Nuclear protein Extraction Kit (without detergent)

Item No. : EX2480
Specification: 50T/100T
Validity: Stored at 2-8°C, valid for 1 year.

Product content:

Name	50T	100T	Storage conditions
Component A: Nuclear protein extract Solution A	30mL	60mL	Store at 2-8℃
Component B: nuclear protein extract solution B	10mL	20mL	Store at 2-8°C
Component C: protease inhibitor mixture	250μL	500μL	Store at -20℃

Note:

1. Protease inhibitors can also be stored at 2-8℃ before use without open lid. Store at -20℃ after opening the lid for use.
2. The protease inhibitor is solid at 2-8°C. Take it out of the refrigerator and return to room temperature or 37°C water bath for a short time. When it becomes liquid, centrifuge it to the bottom of the tube and then open the lid.
3. Please use the reagent as soon as possible after unpacking!

Product Introduction:

Nuclear protein extraction kit provides a complete set of reagents, suitable for extracting nuclear protein and cytoplasmic protein from a variety of primary or successive animal cells and various animal solid tissues, such as brain, spinal cord, nerve junction or fiber, fat, liver, digestive tract, kidney, heart, muscle, blood vessel, connective tissue and other animal tissues. The extraction process is simple and convenient, and can be completed within 1 hour. The prepared nucleoprotein and cytoplasmic protein not only have high purity, maintain natural activity, but also have little cross-contamination.

This kit contains a unique formula that effectively dissolves the nuclear membrane components of cells. The kit contains a protease inhibitor mixture that prevents protease from degrading the protein and ensures the extraction of high purity proteins.

The proteins extracted from this kit can be used for Western Blotting, transcriptional activity analysis, Gel shift gel block assay, immunocoprecipitation, enzyme activity determination and other protein studies.

All components of this kit do not contain detergent components, and the components of the final obtained protein sample have no influence on the downstream applications of NI column purification, molecular sieve, ion exchange, affinity purification, etc.

The protein extraction components of this kit do not contain detergent components that cannot be removed by dialysis, and do not contain SDS, TritonX-100, chaps and other components that may affect the mass spectrometry experiment. After dialysis or desalting treatment, the final protein sample will not contain detergent, high concentration salt and other effects. Basically, it can meet any downstream proteomic related experimental research.

This kit does not contain EDTA and is compatible with downstream applications such as metal chelation and chromatography.

The protein extracted by this kit is an active protein with natural protein conformation.

The protein samples extracted by this kit contain high concentration of salt components and cannot be directly used for 2D electrophoresis. If the downstream experiments need to be directly used for isoelectric focusing or two-dimensional electrophoresis, please use the kit of other product numbers. The final sample can also be desalted and then used for 2D electrophoresis with the desalting column.

Applicable samples: cells, tissues.

Self-prepared reagents and instruments:

Centrifuge, oscillator, homogenizer, vortex mixer, pipette, refrigerator, ice box, PBS buffer (pH7.4, 10mM phosphate buffer solution (1X) commonly used in laboratories) (phosphate buffer saline/Dulbecco's PBS: About 8mM Na2HPO4, 2mM KH2PO4, 137mM NaCl and 3mM KCl), protein quantification kit, centrifuge tube, suction tip, disposable gloves

Product Features:

1. Easy to use, extract protein from cells and tissues without grinding, repeated freeze-thaw, ultrasonic crushing and other pre-treatment.
2. The time of protein extraction is reduced to 30 minutes to 1 hour.
3. Containing protein stabilizer, the extracted protein is stable.
4. The background interference is low when the protein concentration is detected by UV.
5. The protein extract contains a variety of effective components, which can fully release cytoplasmic protein and nuclear protein, and can bind the released protein to prevent precipitation.
6. Protease inhibitor inhibits the degradation of protein, and the formula of protease inhibitor is optimized. The protease inhibitor mixture consists of 6 independent protease inhibitors AEBSF, Aprotinin, Leupeptin, Pepstatin A, Bestatin, E-64; Each inhibitor can specifically inhibit one or several protease activities. The composition of the mixture is optimized so that it can inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

How to use:

First, use precautions:

1. The reagent in the rotating cap centrifuge tube should be centrifuged briefly before opening the cap, and the liquid on the inner wall of the cap should be thrown to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20°C refrigerator. The sample must be kept at a low temperature during the whole process.
3. If the solution of protease inhibitor precipitates during storage, it will not affect the use, and it will be used normally after dissolution.
4. If the kit cannot be used up in a short time, the protease inhibitor mixture should not be added to the extraction solution all at once.
5. Other protease inhibitor products can be added as needed for your own experiment.
6. Lamin B, TBP and Histon H3 can be selected as the internal parameters of Western experiments.

