Mitochondria Isolation Kit (MITOISO)

Cat. No. 8268
100 reactions

Introduction

As cellular power plants, mitochondria are the subcellular organelles found in most eukaryotic cells. They contain the enzymes for tricarboxylic acid cycle and protein complexes for electron transport chain. Mitochondrial dysfunction has been implicated in many human diseases relating to cancer, diabetes, cardiac failures and neurodegenerative disorders. Therefore, there is an increasing need for an effective method to isolate intact and functional mitochondria from fresh or frozen tissues and cultured cells. ScienCell's Mitochondria Isolation Kit has been designed to provide the optimal yield of intact and enzymatically active mitochondria. The basic principles of mitochondria isolation using this kit include three stages: 1) mechanical rupturing of tissues or cells 2) removing cellular debris and nuclei by low speed centrifugation and 3) harvesting mitochondria by high speed centrifugation.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8268a	1	Mitochondria isolation buffer A (2X)	60mL	4°C
8268b	1	Mitochondria isolation buffer B	25mL	4°C
8268c	1	Lysis buffer	10mL	-20°C
8268d	1	Protease inhibitor cocktail (20X)	500µL	-20°C

Product Use

This product is for research purposes only and not for use in animals, humans or diagnostic procedures.

Quality Control

Enzymatic activities of isolated mitochondria are measured by using ScienCell's mitochondrial cytochrome c oxidase activity assay kit (ScienCell™ Cat.No.8278). Percent of intact outer mitochondrial membrane is ~88% from rodent liver and ~85% from human dermal fibroblasts-fetal (ScienCell™ Cat.No.2300), as validated by cytochrome c oxidase activity kit.

Sample	Material	Estimated Yield	Strokes
Fibroblasts	$1x10^7$cells	~35 µg	10-15
Rodent Liver	0.1 g	~1.2mg	10-15
Rodent White Fat	0.5 g	~200 µg	20-30

Reagents and Equipment Supplied by User

1. Dounce homogenizer teflon or Polytron tissue disruptor
2. Bench-top centrifuge refrigerated at 2-8ºC
3. Ultrapure water (ScienCell™ Cat. No.0600)
4. Phosphate buffered saline (PBS) ($Ca^{2+,}$ Mg^{2+} free) (ScienCell™ Cat. No. 0303)
5. Microcentrifuge tubes (1.5mL)

Shipping

Dry ice.

Procedure

Isolating Mitochondria from Cultured Cells

Isolation yield depends on cell type and starting material. All steps are carried out at 4°C using chilled buffers, homogenizer and centrifuge and tubes. Excess homogenization may compromise the mitochondrial integrity.

1. Harvest $\geq 1x10^7$ cells by centrifuging at 500 x g for 5 minutes at 4°C. Adherent cells can be collected by using a cell scraper or by trypsinization (not supplied).
2. Wash cells with 5 mL ice-cold PBS. Centrifuge at 500 x g for 5 minutes at 4°C. Discard the supernatant.
3. Add 1 mL of 1x Mitochondria Isolation Buffer A and homogenize using Polytron tissue disruptor at moderate speed for 20 seconds or Dounce homogenizer teflon for complete homogenization. Keep the homogenate on ice.
4. Transfer the homogenate to a 1.5mL Eppendorf tube and centrifuge the sample at 1000 x g for 5 minutes at 4°C. Discard the pellet.
5. Centrifuge the supernatant at 10,000 x g for 20 minutes at 4°C. Transfer supernatant (cytosolic fraction) into a new 1.5mL Eppendorf tube and collect the pellet (mitochondria fraction).

   Optional: If more pure mitochondria is needed, resuspend the pellet with 1mL 1xMitochondria Isolation Buffer A and centrifuge it for another 10,000 x g for 20 minutes at 4°C and collect the pellet.
6. If intact mitochondria are required, resuspend the pellet in 50 µL mitochondria isolation buffer B and keep it on ice before proceeding further. Sample is now ready for mitochondrial enzyme activity assay. If mitochondrial protein lysate is desired, resuspend the pellet in 50µL lysis buffer with 1x protease inhibitor.
7. Check protein concentration with the Pierce BCA Protein Assay Kit or Bio-Rad protein assay(not supplied). Aliquots of mitochondria should be stored at -80°C.

Isolating Mitochondria from Tissues

Isolation yield depends on tissue type and starting material. All steps are carried out at 4°C using chilled buffers, homogenizer and centrifuge and tubes. Fresh or frozen tissue can be used (frozen tissue would reduce the percent of intact outer membrane of mitochondria).

1. Weigh 0.1 g tissue and wash it twice with 5 mL ice-cold PBS.
2. Add 1 mL of 1x Mitochondria Isolation Buffer A and homogenize using Polytron tissue disruptor at moderate speed for 20 seconds or Dounce homogenizer teflon for complete homogenization. Keep the homogenate on ice.
3. Transfer the homogenate to 1.5mL Eppendorf tube and centrifuge the sample at 1000 x g for 5 minutes at 4°C.
4. Transfer supernatant to a clean 1.5mL Eppendorf tube and centrifuge at 10,000 x g for 20 minutes at 4°C.

Transfer supernatant (cytosolic fraction) into a new tube and collect the pellet (mitochondria fraction).

Optional: If more pure mitochondria is needed, resuspend the pellet with 1mL 1xMitochondria Isolation Buffer A and centrifuge it for another 10,000 x g for 20 minutes at 4°C and collect the pellet.

5. If intact mitochondria are required, resuspend the pellet in 100 µL mitochondria isolation buffer B and keep it on ice before proceeding further. Sample is now ready for mitochondrial enzyme activity assay. If mitochondrial protein lysate is desired, resuspend the pellet in 100µL lysis buffer with 1x protease inhibitor.
6. Check protein concentration with the Pierce BCA Protein Assay Kit or Bio-Rad protein assay (not supplied). Aliquots of mitochondria should be stored at -80°C.

Reference:

Trounce IA, Kim YL, Jun AS, Wallace DC. 1996. Assessment of mitochondrial oxidative phosphorylation in patient muscle. biopsies, lymphoblasts, and transmitochondrial cell lines. Methods Enzymol 264: 484–509.