein Assay kit. Bradford protein assay method should not be used.

Background

Nuclear and Cytoplasmic Protein Extraction Kit is a kit commonly used in molecular biology to extract nuclear and cytoplasmic proteins from cells or tissues. The separation and extraction process takes about 90 minutes. The protein product obtained can be used to study the localization and distribution of protein in the cell. The isolated nuclear protein can also be used for transcriptional regulation research, and can also be used for subsequent experiments such as Western blot, EMSA, footprinting, reporter gene detection and enzyme activity determination.

This product is based on cytoplasmic protein extraction reagents A and B. Under low osmotic pressure, cells can absorb water and burst, the cell membrane is destroyed, cytoplasmic protein is

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released, and the nucleus can be collected by centrifugal precipitation, so as to separate cytoplasmic protein and nuclear protein. The nuclear protein is then purified using a high-salt nuclear protein extraction reagent C.

For cell samples, the kit can extract 50 samples if the cell number of each sample is less than 2 million; For tissue samples, the kit can extract 50 samples if the weight of each sample is less than 30mg; and 37 samples can be extracted if each sample is about 30-60mg.

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