ExKine™ Mitochondrion Extraction Kit (Cultured Cells)

Item NO.	Product Name
KTP4003	ExKine™ Mitochondrion Extraction Kit (Cultured Cells)

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201809

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INTRODUCTION

Background & 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.

The Mitochondria Isolation Kit enables rapid 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.

Storage/Stability

Refer to list of materials supplied for storage conditions of individual components. Stable for at least 12 months at recommended temperature from date of shipment. Gel pack with blue ice.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity		Storage conditions
	50T	200T	
Lysis Buffer A (5×)	7.5 mL	30 mL	4°C
Lysis Buffer B	0.5 mL	2 mL	4°C
Lysis Buffer C	12.5 mL	50 mL	4°C
Storage Buffer	15 mL	50 mL	-20°C

Other supplies required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Phosphate-buffered saline (PBS)
- Glass tissue homogenizer
- Cell scrapers

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Note: Perform all steps at 2–8 °C. Use precooled buffers and equipment. Ensure all the solutions are defrosted and homogeneous.

Reagent Preparation

Lysis Buffer A (5×): Diluting with sterile, deionized water. Immediately before use, add protease inhibitors. Keep on ice while using.

Storage Buffer: Aliquot and store unused stock solutions at -20°C. Keep on ice while using.

Other Reagents: Keep on ice while using.

Recommended procedures

A. Cell Culture Preparation

1. For adherent cells, harvest 2×10^7 cells with cell scrapers and then centrifuge at 500 g for 5 minutes. For suspension cells, harvest by centrifuging at 500 g for 5 minutes. A 100 mm diameter culture dish of freshly confluent cells of a typical adherent cell line should contain about 0.5 to 3 x 10^7 cells per dish.

2. Wash cells by suspending the cell pellet with ice-cold PBS. Centrifuge at 500 g for 2-3 minutes 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 to the cell pellet. Vortex cells for 10 sec at half maximal speed.

4. Incubate for 2 min on ice. Proceed to procedure B or C.
Note: Do not exceed the 2 minutes incubation

B. Isolation of Mitochondria using Reagent-based Method

1. Add 10 µL of Lysis Buffer B to cell lysate. Vortex at maximum speed for 5 seconds.

2. Incubate the tube on ice for 5 minutes, vortexing at maximum speed every minute.

3. Add 250 µL of Mitochondria Isolation Reagent C. Invert tube several times to mix.
Note: Do not vortex.

4. Centrifuge tube at 600 g for 10 minutes at 4ºC.

5. Collect the supernatant in a new tube and centrifuge at 11,000 x 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 3000 g for 15 minutes. The supernatant is cytosol fraction.

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

C. 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 Mitochondria Isolation Reagent C. Invert tube several times to mix.
Note: Do not vortex.

3. Centrifuge tube at 600 g for 10 minutes at 4^{o}C.

4. Collect the supernatant in a new tube and centrifuge at 11,000 x 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 3000 g for 15 minutes. The supernatant is cytosol fraction.

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