USER MANUAL

Mitochondria Extraction Kit

IMGENEX

Innovations in Functional Genomics

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Mitochondrial Extraction Kit

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Research purposes only. Not for diagnostic or human use.

BACKGROUND

The procedure described here for the isolation of mitochondria is based on the principle of differential centrifugation. The high density nuclei are first removed by low-speed centrifugation on a sucrose cushion. The supernatant containing the mitochondria is subjected to high speed centrifugation to retrieve the mitochondria. This kit contains reagents required for the isolation of mitochondria from 10 grams of mammalian tissues (liver, muscle, etc.) or 100 reactions for cells grown in tissue culture plates. The procedure describes isolation of mitochondria from 1 gram of starting tissue material (mouse liver) or 5 x 10^7 HeLa cells. The Mitochondrial and Cytosolic fractions can be used for studying apoptotic and signal transduction pathways to detect translocation of any factors of interest within the two fractions by western blotting, ELISA, or other assay.

FEATURES

The IMGENEX Mitochondrial Extraction Kit can be used to isolate cytosolic and mitochondrial fractions from the same cell or tissue preparations.

KIT COMPONENTS AND STORAGE

Store the components at recommended temperature as indicated below. Stable for 6 months when stored correctly.

Reagents (4°C storage)		Quantity
KC-410	5X Homogenization Buffer	50 ml
KC-411	5X Suspension Buffer	2 x 50 ml
KC-412	Mitochondrial Staining Solution	1 ml
KC-413	Mitochondria Lysis Buffer	50 ml
KC-117	10X PBS	2 x 50 ml
Reagents (-20°C storage)		
KC-404	1M DTT	100 µl
KC-406	100X Protease Inhibitor Cocktail (PIC)	100 µl

Addition Equipment Required

- Sterile Water
- Standard balance (tissues)
- High-speed centrifuge and compatible centrifuge tubes
- Teflon homogenizer
- Cell scraper
- Microcentrifuge tubes

BUFFER PREPARATION

1X Homogenization Buffer:	10 ml of 5X Homogenization Buffer 40 ml of sterile water.
1X Suspension Buffer:	10 ml of 5X Suspension Buffer 40 ml of sterile water.
1X PBS:	5 ml of 10X PBS 45 ml of sterile water.

Complete Mitochondrial Lysis Buffer: (for 10 rxn, ammend the formulation for the number of rxns) Prepare the amount of Complete Mitochondria Lysis Buffer required for your experiment by adding 10 µl of 100X PIC and 1 µl of 1M DTT to 1 ml of Mitochondrial Lysis Buffer. Some protease inhibitors lose their activity within 24 hours of dilution. Therefore, we recommend using the PIC just before starting the experiment.

PROCEDURE

A. Preparation of Mitochondrial Extract from Cells (5 x 10^7 HeLa Cells)

1. Wash cells with 5 ml of cold 1X PBS.
2. Add 10 ml of cold 1X PBS and scrape the cells from the dish using a cell scraper. Transfer the cells to a pre-chilled 15 ml centrifuge tube and spin at 1,200 rpm for 5 min at 4^oC.
3. Remove supernatant and wash cell pellet by resuspending in 5 ml of cold PBS. Spin at 1,200 rpm for 5 min at 4^oC and remove super natant.
4. Add 1 ml of cold 1X Homogenization Buffer. Resuspend cell pellet by pipetting up and down with a pipette. Incubate on ice for 15 min, then transfer to a pre-chilled pestle homogenizer.

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5. Homogenize cells using 30-50 strokes with the homogenizer.

   Transfer the supernatant to a pre-chilled microcentrifuge tube.

6. Spin down the lysate at 3,000 rpm for 20 min at 4°C. The pellet is the nuclei, cellular debris and intact cells, whereas the supernatant contains the cytosol and mitochondria.

7. Transfer the supernatant to a pre-chilled microcentrifuge tube.

8. Spin the supernatant again at 3,000 rpm for 10 min at 4°C to remove any residual nuclei and transfer the supernatant to a pre-chilled microcentrifuge tube.

9. Spin the supernatant at 12,000 rpm for 30 min at 4°C. The resulting supernatant is the cytosolic fraction and the pellet is the mitochondrial fraction.

10. Transfer supernatant (cytosolic fraction) into a microcentrifuge tube and store at 4°C (you may centrifuge again to remove residual mitochondria).

11. Resuspend mitochondrial pellet in 4 ml of 1X Suspension Buffer. Centrifuge at 12,000 rpm for 10 min at 4°C and discard supernatant.

12. Resuspend pellet with 1 ml of 1X Suspension Buffer.

13. Mix 10 µl of mitochondrial suspension from step 12 with 10 µl of Mitochondrial Staining Solution and observe under microscope. The number of mitochondria can be counted using a standard hemocy tometer.

14. Spin the mitochondrial suspension at 12,000 rpm for 10 min at 4°C and discard the supernatant.

15. Lyse the mitochondrial pellet by adding 100 µl of Complete Mitochondrial Lysis Buffer and incubate on ice for 30 min. After incubation, vortex for 10 s to mix thoroughly. Then centrifuge for 5 min at 12,000 rpm at 4°C.

