

Platelet Mitochondrial Extraction Kit

Cat: EX2850

Size: 50T/100T

Storage: 2-8°C storage, reagent bottle after opening the lid of each component according to the required conditions of storage, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Platelet washing solution A	25mL	50mL	2-8°C
Component B: Mitochondrial extract solution B	25mL	50mL	2-8°C
Component C: Mitochondrial extract solution C	25mL	50mL	2-8°C
Component D: Mitochondrial preservation solution D	25mL	50mL	2-8°C

Note:

1. Long-term use can be frozen at -20°C.
2. Please use the reagent as soon as possible after unpacking!

Introduction:

Platelet mitochondrial extraction kit is suitable for extracting fully functional mitochondria from mammalian platelets.

The mitochondria extracted by this kit can be used for various downstream activity experiments, mitochondrial membrane potential detection, mitochondrial protein extraction and other experiments.

Product Features:

1. Easy to use, no special instruments are required.
2. Mitochondria have high yield and good purity.

Self-prepared reagents and instruments:

Centrifuge, oscillator, vortex mixer, pipette, refrigerator, ice box, Tyrode's solution or CGS buffer/saline/PBS, etc., centrifuge tube, suction tip, disposable gloves

Protocols:

First, notes for use:

1. The reagent in the rotating cap centrifuge tube should be centrifuged briefly before opening the cap, and the liquid on the inner wall of the cap should be thrown to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20°C refrigerator. The sample must be kept at a low temperature during the whole process.

Second, platelet mitochondrial extraction:

1. Take 1-5mL anticoagulant blood and centrifuge at room temperature 200×g for 10min.

[Note]:

- 1) Centrifugal force in 180-220×g can be, not greater than 250×g.
- 2) It's best to get as fresh a blood sample as possible.

2. Collect the upper plasma and discard the lower blood cells to precipitate.

[Note]:

- 1) If a white membrane forms between the plasma layer and the red blood cell layer, carefully absorb the white membrane layer and add the upper plasma.
3. Centrifuge the upper plasma at room temperature 3000×g for 20min. Discard the supernatant and retain the precipitation.
4. Add 500μL platelet washing solution A to the precipitate and wash once, centrifuge 3000×g for 15min. Discard the supernatant and retain the precipitation.
5. Add 500μL of cold mitochondrial extract solution B to the precipitation, mix thoroughly, and oscillate at 4°C for 20-30min.

[Note]:

- 1) Use the lower speed of the oscillator/shaker to keep the liquid shaking slightly.
- 2) No oscillating conditions can not oscillate, slightly extend the processing time can be, every few minutes in the middle of the pipette blow to mix.
- 3) The precipitation volume of extract solution B after treatment should be reduced, otherwise the extract solution B oscillation treatment time should be extended.
6. Mix well and centrifuge at 4°C, 2000×g, for 5min. Discard the precipitation and collect the supernatant.
7. Centrifuge the supernatant at 4°C, 12000×g, for 20min. Discard the supernatant and collect the precipitation.
8. Add 500μL cold reagent C to the precipitate and mix well.
9. Centrifuge at 4°C, 12000×g, for 20min. Discard the supernatant and leave to precipitate.
10. The precipitation was re-suspended with mitochondrial preservation solution D.

[Note]:

- 1) The mitochondrial preservation solution D can be used for resuspension, and the appropriate buffer solution can be used for resuspension and preservation according to the needs of downstream experiments or directly used for downstream experiments.
11. That is, platelet mitochondrial samples are obtained and stored in the refrigerator for later use or directly used in downstream experiments.

Analysis of common problems:

1. How to preserve the extracted mitochondria?
Short time storage in 4°C refrigerator storage; Long-term storage store at -80°C.
2. How to extract mitochondrial protein?
If mitochondrial proteins need to be extracted, re-suspension precipitation is not required with the reagent D mitochondrial preservation solution. According to the downstream experimental application, an appropriate amount of protein lysate (such as RIPA) can be directly added to the precipitation for cleavage.

Notes:

1. Before the formal experiment, please select a few samples for 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. Do not 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 result in false results.
5. 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.
6. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
7. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
8. This product is for scientific use only. Do not use for medicine, clinical diagnosis or therapy, food or cosmetics. Do not store in ordinary residential areas.
9. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.