Extraction kit

Item number: EX2610

Specification: 50T/100T

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

Product content:

Name	50T	100T	Storage conditions
Component A: Mitochondrial extract Solution A	20mL	40mL	Store at 2-8°C
Component B: Mitochondrial extract B	20mL	40mL	Store at 2-8°C
Component C: Mitochondrial preservation solution C	20mL	40mL	Store at 2-8°C

Note:

1. If the extract is not used for a long time, it can be stored at -20℃, and it can be stored at 4℃ if it is used several times within 15 days.
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. This kit can extract mitochondria in 40 minutes with a simple and rapid method.

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

Mitochondria extracted from this kit can be used in 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 to do 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. 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.

2. Cell mitochondrial extraction:

1. Take 1-2×10^7 cells, centrifuge at 4℃, 500×g for 2-3 minutes, carefully absorb the medium, blot as much as possible, and collect cells;
2. Wash twice with cold PBS, and blot the supernatant as much as possible after each wash.
3. Add 400μL of cold reagent A to the cell precipitation and put it on ice for 5 minutes.
4. Homogenize the cells with a Dounce homogenizer for 30-40 minutes, then centrifuge them at 500×g at 4℃ for 5 minutes.
5. Inhale the supernatant into another pre-cooled clean centrifuge tube and discard the precipitation.
6. Centrifuge the supernatant at 4℃, 1000×g, for 10 minutes. Discard the precipitation and collect the supernatant.
7. Centrifuge the supernatant at 4℃, 11000×g, for 15 minutes. Discard the supernatant and collect the precipitation.
8 Add 400μL of cold reagent B to the precipitate and mix gently.
9. Centrifuge at 4℃, 11000×g, for 15min.
10. Discard the supernatant and precipitate with mitochondrial preservation solution C.
11. That is, the mitochondrial samples were obtained, which were stored in the refrigerator or directly used for downstream experiments.

Points for attention:

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 accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.