BioChain®
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User's Manual and Instructions

Mitochondria Isolation Kit for Tissue and Cultured Cells

Catalog Number: KC010100

Introduction

Mitochondria are the eukaryotic subcellular organelles that contain the enzymes of the citric acid cycle, the electron transport chain, and oxidative phosphorylation. As new discoveries of human disorders are being related to mitochondrial dysfunction, there is an increasing need for an effective method to isolate intact mitochondria from tissues and cultured cells. The key steps when isolating mitochondria from any tissue or cell are always the same: (i) rupturing of cells by mechanical and/or chemical means and (ii) differential centrifugation at low speed to remove debris and extremely large cellular organelles followed by centrifugation at a higher speed to isolate mitochondria which are collected. The procedures described in this manual are easy and fast, and has been designed to provide the highest possible yield of intact and enzymatically active mitochondria. The enzymatic activity and integrity of isolated mitochondria has been measured using BioChain's Mitochondria Activity Assay (Cytochrome C Oxidase Activity Assay) Kit. The activity of mitochondria may be measured by assaying cytochrome c oxidase activity (using Mitochondria Activity Assay (Cytochrome C Oxidase Activity Assay) kit, Catalog # KC310100).

The isolated mitochondria can be used for numerous downstream applications, including apoptosis studies, metabolic studies and mitochondria diseases studies. And this kit can also be used for isolating mitochondria proteins for proteomics research, western blottings and ELISA.

Features

- **Easy and fast** – Procedures can be performed within an hour, no ultracentrifuge needed.
- **Reliable** – super-quality and highly reproducible isolation of mitochondria
- **Efficient** – provide the highest possible yield of intact and enzymatically active mitochondria

Applications

- Isolating intact and enzymatically active mitochondria from tissues and cultured cells
- Isolating mitochondria proteins from tissues and cultured cells.

Description

Components in this kit are prepared with pure chemicals according to our proprietary technology. BioChain's Mitochondria Isolation Kits are designed to isolating intact and active mitochondria in a fast and easy way. One kit is consisted of reagents enough for performing 100 isolations (enriching mitochondria from 10 – 20 g tissue or from $1x10^{10}$ – $5x10^{10}$ cells).

Quality Control

1 kit of this lot has been tested to go through the complete isolation procedure. Yield, Activity and Integrity of mitochondria isolated using this lot are comparable to those obtained with control (previous) lot.

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Components
Mitochondria Isolation Kit: Reagents are sufficient for 100 isolations.
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Item	Amount	Part No.
1. Mitochondria Isolation Buffer (5x)	50 ml	KC010100-1
2. Mitochondria Storage Buffer	10 ml	KC010100-2
3. Lysis Buffer	10 ml	KC010100-3
4. Protease Inhibitor cocktail (50x)	200 µl	KC010100-4

Reagents and Equipments Required but not Supplied in this Kit:
1. homogenizer (polytron tissue disruptor or dounce tissue grinder)
2. bench-top centrifuge refrigerated at 2-8°C.
3. ultrapure water
4. PBS (Ca^{2+}, Mg^{2+} free)

Storage and Stability
Store at -20°C (The Lysis Buffer can be stored at 2-8°C after it is thawed). The kit is stable for one year when handled properly.

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Protocol

I. Prepareation of Reagents

Use ultrapure water for the preparation of reagents.

Solution	Preparation	Storage	Notes
1x Mitochondria Isolation Buffer	Dilute an aliquot of 5x mitochondria isolation buffer 5-fold with water.	2-8°C	5x mitochondria isolation buffer may be refrozen at -20°C. Precipitates may form during the storage and can be redissolved by warming up to 50°C.
Mitochondria Storage Buffer	Divide mitochondria storage buffer into working aliquots.	-20°C	
Lysis Buffer with 1x protease inhibitor cocktail	Add 20 µl of 50x protease inhibitor cocktail to 1 ml of lysis buffer.	-20°C	Divide 50x protease inhibitor cocktail to working aliquots and store at -20°C. Store the Lysis Buffer (without protease inhibitor) at 4°C. Store the Lysis Buffer with protease inhibitor at -20°C.

II. Suggested Starting Materials

Sample	Starting materials
Tissue	100 – 200 mg
Cultured cells	$5x10^{7}$ – $1x10^{8}$

III. Isolating Intact Mitochondria from Tissue

Isolation yields are dependant upon the type, amount and freshness of the tissue. All steps are carried out at 4°C using chilled buffers, homogenizer pestles and centrifuges. The tissue should be obtained fresh and kept on ice and the isolation should start within 2 hours of sacrifice. We recommend starting with 100 – 200 mg tissue. Although this procedure may be up/downscaled.

