Mitochondria Extraction Kit, for Tissue

Cat #: D-AKE4004

Size: 50T

Storage: Stored at -20°C for 12 months

Assay Principle

Mitochondria is a double-membrane-bound organelle found in most eukaryotic cells. The function of mitochondria is to provide cellular energy. Moreover, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorder syndrome, heart failure and autism. Mitochondria may play an important role in these cellular processes. Mitochondrion Extraction Kit (Tissue) enables rapid and crude isolation of intact mitochondria from animal tissues from both soft and hard tissues using differential centrifugation. This crude mitochondrial preparation is often enough for most applications, such as study of mitochondrial respiration, mitochondria membrane potential, apoptosis, mtDNA and mtRNA, and mitochondrial protein profiling etc.

Materials Supplied and Storage Conditions

Kit components	Size（50T）	Storage conditions
Lysis Buffer A (5×)	20 mL	-20°C
Lysis Buffer B (5×)	20 mL	-20°C
Storage Buffer	3 mL	-20°C

Materials Required but Not Supplied

·Vortexer, centrifuge tube

·Microscope, Cell scraper

·Precision Pipettes, Disposable Pipette Tips

·Phosphate buffered saline (PBS)

·Dounce homogenizer (for Tissue Samples)

·0.25 mg/mL trypsin

Reagent Preparation

Lysis Buffer A (1×): Dilute Lysis Buffer A (5×) with sterile deionized water. Before use, immediately add protease inhibitors and place on ice. Store at -20°C.

Lysis Buffer B (1×): Dilute Lysis Buffer B (5×) with sterile deionized water. Before use, immediately add protease inhibitors and place on ice. Store at -20°C.

Storage Buffer: Ready to use as supplied. The solution can also be stored at -20°C after aliquoting to avoid repeated freezing and thawing. Before use, place on ice.

Assay Procedure

Note: Perform all steps at 2-8°C. Use precooled buffers and equipment. Ensure all the solutions are defrosted and homogeneous. For skeletal muscle use Lysis Buffer B (1×). For other tissues, use Lysis Buffer A (1×) first.

I-A For soft tissues (liver or brain)

1. Prepare a fresh tissue sample (obtained within one hour of sacrifice) and wash the sample twice with ice-cold PBS.

Note: Do not freeze.

2. Cut 100 mg of tissue into very small pieces and wash the sample once with 1 mL ice-cold Lysis Buffer A (1×).
3. Add fresh 1 mL ice-cold Lysis Buffer A (1×) and transfer to a pre-cooling Dounce homogenizer.

4. Homogenize the sample on ice (usually 10-20 strokes).

5. Transfer the homogenate to a new tube and centrifuge the sample at 600 g for 10 min. Collect the supernatant in a new tube.

Note: For a more purified mitochondrial fraction, this step can be repeated once. The BSA (delipidated, final concentration 2 mg/mL) can be added to the Lysis Buffer A (1×) to remove lipids, which may be present in the tissue.

6. Centrifuge at 11,000 g for 10 min at 4°C.

Note: To obtain a more purified fraction of mitochondria, with >50% reduction of lysosomal and peroxisomal contaminants, this step can be changed to centrifuge at 3,000 g for 15 min. The supernatant is cytosol fraction.

7. Remove the supernatant and resuspend the pellet in 1 mL ice-cold Lysis Buffer A (1×). Repeat steps 5 and 6.

8. Suspend the pellet (purified mitochondria) in Storage Buffer (40 µL per 100 mg tissue). Freeze and aliquot at -80°C until use.

Note: It is expected the protein concentration of the sample should be approximately 10-25 mg/mL.

I -B For hard tissues (skeletal muscle)

1. Prepare a fresh tissue sample (obtained within 1 h of sacrifice) and wash the sample twice with ice-cold PBS.

Note: Do not freeze.

2. Cut 100 mg of tissue into very small pieces. Centrifuge the sample at 600 g for 30 s, and then discard the supernatant.

3. Suspend the sample in 1 mL 0.25 mg/mL trypsin in a new 2 mL Eppendorf tube.

4. Incubate on ice for 5-10 min.

Note: The incubation time varies in different tissues.

5. Spin down the tissue for a few seconds in the centrifuge and remove the supernatant.

6. Wash the sample once with 1 mL ice-cold Lysis Buffer B (1×) and spin down the tissue for a few seconds in the centrifuge.

7. Remove the supernatant and add fresh 1 mL ice-cold the appropriate Lysis buffer.

8. Transfer the sample to a pre-cooling Dounce homogenizer and homogenize the sample on ice (usually 20-30 strokes).

9. Transfer the homogenate to a new tube and centrifuge the sample at 600 g for 10 min.

Note: For a more purified mitochondrial fraction, this step can be repeated once.

10. Collect the supernatant in a new tube and centrifuge at 11,000 g for 10 min at 4°C.

Note: To obtain a more purified fraction of mitochondria, with >50% reduction of lysosomal and peroxisomal contaminants, this step can be changed to centrifuge at 3,000 g for 15 min. The supernatant is cytosol fraction.

11. Remove the supernatant and resuspend the pellet in 1 mL ice-cold Lysis Buffer B (1×). Repeat steps 9 and 10.

12. Suspend the pellet (purified mitochondria) in Storage Buffer (40 µL per 100 mg tissue). Freeze and aliquot at -80°C until use.

Note: It is expected the protein concentration of the sample should be approximately 10-25 mg/mL.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.