2. Bacterial protein extraction:

1. Extraction solution preparation:

Add 1μL protease inhibitor mixture and 1μL phosphatase inhibitor for every 500μL of cold protein extract A.

Add 1μL protease inhibitor mixture and 1μL phosphatase inhibitor into every 200μL cold protein extract B. Mix well and set aside on ice.

[Note] :

1) Prepare the protein extract according to the number of samples to be processed, the protease inhibitor mixture cannot be added to the extract all at once. Phosphatase inhibitors may not be added all at once.
2) If the extract with a protease inhibitor is not used up within a week, the protease inhibitor needs to be added again before being used again.
3) The protein extract used in the following steps is a protease inhibitor-containing extract prepared for this step.

2. Take 5-10×10^6 cells, centrifuge at 4°C, 500×g force for 5 minutes, carefully absorb the medium, blot as dry as possible, and collect the cells.

3. Wash the cells twice with cold PBS, draining the supernatant as much as possible after each wash.

4. Every 20μL volume of cell precipitation (about 20mg, 2×10^6 cells), add 200-300μL of cold extract solution A, and mix with high-speed vortex oscillation or blow mixing, and oscillate at 2-8°C for 10-30 minutes.

【 Note 】:

1) After the treatment of extract A, the cell volume should be reduced, otherwise the oscillating treatment time of extract A should be extended.
2) If it is necessary to collect cytoplasmic protein at the same time, the amount of reagent A can be slightly reduced to improve the concentration of the obtained cytoplasmic protein sample.
3) Use the low speed of the oscillator/shaker to allow the extract to shake slightly.
4) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with the pipette blow mixing can be.

5. Then centrifuge at 4℃, 2000×g, for 5 minutes.

6. Inhale the supernatant into another pre-cooled clean centrifuge tube to get the cytoplasmic protein. Please store it on ice or in the refrigerator at -80°C for later use.

7. Add 80-200μL of cold extract solution B to the precipitation and swirl at high speed for 15 seconds.

[Note] :

1) Extract A must be drained as much as possible before adding extract B. Otherwise, the purity of nuclear protein will be affected.
2) You can also wash the precipitate once with PBS. PBS re-suspended precipitation is centrifuged at 12000g for 5 minutes.
3) The amount of extract B was adjusted according to the number of experimental cells, and more cells were added. It can also be adjusted according to the actual situation of the final protein concentration in the pre-experiment.

8. Oscillate at 2-8°C for 30-40 minutes.

[Note] :

1) Use the lower speed of the oscillator/shaker, so that the extract can shake slightly.
2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with the pipette blow mixing can be.
3) In this step, a small amount of transparent glue sometimes appears in the treatment products of the protein extract, which is a normal phenomenon. The transparent glue is a protein complex containing genomic DNA, etc. The supernatant can be directly centrifuged for subsequent experiments without detecting specific proteins that bind particularly closely to genomic DNA. If it is necessary to detect the protein closely bound to the genome, it can be treated by ultrasound, 300w/ 10sec interval of 10 seconds, ultrasound for 3 minutes, and then centrifuge the supernatant for follow-up experiment. The detection of some common transcription factors, such as NF-kappaB, p53, etc., does not require ultrasound treatment. If the introduction of other exogenous proteins does not affect the downstream experiment, DNase treatment can also be added.

9. Centrifuge at 4°C, 12000×g, for 10min.

10. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the nuclear protein.

11. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiment.

[Note] :

1) BCA method is recommended for protein quantification.

2) The protein sample is stored at -80℃ for one year without problem. Be careful not to be hydrolyzed off by protease and not to be contaminated by bacteria.

III、Histone extraction:

1. Extraction solution preparation:

Add 1μL protease inhibitor mixture and 1μL phosphatase inhibitor for every 500μL of cold protein extract A.

Add 1μL protease inhibitor mixture and 1μL phosphatase inhibition to every 200μL of cold protein extract B

Mixture, mix well and set aside on ice.