1. Weigh 100 – 200 mg tissue and wash it twice with 10 ml ice-cold PBS. Mince the tissues to smaller pieces.
2. Add 2 ml of 1x Mitochondria Isolation Buffer and homogenize using polytron tissue disruptor at moderate speed (e.g. speed 4) for 20 sec. Let it stand on ice for 5 sec, repeat homogenization twice. Keep the homogenate on ice.
3. Transfer the homogenate to 2 ml Eppendorf tube and centrifuge the sample at 600 g for 10 min at 4°C. Discard the pellet.
4. Centrifuge the supernatant at 12,000 g for 15 min at 4°C. Collect the pellet.
5. Resuspend the pellet in 0.5 ml 1x Mitochondria Isolation Buffer.
6. Repeat step of 3 and 4.
7. Resuspend the pellet in 50 – 100 µl mitochondria storage buffer and keep it on ice before downstream processing.
8. If mitochondria protein lysate is desired, resuspend the pellet in 100 µl Lysis Buffer with 1x protease inhibitors. The mitochondria protein concentration can be measured at this step. The expected concentration should be about 0.5 – 1 mg/ml. Store the lysate at -80°C.

IV. Isolating Intact Mitochondria from Cultured Cells

This kit can also be used for isolating intact mitochondria from cultured cells. But due to the low bioenergetic requirement of the cells, cells usually contain little mitochondria, therefore it can be difficult to isolate mitochondria in large quantities. We recommend starting with $5x10^7$ – $1x10^8$ cells. Although this procedure may be up/downscaled. All steps are carried out at 4°C using chilled buffers, homogenize grinders and centrifuges.

1. Cells are collected by centrifuging at 600 g for 5 min at 4°C. In the case of adherent cells they can be collected with a cell lifter or trypsinization).
2. Wash cells with 10 ml ice cold PBS. Centrifuge at 600 g for 5 min at 4°C. Discard the supernatant.
3. Resuspend the pellet in 1 ml 1x Mitochondria Isolation Buffer and transfer into a pre-chilled Dounce tissue grinder. Perform the homogenization on ice. We recommend 30 – 40 strokes with grinder. However, the efficiency of homogenization may depend on cell type. Excess homogenization may compromise the mitochondria integrity.
4. Transfer the homogenate to 2 ml Eppendorf tube and centrifuge the sample at 600 g for 10 min at 4°C. Discard the pellet.
5. Centrifuge the supernatant at 12,000 g for 15 min at 4°C. Collect the pellet.
6. Resuspend the pellet in 50 – 100 µl mitochondria storage buffer and keep it on ice before downstream processing.
7. If mitochondria protein lysate is desired, resuspend the pellet in 100 µl Lysis Buffer with 1x protease inhibitors. The mitochondria protein concentration can be measured at this step. The expected concentration should be about 0.5 – 1 mg/ml. Store the lysate at -80°C.

V. (Optional) Isolating Cytosol and Nuclear Protein Fraction
This is an optional protocol using this kit for Cytosol and Nuclear protein isolation, please be aware, by using this protocol, the reagent will not be enough for the main purpose of mitochondria isolation

1. Weigh 100 – 200 mg tissue and wash it twice with 10 ml ice-cold PBS. Mince the tissues to smaller pieces.

2. Add 1 ml of 1x Mitochondria Isolation Buffer with 1x protease inhibitors and homogenize using polytron tissue disruptor at moderate speed (e.g. speed 4) for 20 sec. Let it stand on ice for 5 sec, repeat homogenization twice. Keep the homogenate on ice.

3. Transfer the homogenate to 2 ml Eppendorf tube and centrifuge the sample at 600 g for 10 min at 4°C. Carefully transfer the supernatant to another tube for later use.

4. Resuspend the pellet in 200 µl Lysis Buffer with 1x protease inhibitors. This is the nuclear fraction.

5. Centrifuge the supernatant from step 3 at 12,000 g for 15 min at 4°C. Transfer the supernatant to another tube. The supernatant is the cytosol fraction.

6. Resuspend the pellet in 100 µl Lysis Buffer with 1x protease inhibitors. This is the mitochondria fraction.

7. Store all three fractions at -80°C.

Related Products
Mitochondria Activity Assay (Cytochrome C Oxidase Activity Assay) Kit, Compartment Protein Isolation Kit

References

1. Storrie, B. and Madden, E.A., Methods Enzymol. 182, **203** (1990).
2. Rabilloud, T., et al, Electrophoresis, **19**, 1006 (1998).
3. Lopez M.F., et al, Electrophoresis, **21**, 3427 (2000).