Tissue mitochondrial extraction kit

Article number: EX2620
Specification: 50T/100T
Validity: 2-8℃ storage, valid for one year.

Product content:

Name	50T	100T	Storage conditions
Component A: Mitochondrial extract Solution A	50mL	100mL	Store at 2-8°C
Component B: Mitochondrial extract B	25mL	50mL	Store at 2-8°C
Component C: Mitochondrial preservation solution C	10mL	20mL	Store at 2-8°C

Note:

1. The extract is stored at -20℃ when not used for a long time.
2. Please use the reagent as soon as possible after unpacking!

Product Introduction:

Mitochondria (mitochondria) are important organelles producing energy in eukaryotic cells. Energy substances in cells -- fat, sugar and some amino acids are finally oxidized here, and ATP is produced by coupling phosphorylation to supply physiological activities of cells. The study of mitochondrial structure and function is usually carried out on isolated mitochondria, and this kit can extract mitochondria in a simple and rapid method within 40 minutes.

This kit can be used to extract mitochondria from soft and hard tissue samples of various animal entities. It is best used for the mitochondrial extraction of fresh samples, because the mitochondria of frozen samples may be destroyed during cryopreservation, and the extraction recovery rate may be greatly reduced.

The mitochondria extracted from this kit can be used for various downstream applications such as mitochondrial function research and protein extraction.

Self-prepared reagents and instruments:

Centrifuge, oscillator, homogenizer, vortex mixer, pipette, refrigerator, ice box, PBS buffer, centrifuge tube, suction head, disposable gloves

How to use:

First, use precautions:

1. Before the formal experiment, please select several samples for pre-experiment 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 in the process of 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. It is best to homogenize the sample using standard Dounce homogenizers. If standard Dounce homogenizers are not available, ordinary 1ml glass homogenizers may also be used. However, mitochondrial recovery will be reduced.
5. Homogenization with Dounce homogenizer is a key step in mitochondrial extraction. Therefore, it is advisable to determine the optimal number of homogenization times for different tissue samples through pre-experiments before homogenization. After 5-10 homogenization, 2-3μL of homogenate should be added to the slide, and then the number of intact cells should be reduced to less than 20% under a phase contrast microscope. This microscopical procedure is not necessary,

but may affect the recovery of mitochondria. In experiments where samples are extremely difficult to obtain, microscopy must be performed.

2. Tissue mitochondrial extraction:

1. Take 50-100mg fresh animal tissue sample and wash it with PBS.

[Note] :

● Because some samples have less mitochondrial content, a sub-larger number of samples may be required to guarantee the yield.

2. Cut as much as possible with scissors and wash twice with cold PBS.

[Note] :

● Centrifuge 1000 x g for 5 minutes.

3. Add 500-1000μL of cold reagent A and put on ice for 10 minutes.
4. Homogenize 30-40 times with a Dounce homogenizer.

[Note] :

● Use the loose mallet of the Dounce homogenizer for initial homogenization 10-20 times, then use the tight mallet for homogenization 20-30 times.

● Each next round trip is once.

5. Then centrifuge at 4°C, 500×g, for 5 minutes. Discard the precipitation and collect the supernatant.
6. Centrifuge the supernatant at 4°C, 1000×g, for 10 minutes. Discard the precipitation and collect the supernatant.
7. Centrifuge the supernatant at 4°C, 2000×g, for 10 minutes. Discard the precipitation and collect the supernatant.
8. Centrifuge the supernatant at 4°C, 11000×g, for 20 minutes. Discard the supernatant and leave to precipitate.
9. Add 500μL of cold reagent B to the precipitate and mix well.
10. Centrifuge at 4°C, 11000×g, for 20 minutes. Discard the supernatant and leave to precipitate.
11. The precipitates were re-suspended with mitochondrial preservation solution.

[Note] :

● The preservation solution provided in the kit may not be used.

● According to the needs of the experiment, it can be stored with its own other buffer or directly used for downstream experiments such as protein cleavage/mitochondrial staining.

● The preserved mitochondria in the mitochondrial preservation solution can be centrifuged at 12000×g for 15 minutes to collect and precipitate again.

12. That is, the mitochondrial sample can be stored in the refrigerator or directly used for downstream experiments.

[Note] :

● It can be used within 3 days and stored at 4℃. Long-term storage must be frozen below -20℃.

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.
5. If the reagent comes into contact with skin or eyes, it should be rinsed with water immediately.