[Note] :

1) Prepare the protein extract according to the number of samples to be processed, the protease inhibitor mixture cannot be added to the extract all at once. Phosphatase inhibitors may not be added all at once.
2) If the extract with a protease inhibitor is not used up within a week, the protease inhibitor needs to be added again before being used again.
3) The protein extract used in the following steps is a protease inhibitor-containing extract prepared for this step.

2. Take appropriate tissue samples and cut them as much as possible with surgical scissors. Add 200-300μL cold extract A to every 20mg sample and homogenize them with a tissue homogenizer until there are no visible solids.

3. Oscillate the homogenate at 2-8℃ for 10-30 minutes.

[Note] :

1) The cell volume should be reduced after the treatment of extract A, otherwise the oscillating treatment time of extract A should be extended.
2) If it is necessary to collect cytoplasmic protein at the same time, the amount of reagent A can be slightly reduced to improve the concentration of the obtained cytoplasmic protein sample.
3) Use the low speed of the oscillator/shaker to allow the extract to shake slightly.
4) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with the pipette blow mixing can be.

4. Then centrifuge at 4℃ and 2000×g force for 5 minutes.

5. Inhale the supernatant into another pre-cooled clean centrifuge tube to get the cytoplasmic protein. Please place on ice or -80℃ ice

Keep in the box for later use.

6 Add 80-200μL cold protein extract B to the precipitate and swirl at high speed for 15 seconds.

[Note] :

1) Extract A must be drained as much as possible before adding extract B. Otherwise, the purity of nuclear protein will be affected.
2) You can also wash the precipitate once with PBS. PBS re-suspended precipitation is centrifuged at 12000g for 5 minutes.
3) The amount of extract B was adjusted according to the number of experimental cells, and more cells were added. It can also be adjusted according to the actual situation of the final protein concentration in the pre-experiment.

7. Oscillate at 2-8℃ for 30-40 minutes until there is no obvious precipitation.

[Note] :

1) Using the lower speed of the oscillator/shaker, the extract can be shaken slightly.
2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with the pipette blow mixing can be.
3) In this step, a small amount of transparent glue sometimes appears in the treatment products of the protein extract, which is a normal phenomenon. The transparent glue is

a protein complex containing genomic DNA, etc. The supernatant can be directly centrifuged for subsequent experiments without detecting specific proteins that bind particularly closely to genomic DNA. If it is necessary to detect the protein closely bound to the genome, it can be treated by ultrasound, 300w/ 10sec interval of 10 seconds, ultrasound for 3 minutes, and then centrifuge the supernatant for follow-up experiment. The detection of some common transcription factors, such as NF-kappaB, p53, etc., does not require ultrasound treatment. If the introduction of other exogenous proteins does not affect the downstream experiment, DNase treatment can also be added.

8. Centrifuge at 4°C, 12000×g, for 10 min.
9. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the nuclear protein.
10. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiment.

[Note] :
1) BCA method is recommended for protein quantification.
2) The protein sample is stored at -80°C for one year without problem. Be careful not to be hydrolyzed off by protease and not to be contaminated by bacteria.

Analysis of common problems:

1. Low nuclear protein concentration?

The abundance of nuclear protein is low, and a sufficient number of cells is needed to extract it, and the number of cells is as large as possible when conditions permit.

Note that when treated with protein extract solution B, the cleavage is not complete, resulting in low protein concentration. As long as the treatment time of reagent B is extended appropriately. It is best to deal with it under the condition of continuous oscillation, without an oscillator, it can also be mixed with a suction head at intervals of a few minutes, until there is no obvious precipitation.

If it is necessary to detect proteins that are particularly tightly bound to the genome, ultrasound treatment is required.

2. What methods are used to quantify proteins?

BCA method is recommended. The Bradford method is not suitable because reagent A contains components that interfere with the Bradford method, resulting in inaccurate quantification. If dialysis has been performed or the buffer system has been replaced with a desalting column, the Bradford method can be used for quantification.

3. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the protein structure, does not disrupt the original interaction between the proteins, and the proteins maintain their natural conformation and activity.

What to note:

1. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
2. It is best to use disposable suction heads, tubes, bottles, or glassware, and reusable glassware must be washed and thoroughly removed of residual cleaners before use.
3. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
4. Avoid skin or mucous membranes coming into contact with the reagent.
5. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.