Cell Protein Rapid Extraction Kit (centrifugal column method)

Item No. : EX2400

Specification: 50T/100T

Validity: 2-8℃ storage, valid for one year.

Product content:

Components	Name	50T	100T	Storage conditions
Component A	Protein extract Liquid A	25mL	50mL	Store at 2-8℃
Component B	Protease inhibitor mixture B	100μL	200μL	Store at -20℃
Component C	Protein stabilizer C	100μL	200μL	Store at 2-8℃
Component D	Egg white centrifugal column	50 sets	100 sets	Store at RT

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 a solid state at low temperature of 2-8℃, and is returned to room temperature or 37℃ 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 Golgi membrane protein extraction kit provides a complete set of reagents suitable for the extraction of Golgi membrane protein from various plant tissue samples. The extraction process is simple and convenient and can be completed within 1 hour. The extracted membrane proteins are not only pure, maintain natural activity, and have little cross-contamination.

This kit contains a unique formula 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 protein extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, enzyme activity determination, etc.

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.

Bring 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.
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, 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. Protease inhibitor at 2-8℃ is a solid state, after taking out from the refrigerator, return to room temperature or 37℃ for a short time water bath, become a liquid state, centrifuge to the bottom of the tube and then open the lid.
3. All reagents used in the experiment must be pre-cooled; All utensils must be pre-cooled in a -20℃ 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.

Two, operation steps

1. Extraction solution preparation:

Add 2μL protease inhibitor mixture and 2μL protein stabilizer to every 500μL protein extract, mix well and set aside on ice.

2. Take 5×10^6 cells, centrifuge at 4℃, 2500×g for 5 minutes, carefully absorb the medium, blot as dry as possible, and collect the cells.
3. Wash the cells twice with cold PBS, sucking up as much supernatant as possible after each wash.
4. Every 5×10^6-1×10^7 cells (about 50mg cells /50μL cell volume), add 500μL of cold total protein extraction solution, blow and mix well, and shake at 4℃ for 20-30 minutes until the cells are fully lysed without obvious cell precipitation.
5. Put on a liquid collection tube, inhale the extracted solution into the protein centrifuge tube, and

centrifuge at 4℃, 10000g, for 5 minutes.

6. The total cell protein was obtained by collecting the liquid in the tube.
7. The protein extracts were quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiments.

Analysis of common problems:

1. Slow cell lysis

In order to fully ensure the activity of the extracted protein, the extract adopts a unique formula of protective protein, which has mild cracking ability and wide downstream application. The cracking time can be extended appropriately.

2. Low protein concentration?

Handling part of the cell sample may not fully lyse, resulting in low protein concentrations. Just extend the processing time of reagent A appropriately. 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.

3. What is the method of quantifying the protein?

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.

4. 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.