Tissue mitochondrial protein extraction kit

Article number: EX2250

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Component A: Mitochondrial extract Solution A	25mL	50mL	Store at 2-8°C
Component B: Mitochondrial extract B	20mL	40mL	Store at 2-8°C
Component C: Protein extract C	10mL	20mL	Store at 2-8°C
Component D: Protease inhibitor mixture D	100μL	200μL	Store at -20°C

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°C. Take it out of the refrigerator and return to room temperature or 37°C 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:

Tissue mitochondrial protein extraction kit provides a complete set of reagents, suitable for extracting mitochondrial protein from various solid tissues, such as brain, spinal cord, nerve junction or fiber, fat, liver, digestive tract, kidney, heart, muscle, blood vessel, connective tissue and other animal tissues. 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 protein 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 experiment, enzyme activity determination, etc.

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、The time of protein extraction is reduced to 30 minutes 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、The protein extract contains a variety of effective components, which can fully release cytoplasmic protein and nuclear protein, and can bind the released protein to prevent precipitation.

6、Protease inhibitor inhibits the degradation of protein, and the formula of protease inhibitor is 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. 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. All reagents in the process of 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.

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

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

5. It is best to use Dounce homogenizer to homogenize. If there is no Dounce homogenizer, ordinary 1ml glass homogenizer can also be homogenized, but the recovery rate of mitochondrial protein will decrease.

2. Operation steps

1. Preparation of extraction liquid:

According to the number of samples, add 2μL protease inhibitor mixture into every 200μL of cold protein extract solution C, mix well and put on ice for later use.

2. Take 50-100mg of fresh tissue sample and cut it up as much as possible with surgical scissors.

3 Wash twice with cold PBS, sucking up as much supernatant as possible after each wash.

4 Add 400-500μL of cold reagent A and put on ice for 10 minutes.

5. Use a Dounce homogenizer to homogenize for 30-40 strokes. Then centrifuge at 4°C, 500×g, for 5 minutes.

6. Inhale the supernatant into another pre-cooled clean centrifuge tube and discard the precipitation.

7. Centrifuge the supernatant at 4°C, 1000×g, for 10 minutes. Discard the precipitate and leave the supernatant.

8. Centrifuge the supernatant at 4°C, 10000×g, for 20 minutes. Discard the supernatant and leave to precipitate.

9. Add 200-400μL of cold reagent B to the precipitate and mix gently.

10. Centrifuge at 4℃, 10000×g, for 20min. Discard the supernatant and leave to precipitate.

11. Add 80-150μL protein extract C to the precipitate and mix well. Set at 4℃ and shake for 20-30 minutes.

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

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

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

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 processing time of reagent C is extended 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. Adequate loading of cell or tissue sample is required.

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.