Plant mitochondrial protein extraction kit

Item No. : EX2260

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Mitochondrial extract Solution A	50mL	100mL	Store at 2-8℃
Mitochondrial extract solution B	50mL	100mL	Store at 2-8℃
Mitochondrial protein extract solution C	15mL	30mL	Store at 2-8℃
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:

mitochondria are organelles covered by two layers of membranes in eukaryotic cells, and are an important organelle structure for producing energy in cells, and are the main place for cells to perform aerobic respiration. The energy substances in the cell -- fat, sugar and some amino acids are finally oxidized here, and ATP is produced by coupling phosphorylation to supply the physiological activities of the cell. The study of mitochondrial structure and function is usually carried out in isolated mitochondria.

The plant mitochondrial protein extraction kit provides a complete set of reagents, which can be extracted in a simple and rapid way within 1 hour.

This kit contains a unique formulation that effectively dissolves mitochondrial membrane components.

The kit contains a protease inhibitor mixture that prevents 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.

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

Directions 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, protease inhibitor at 2-8℃ is a solid state, from the refrigerator to return to room temperature or 37℃ for a short time water bath, into a liquid state after centrifugation to the bottom of the tube and then open the lid.

3. All reagents in 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.

4. If the solution of protease inhibitor precipitates during storage, it will not affect the use, and it will be used normally after dissolution.

5, if the kit can not be used up in a short time, the protease inhibitor mixture can not be added to the extraction solution at one time.

6, you can add other protease inhibitor products according to your own experimental needs.

Second, operation steps

1. Preparation of extraction liquid:

Add 1μL protease inhibitor into every 200μL pre-cooled protein extract C, mix well and put on ice for later use.

2. Take 200-500mg of fresh plant sample leaves, wash and dry with PBS, and then remove the leaves and thick veins. Use surgical scissors to cut up as much as possible.

3. Add 1mL of extract A and fully homogenize with A homogenizer or add appropriate amount of extract A and fully homogenize with a homogenizer /Dounce homogenizer.

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

5. Centrifuge the filtrate at 500×g for 5 minutes, discard the precipitation, and take the supernatant.

6. Centrifuge the supernatant at 800×g for 5 minutes, discard the precipitation, and collect the supernatant.

7. Centrifuge the supernatant at the condition of 2000×g for 5 minutes, discard the precipitation and collect the supernatant.

8. Centrifuge the supernatant at 12000×g for 20 minutes. Discard the supernatant and collect the precipitate.

9. Re-suspend the precipitate with 500μL reagent B.

10. Centrifuge the suspension with 12000×g force for 20 minutes, discard the supernatant, and collect the precipitation.

11. Add 100-200μL mitochondrial protein extract C to the precipitation and mix thoroughly.

12. Oscillate at 4℃ for 20-40 minutes.

13. Centrifuge at 4℃, 14000×g, for 15 minutes.

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

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

Analysis of common problems:

1. Low protein concentration?

Some samples may not be fully lysed when processed, resulting in low protein concentrations. As long as the processing time of reagent C is properly extended ie

Yes. It is best to handle under the condition of continuous oscillation, without an oscillator can also be mixed by blowing with a suction head at intervals of several minutes.

2. 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.

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 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.