Plant Endoplasmic reticulum Protein Extraction Kit

Item No. : EX2060
Specification: 50T/100T
Validity: 2-8°C storage, valid for one year

Product content:

Name	50T	100T	Storage conditions
Reagent A: Plant endoplasmic reticulum protein extract A	25mL	50mL	Store at 2-8°C
Reagent B: Plant endoplasmic reticulum protein extract B	25mL	50mL	
Reagent C: Plant endoplasmic reticulum protein extract C	50mL	100mL	
Reagent D: Plant endoplasmic reticulum protein extract solution D	15mL	30mL	
Reagent E: protease inhibitor mixture	100μL	200μL	Store at -20°C

Note: Storage conditions for each component:
The protein extract is stored at 2-8°C
Store protease inhibitor -20°C.
Extract liquid A is not required to be stored at -20°C for a long time.

1. The protease inhibitor can also be stored at 2-8°C before use. Store at -20°C after opening the lid for use.
2. The protease inhibitor is a solid state at low temperature of 2-8°C, and is returned to room temperature or 37°C water bath for a short time after taking out of the refrigerator, and becomes a liquid state, centrifuge 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 endoplasmic reticulum protein extraction kit provides a full set of reagents, suitable for extracting endoplasmic reticulum 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 the prepared ER proteins not only have high purity, maintain natural activity, but also have little cross-contamination.

This kit contains a unique formula that effectively dissolves plant ER 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 proteins 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 assay, and enzyme activity determination.

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

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

The protein samples extracted from this kit contain a high concentration of salt components and cannot be used directly for 2D electrophoresis. The final sample can also be desalted and then used for 2D electrophoresis.

This kit is extracted by enzyme method. Compared with non-enzyme method, the recovery rate of protein extracted by enzyme method is slightly higher, and the protein purity is high, but it takes a long time. The non-enzymatic extraction process is simple and convenient, fast, can be completed within 1 hour, less cross contamination, maintain natural activity, but the protein recovery is lower

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than the enzymatic method. If you need a faster extraction kit, you can choose a non-enzymatic extraction kit, if there is no requirement for extraction speed and protein activity, you can choose an enzymatic extraction kit. Please select the kit according to your actual needs.

Bring your own reagents and instruments:
Centrifuge, oscillator, homogenizer/homogenizer, vortex mixer, pipette, refrigerator, ice box, 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 inhibitor inhibited protein degradation, and the formulation of protease inhibitor was 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. 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 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 is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
4. You can add other protease inhibitor products according to your own experimental needs.

Two, operation steps

1, extraction liquid preparation:

Add 2μL protease inhibitor mixture to every 300μL extract solution D, mix well and put on ice for later use.

Note: Prepare the protein extract solution according to the number of samples to be processed. The protease inhibitor mixture cannot be added to the extract solution all at once.

If the extract with a protease inhibitor is not used up within one week, the protease inhibitor should be added again before re-use.

2. Take a 200-500mg plant tissue sample that has been washed and dried, and leaves and thick veins removed, and cut it up as much as possible with surgical scissors.

Note: A sharp blade can also be used to cut the leaf sample into as small squares (1-2mm) as possible.

3. Add 500μL of reagent A and mix well.

Note: The leaf tissue can be frozen in liquid nitrogen and slightly mashed into A fine powder before adding reagent A.

4. Let stand at 30°C for 15 minutes.
5. Centrifuge at 2000×g for 10 minutes, discard the supernatant and collect the precipitation.

6. Add 0.5mL of extract B to the precipitate and mix thoroughly.

Note: The cultured cells were centrifuged at 1000xg for 5min. The medium was carefully absorbed and dried as much as possible. After the cells were collected, the cells were washed twice with PBS, and then the extract B was added directly for re-suspension.

1mL of extract solution B was added approximately every 300μL of cell volume.

Adjust the amount of extract liquid according to the actual sample volume, the general reagent is 2-3 times the sample volume, and the sample can be fully submerged.

7. The suspension of the extract solution B sample is placed in an oscillator at 37℃-45℃ or room temperature and oscillated for 24-72 hours.

Note: Using the low speed of the oscillator/shaker, the extract can shake slightly.

No oscillating conditions can also not oscillate, in the middle of every few hours with a pipette blow mixing can be.

Select the right temperature according to the downstream ribosome application, and the preparation speed is the fastest when the highest temperature is 55℃. However, too high temperature will affect the sample activity, please select the temperature according to the downstream application.

The time required for processing varies greatly between different types of plant samples. Arabidopsis is easier to handle, the fresh leaves are easier to handle, and the processing time is shorter for younger samples. Young Arabidopsis leaves can be processed in as little as 4 hours.

Some plant types with thicker cell walls may require longer processing. Treatment can be extended beyond 72 hours to improve yield.

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

Note: If conditions permit, it is best to use 100μm cell screen to filter extract B treatment solution, collect filtrate and then centrifuge.

No cell screen can be left unfiltered.

9. Add 1000μL reagent C to the precipitate to re-suspend the precipitate and mix well.

10. Fully homogenize with a homogenizer or a Dounce homogenizer.

Note: Generally homogenate 30-40 times with a Dounce homogenizer.

Each round trip is once.

11. Oscillate the homogenate at 4°C for 20-30 minutes.

Note: Use the low speed of the oscillator/shaker to keep the liquid shaking slightly.

No low temperature oscillation conditions can not be oscillated, at 2-8°C, slightly extend the processing time, every few minutes in the middle of the pipette blow mix.

12. Centrifuge at 500×g for 5 minutes, discard the precipitation, and collect the supernatant.

13. Centrifuge at 1000×g for 10 minutes, discard precipitation and collect supernatant.

14. Centrifuge 20000×g supernatant for 10 minutes. Discard the precipitate and remove the supernatant.

15. Centrifuge the supernatant 30,000-50000 ×g for 45 minutes. Discard the supernatant and leave to precipitate.

Note: If conditions permit, centrifugal force can be increased to 100000×g, which is conducive to recovery of ER vesicles.

16. Add 100-300μL of extract solution D to the precipitate and mix well.

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

Note: Use the low speed of the oscillator/shaker to keep the liquid shaking slightly.

No low temperature oscillation conditions can not be oscillated, at 2-8°C, slightly extend the processing time, every few minutes in the middle of the pipette blow mix.

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

19. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain endoplasmic reticulum protein.
20. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.

Note: It is recommended to use BCA method for protein quantification.

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

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. As long as the homogenization times are appropriately increased, and the processing time of reagents B and C and D is appropriately extended. It is best to handle under the condition of continuous oscillation, and no oscillator can also be blown with a suction head at intervals of several minutes Beat and mix well.

2. What method is used to quantify the protein?

BCA method is recommended. Bradford method is not suitable, because reagent D contains components that interfere with 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. Before the formal experiment, please select a few samples for 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. Do not 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 result in false results.
5. 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.
6. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.