



TECHNICAL MANUAL

Mitochondrial Extraction Kit

- **SKU CODE:** MAES0254
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Intended use

This kit can be used to extract mitochondria from animal tissue.

2. Detection principle

The principle of mitochondrial separation and purification: after rupturing cells mechanically, the debris and large organelles are removed by low-speed differential centrifugation, then purified mitochondria will be obtained by high-speed differential centrifugation.

3. Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 1 (100 Assays)	Storage
Reagent 1	Lysis Buffer	30 mL × 1 vial	60 mL × 1 vial	-20°C, 12 months
Reagent 2	Mito-Wash Buffer	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Store Buffer	7 mL × 1 vial	15 mL × 1 vial	-20°C, 12 months

4. Materials prepared by users

Instruments:

High-speed freezing centrifuge, 5 mL Glass homogenizer

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

5. Reagent preparation

Place all reagents in ice water for pre-cooling for at least 15 min after they have returned to the solution state before use.

6. Operation procedure

1. Weigh 0.05 g~0.1 g of fresh tissue and wash with PBS (0.01 M, pH 7.4) at 2-8°C to remove blood. Blot the water with absorbent paper. Cut the tissue into pieces with scissors and place them into a pre-cooled 5 mL glass homogenizer. Add 0.5 mL of

pre-cooled lysis buffer. Grind the tissue up and down in the ice bath for about 30-40 times.

2. Transfer tissue homogenate to a 2 mL pre-cooled EP tube, centrifuge at 500×g at 4°C for 5 min.
3. Transfer the supernatant to a new 2 mL pre-cooled EP tube, centrifuge at 500×g at 4°C for 5 min.
4. Transfer the supernatant to a new 2 mL pre-cooled EP tube, centrifuge at 15000×g at 4°C for 15 min.
5. Discard the supernatant, resuspend the precipitate with 0.2 mL of pre-cooled mito-wash buffer, then centrifuge at 11000×g at 4°C for 10 min.
6. Discard the supernatant, resuspend the precipitate with 50-100 µL of pre-cooled store buffer. The prepared mitochondrial extract can be used immediately or stored at -70°C, avoiding repeated freeze-thaw.

7. Notes

1. All steps of mitochondria isolation were performed on ice or at 4°C, and the solutions should be placed in ice water for pre-cooling before use.
2. Use a glass homogenizer with tight clearance.
3. The reagents must be stored strictly according to the preservation conditions in the table above.
4. The reagents in different kits cannot be mixed with each other.

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