Plant nuclear protein/cytoplasmic protein extraction kit - non-enzymatic method

Item No. : EX2232

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	50mL	100mL	Store at 2-8℃
Component B: Plant nuclear protein extract B	10mL	20mL	Store at 2-8℃
Component C: Plant cytoplasmic protein extract C	2.5mL	5mL	Store at 2-8℃
Component D: protease inhibitor mixture	250μL	500μL	Store at -20℃

Note:

1. Protease inhibitors can also be stored at 2-8°C before use without open lid. Store at -20°C 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 and cytoplasmic protein extraction kit provides a full set of reagents, suitable for extracting nuclear protein and other cytoplasmic proteins from a variety of plant cells and a variety of 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 extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel blocking experiment, enzyme activity determination, etc.

The proteins extracted by this kit are active proteins with natural protein conformation.

Prepare your own reagents and instruments:

Centrifuge, oscillator, vortex mixer, pipette, refrigerator, ice box, PBS buffer, 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 of which can specifically inhibit 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. Please centrifuge the reagent in the rotating cap centrifuge tube briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when opening the cap. 2. All reagents in the process of the experiment must be pre-cooled; All appliances must be pre-cooled in the -20℃ 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, you can add other protease inhibitor products according to your own experimental needs.

5, centrifuge speed has relative centrifugal force (RCF, ×g) and speed per minute (RPM) two ways of expression, some centrifuges set RPM and ×g display switch, but some centrifuges do not automatically switch function. Need to use the following formula for conversion:

$g=r\times1.118\times10^{-5}\times rpm^2$ (r is the effective centrifuge radius, the length in centimeters from the centrifuge axis to the center of the bottom of the centrifuge collection tube)

For example, if the rotational speed is 3000rpm and the effective centrifugal radius is 10cm, then the relative centrifugal force (RCF, ×g) is $=10\times1.118\times10^{-5}\times3000^2=1006.2$ (×g).

2. Operation steps:

1. Preparation of extraction liquid:

Add 1μL protease inhibitor to every 500μL of cold protein extract solution A; Add 1μL protease inhibitor mixture into every 200μL protein extract solution B, mix well and put on ice for later use.

2. Take 200-500mg of fresh plant leaf samples, wash and dry them with PBS or pure water, remove the leaf stems and thick veins, and cut them as much as possible with surgical scissors.

3. Add 500-1000μL extract liquid A and then fully homogenize with a homogenizer or a homogenizer.

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

5. Centrifuge the filtrate at 4°C, 800×g, for 5 minutes.

6. Collect the **precipitate (I)** and transfer the **supernatant (I)** into another clean centrifuge tube.

7. Add **100-200μL cold extract B to precipitate (I)**, swirl at high speed for 5 seconds, and mix thoroughly.

8. Oscillate at 4°C for 20-40 minutes.

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

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

11. Add 50μL of cytoplasmic extract solution C to the **supernatant (I)** in step 6, vortex for 10 seconds at high speed, and mix thoroughly.

12. Oscillate at 4°C for 5 minutes.

13. Centrifuge at 4°C, 12000×g, for 5 minutes.

14. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain cytoplasmic protein.

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

Analysis of common problems:

1. Low protein concentration?

Plant nucleoprotein abundance is relatively low, when conditions allow, as much as possible to increase the sample size.

Some samples may not be fully lysed when processed, resulting in low protein concentration. Just appropriately increase the number of homogenates of reagent A, and

Appropriately extend the processing time of reagent B. It is best to deal with it under the condition of continuous oscillation, and it can be separated by a few minutes without an oscillator

The clock is blown and mixed with a suction head.

2. In what way is the protein quantified?

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.

4. Is there a gelatinous precipitate during extraction?

A small amount of transparent glue sometimes appears in the treatment products of protein extract, which is a normal phenomenon. The transparent glue may contain genes

A complex of 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.

Points to note:

1. Before the formal experiment, please select several samples for pre-experiment to optimize the experimental conditions and achieve the best experimental results.
2. The reagent in the screw cap trace reagent tube should be centrifuged briefly before opening the cap, and the liquid on the cap and inner wall should be centrifuged to the bottom of the tube to avoid the loss of the reagent when opening the cap.
3. It is prohibited to mix with other brands of reagents, otherwise it will affect the use effect.
4. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may lead to wrong results.
5 It is best to use disposable suction heads, tubes, bottles or glassware, reusable glassware must be cleaned before use
And thoroughly remove residual cleaners.
6. After the completion of the experiment, all samples and utensils in contact should be disposed of in accordance with the prescribed procedures.