Plant Chloroplast Membrane Protein Extraction Kit - Non-enzymatic Method

Cat: EX1330
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

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Reagent A: Chloroplast Extract A	100mL	200mL	2-8℃
Reagent B: Chloroplast Extract B	25mL	50mL	
Reagent C: Membrane Protein Solution C	10mL	20mL	
Reagent D: Protease Inhibitor Mixture	100μL	200μL	-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!

Introduction:

Chloroplasts are unique organelles for energy conversion in plant cells, and photosynthesis is carried out in chloroplasts. Because of this important function, chloroplasts have been the important research objects in cell biology, genetics and molecular biology. Chloroplasts are the larger organelles in plant cells. Transmembrane proteins undertake various biological functions and play an important role in various developmental processes of organisms. The preparation of membrane protein samples needs to be fully considered for downstream applications such as gel analysis and mass spectrometry, so the preparation of membrane protein samples becomes an insurmountable challenge.

Chloroplast membrane protein extraction kit can be used to extract chloroplast membrane protein from various plant samples with a simple and rapid method. The extraction process is simple and convenient, and high quality chloroplast membrane protein can be extracted within one hour. The kit contains protease inhibitor mixture and phosphatase inhibitor mixture, which prevents protease from degrading the protein and provides guarantee for extracting high quality protein.

The kit contains a unique formulation that effectively dissolves the membrane 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.

Self-prepared 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 inhibitors inhibited protein degradation, and the formulation of protease inhibitors was optimized. The protease inhibitor mixture consists of 6 separate protease inhibitors; Each inhibitor specifically inhibits one or several protease activities. The composition of the mixture is optimized so that it can inhibit almost all important protease activities.

Protocols:

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 must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
4. The temperature conditions in the following steps must be followed during the operation, otherwise the recovery of membrane protein will be seriously affected.
5. The extraction solution B must be kept at 2-8℃ before use, otherwise the downstream membrane protein extraction will not be easy to delaminate.
6. Boiling should be avoided in loading buffer during electrophoresis of membrane proteins.
7. The SDS content of loading buffer can be increased during membrane protein electrophoresis.
8. Do not mix with other brands of reagents, otherwise the effect will be affected.
9. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.

Second, protein extraction

1. Extraction liquid preparation:
   Add 2μL protease inhibitor mixture into every 500μL cold protein extract B, mix well and put on ice for later use.
2. Take 500mg-1g fresh plant leaf sample, wash and dry with pure water, remove leaf stem and thick veins, and cut as much as possible with surgical scissors.
3. Add 2mL of extract A fully homogenize with a tissue blender/homogenizer /Dounce homogenizer.
4. Strain the homogenate through a 100μm cell screen.
5. Centrifuge the filtrate at 500×g force for 3min. Transfer the supernatant into another clean centrifuge tube.
6. Centrifuge the supernatant 3000×g force for 10min. Discard the supernatant and collect the precipitate.
7. Add 500μL protein extract B to the precipitate and mix well. Oscillate at 2-8℃ for 1h.
8. Centrifuge the extract at 4℃, 12000×g, for 5min, and remove the supernatant.

9. Water bath at 37℃ for 10min.
10. Centrifuge at 37°C, 500×g, for 3min.
11. At this point the solution is divided into two layers, carefully remove the upper layer and retain the lower solution, about 30-50μL.
12. Dissolve the lower layer with 50-100μL cold membrane protein solution to obtain chloroplast membrane protein.
13. The protein extract was quantified and divided into -80℃ refrigerator for reserve or adjusted to the corresponding concentration and directly used in downstream experiments.

Analysis of Common Problems:

1. Low protein concentration?

Some tissue samples may not be fully lysed when treated, resulting in low protein concentration. Just properly extend the reagent AB when treating. Can be. It is best to handle under the condition of continuous oscillation, and it can be mixed with a suction head at intervals of several minutes without an oscillator.

2. What method is used to quantify protein?

It is recommended to use BCA method. 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 destroy the original interaction between proteins, proteins maintain their natural conformation and activity.

4. Is there no band in membrane protein electrophoresis?

The concentration of membrane protein samples is usually low, and protein quantification must be carried out before electrophoresis to ensure that the amount of protein on the electrophoresis is sufficient. After the membrane protein is extracted and fully dissolved with the solution, it can be treated by ultrasound and then quantified. After Loading the protein with Loading buffer, it can be kept at 50℃ for 30min without boiling. The final concentration of SDS in protein Loading buffer can be increased to 3%-10%. The content of membrane protein in some samples is too low, so acetone can be used to precipitate the membrane protein and dissolve the membrane protein in the loading buffer. Generally, clear bands of protein can be produced. The final electrophoresis is low current and low current electrophoresis.

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.