Mitochondria Extraction Kit, for Cell

Cat #: D-AKE4003

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 disorders, heart failure and autism. Mitochondria may play an important role in these cellular processes.

Mitochondria Extraction Kit, for Cell and crude isolation of intact mitochondria from cultured mammalian cells. The kit offers two protocols for the separation of mitochondria from cytosolic components relying on differential centrifugation. First protocol utilizes a reagent-based method allowing multiple samples to be processed concurrently. The second protocol uses traditional Dounce homogenization and provides approximately two-fold more mitochondria.

Materials Supplied and Storage Conditions

Kit components	Size（50T）	Storage conditions
Lysis Buffer A (5×)	7.5 mL	-4°C
Lysis Buffer B	0.5 mL	-4°C
Lysis Buffer C	12.5 mL	4°C
Storage Buffer	15 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)

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 4°C.

Lysis Buffer B: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Lysis Buffer C: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°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.

I Cell Culture Preparation

1. For adherent cells, harvest 2×10^7 cells with cell scrapers and then centrifuge at 500 g for 5 min. For suspension cells, harvest by centrifuging at 500 g for 5 min. A 100 mm diameter culture dish of freshly confluent cells of a typical adherent cell line should contain about 0.5 to 3×10^7 cells per dish.
2. Wash cells by suspending the cell pellet with ice-cold PBS. Centrifuge at 500 g for 2-3 min and discard the PBS.

Note: Use a pipette to carefully remove and discard the PBS, leaving the cell pellet as dry as possible.

3. Add 0.75 mL ice-cold Lysis Buffer A (1×) to the cell pellet. Vortex cells for 10 s at half maximal speed.
4. Incubate for 2 min on ice. Proceed to procedure II or III.

Note: Do not exceed the 2 min incubation.

II-A Isolation of Mitochondria using Reagent-based Method

1. Add 10 µL of Lysis Buffer B to cell lysate. Vortex at maximum speed for 5 s.
2. Incubate the tube on ice for 5 min, vortexing at maximum speed every minute.
3. Add 250 µL of Lysis Buffer C. Invert tube several times to mix. **(Note: Do not vortex)**
4. Centrifuge tube at 600 g for 10 min at 4°C.
5. Collect the supernatant in a new tube and centrifuge at 11,000 g for 10 min at 4°C.

Note: To obtain more purified mitochondria and reduce more than 50% of lysosomal and peroxisome contaminants, this step can be changed to 3,000 g centrifugation for 15 min. The supernatant is the cytoplasmic part.

6. Carefully remove the supernatant, and suspend the pellet in 200 µL Storage Buffer and freeze at -80°C until use, or a buffer suitable for your application.

II-B Isolation of Mitochondria using Dounce Homogenization

1. Transfer cell suspension to Dounce Tissue Grinder. Homogenize cells on ice. Perform enough strokes to effectively

lyse the cells. Each cell type requires an optimization of the number of strokes.

Note: The number of Dounce homogenization strokes necessary for optimal cell lysis will vary depending upon cell line, usually 10-30 strokes.

2. Transfer cell suspension to original tube and add 250 µL of Lysis Buffer C. Invert tube several times to mix. **(Note: Do not vortex)**

3. Centrifuge tube at 600 g for 10 min at 4°C.

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

Note: To obtain more purified mitochondria and reduce more than 50% of lysosomal and peroxisome contaminants, this step can be changed to 3,000 g centrifugation for 15 min. The supernatant is the cytoplasmic part.

5. Carefully remove the supernatant, and suspend the pellet in 200 µL Storage Buffer and freeze at -80°C until use, or a buffer suitable for your application.

Disclaimer

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