Plant nuclear protein extraction kit (proteome test, mass spectrometry) - enzymatic method

Item No. : EX2512
Specification: 50T/100T
Validity: 2-8℃ storage, valid for one year.

Product content:

Name	50T	100T	Storage conditions
Component A: Plant nuclear protein extract A	100mL	200mL	Store at 2-8℃
Component B1: Plant nuclear protein extract B1	22.5mL	22.5mL	Store at 2-8℃
Component B2: Plant nuclear protein extract B2	2.5mL	2.5mL	Store at -20℃
Component C: Nuclear extract solution C	25mL	50mL	Store at 2-8℃
Component D: protease inhibitor mixture	100μL	200μL	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℃. Take it out of the refrigerator and return to room temperature or 37℃ 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:

Plant nuclear protein extraction kit provides a full set of reagents, suitable for extracting nuclear protein from various plant cells and various solid plant tissues, such as leaves, roots, seeds and other plant tissues. The extraction process is simple and convenient, and can be completed within 1 hour. The prepared nuclear protein not only has high purity and natural activity, but also has little cross-contamination.

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

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

The protease inhibitor mixture of this product does not contain AEBSF, which can avoid the Mass Spectrometry peak shift caused by AEBSF. Therefore, the protein samples extracted from this product can be used for mass spectrometry (MS) detection and analysis, proteomics and other related research.

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

EDTA is not present in this kit and is compatible with downstream applications such as metal chelation and chromatography.

The proteins extracted from this kit can also be used in downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, and enzyme activity determination.

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Bring your own reagents and instruments:

Centrifuge, oscillator, homogenizer, vortex mixer, pipette, refrigerator, ice box, 1×PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves

Product Features:

1、Easy to use.

2、Contains protein stabilizer, the extracted protein is stable.

3、The background interference is low when the protein concentration is detected by UV.

4、Protease inhibitors inhibited protein degradation, and the formulation of protease inhibitors was optimized. The protease inhibitor mixture consists of 6 separate protease inhibitors, each of which specifically inhibits one or several protease activities. The optimized composition of this mixture allows it to inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

How to use:

First, use precautions:

1. Before the formal experiment, please select several samples to do pre-experiment, in order to optimize the experimental conditions and achieve the best experimental results
2. Centrifuge the reagent in the screw cap microreagent tube briefly before opening the cap, and centrifuge the liquid on the cap and inside wall to the bottom of the tube to avoid reagent loss when opening the cap.
3. All reagents in the process of the experiment must be pre-cooled; All utensils must be pre-cooled in a -20°C refrigerator. The sample must be kept at a low temperature during the whole process.
4. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
5. 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.
6. Other protease inhibitor products can be added as needed for your own experiment.
7. Do not mix with other brands of reagents, otherwise it will affect the use effect.
8. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.

2. Nuclear protein extraction from plant tissue:

1. Extraction solution preparation:

Mix reagent B1 and reagent B2 to form protein extract solution B, mix well and set aside.

Add 2μL protease inhibitor mixture to protein extract solution B every 500μL, mix well and put on ice for later use.

2. Take 500mg-1g fresh plant leaf samples, wash and dry them with pure water, remove the leaf stems and thick veins, cut them with surgical scissors or sharp blades, and cut them into small pieces 0.5mm * 0.5mm.

3. Add 500μL of extract solution C and mix well.

4. Place the suspension on an oscillator and oscillate at room temperature for 12-72 hours.

5. Centrifuge at 1000×g for 5-10 minutes, discard the supernatant and collect the precipitation.

6. Add 1mL of extract liquid A to the precipitate and fully homogenize it with a homogenizer or with a homogenizer.

7. Filter the homogenate through a 100μm cell screen.

8. Centrifuge the filtrate at 100×g for 3 minutes, discard the precipitation, and collect the supernatant.

9. Centrifuge the filtrate at 2000×g for 10 minutes, discard the supernatant and collect the precipitation.

10. Add 150-250μL of extract solution B to the precipitate and mix thoroughly.

11. Set the oscillator at 2-8℃ and oscillate for 30-45 minutes.

12. Centrifuge at 4℃, 12000×g for 15 minutes.

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

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

15. The protein samples were treated by dialysis or desalting column and then used for downstream experiment.

Analysis of common problems:

1. Low protein concentration?

The abundance of plant nucleoprotein is relatively low, so the sample size should be increased as much as possible if conditions permit.

Some samples may not be fully lysed when processed, resulting in low protein concentration. As long as the number of homogenates of reagent A is appropriately increased and the processing time of reagents A and B is appropriately extended. It is best to handle under the condition of continuous oscillation, and no oscillator can also be mixed with a suction head at intervals of a few minutes.

2. What method is used to quantify the protein?

The 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. Gelatinous precipitate during extraction?

Protein extract treatment products sometimes appear a small amount of transparent glue, is a normal phenomenon. The transparent glue is a complex containing genomic DNA, etc. Without detecting specific proteins that bind particularly closely to genomic DNA, the supernatant can be directly centrifuged for subsequent experiments. 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.

4. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the structure of the protein, does not destroy 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.