Fungal mitochondrial protein extraction kit

Item No. : EX2290

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Mitochondrial extract Solution A	100mL	200mL	Store at 2-8℃
Mitochondrial protein extract B	20mL	40mL	Store at 2-8℃
Protease inhibitor mixture C	100μL	200μL	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:

Fungal mitochondrial protein extraction kit provides a complete set of reagents suitable for extracting mitochondrial proteins from various fungal samples. The extraction process is simple and convenient. The prepared mitochondrial proteins not only have high purity and natural activity, but also have little cross-contamination.

This kit contains a unique formula 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

Product features:

1. Easy to use, extract protein from cells and tissues without grinding, repeated freeze-thaw, ultrasonic crushing and other pre-treatment.
2. Shorten the time of protein extraction 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. Protease inhibitor inhibited protein degradation, and the formula of protease inhibitor was optimized. The protease inhibitor mixture consists of 6 types

The specific protease inhibitors AEBSF, Aprotinin, Leupeptin, Pepstatin A, Bestatin, E-64, each inhibit

Agent can specifically inhibit the activity of one or several proteases. The composition of the mixture is optimized so that it can inhibit almost all important proteases

Protease activity, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase

Et al.

How to use:

I、**Precautions for use**:

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:

According to the number of samples, add 2μL protease inhibitor mixture into every 500μL of cold protein extract B, mix well and put on ice for later use.

2. Take 100mg-500mg fungal sample and wash it once with PBS. Centrifuge to collect thallus precipitates.

3. Add 2mL of extract solution A to the bacteria and homogenize them fully with a homogenizer /Dounce homogenizer.

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

5. Centrifuge the supernatant at 1000×g for 5 minutes, discard the precipitation and collect the supernatant.

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

7. Centrifuge the supernatant at 11000×g for 20 minutes, discard the supernatant and leave the precipitation.

8. Add 100-400μL protein extract B to the precipitation, mix well, and oscillate at 4℃ for 20-30 minutes until there is no obvious precipitation.

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

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

11. 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 mitochondrial samples may not be fully cleaved when treated, resulting in low protein concentrations. As long as the treatment of reagent C is properly prolonged

Can be extended. 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.

Fungal mitochondrial protein is small, as much as possible filamentous fungal sample loading.

2. What is the method of protein quantification?

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.