16. Transfer the supernatant (mitochondrial fraction) into a microcentrifuge tube.

17. Measure the protein concentration of both (mitochondrial and cytosolic) fractions using a Bio-Rad assay with BSA standard.

18. Aliquot the fractions and store at -80°C. Avoid freeze/thaw cycles.

19. 5 x $10^{7)}$ HeLa cell extract yields approximately 50 µg of mitochondrial protein.

B. Preparation of Mitochondrial Extract from Tissue (Mouse Liver)

The protocol described below is optimized for extraction of mitochondria from 1 gram mouse liver. However, this protocol may be used to extract mitochondria from other tissues.

1. Isolate the tissue of interest using standard dissection procedures.
2. Wash the tissue with 10 ml of ice-cold 1X PBS.
3. Mince the tissue with a scalpel or a razor blade.
4. Weigh out the required amount of tissue (1 gram for mouse liver tissue) and transfer to a glass beaker.
5. Add 5 ml of ice cold Homogenization Buffer per gram of tissue.
6. Homogenize the tissue in a Dounce-type homogenizer (preferably with a Teflon pestle) applying 20-30 strokes.
7. Transfer the suspension to centrifuge tubes and centrifuge at 3000 rpm for 10 min at 4°C.
8. Transfer the supernatant to a clean centrifuge tube. The pellet, which is rich in nuclei, may be discarded.
9. Centrifuge the supernatant at 12,000 rpm for 30 min at 4°C.
10. Transfer the supernatant (cytosolic fraction) into a microcentrifuge tube and resuspend the pellet with 5 ml of ice-cold 1X Suspension Buffer.
11. Centrifuge the suspension at 12,000 rpm for 10 min at 4°C.
12. Discard the supernatant and resuspend the pellet in 5 ml (per gram of original tissue) of Suspension Buffer.
13. Mix 10 µl of the suspension with 10 µl of staining solution and observe under a microscope.
14. The number of mitochondria may be counted using a standard hemocytometer.
15. Spin the mitochondrial suspension at 12,000 rpm for 10 min at 4°C and discard the supernatant.
16. Lyse the mitochondrial pellet in 1 ml of Complete Mitochondrial Lysis Buffer by mixing end-over-end for 30 min at 4°C.

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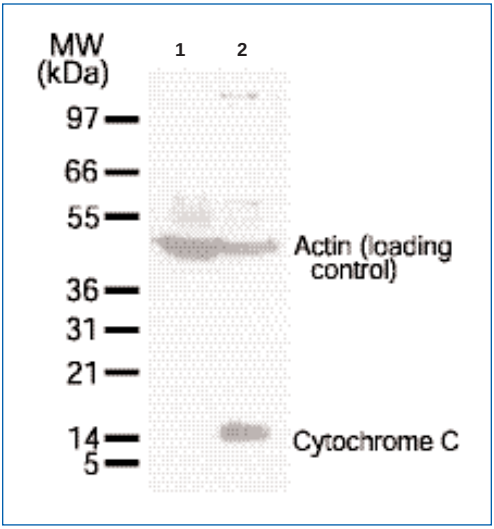
17. Centrifuge the mitochondrial extract at 12,000 rpm for 5 min at 4°C and transfer the supernatant (mitochondrial fraction) into a microcen trifuge tube.
18. Measure the protein concentration of both (mitochondrial and cytosolic) fractions using a Bio-Rad assay with BSA standard.
19. Aliquot the fractions and store at -80°C. Avoid freeze/thaw cycles.
20. One gram of mouse liver extract yields approximately 2.28 mg of mitochondrial protein.



Mouse liver cells were fractionated using IMGENEX's Mitochondrial Extraction Kit. 20 ug of cytosolic (lane 1) and mitochondrial (lane 2) extracts were probed with anti-Cytochrome C antibody (IMG-101), and anti-Actin antibody (loading control, IMG-5142A). IMGENEX's extraction kit efficiently separated the two fractions and no Cytochrome C (specific to mitochondria) was detected in the cytosolic extract.

PRODUCT CITATIONS

1. **Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice.** Ayala JE, DP Bracy, BM Julien, JN Rottman, PT Fueger, and DH Wasserman. Diabetes 56:1025-1033 (2007). Isolation of mitochondria from intacapsular brown fat (mice) using 10082K, Fig 3: Mitochondrial extracts were used for western blot.

2. **UPC2 is highly expressed in pancreatic alpha-cells and influences secretion and survival.** Diao J, EM Allister, V Koshkin, SC Lee, A Bhattacharjee, C Tang, A Giacca, CB Chan, and MB Wheeler. PNAS 105:12057-12062 (2008). Isolation of mitochondrial and cytosolic fractions from mouse pancreatic cell lines, alpha-TC6 and MIN6 using 10082K: Fig 2, mitochondrial and cytosolic fractions were used for western blot.

TROUBLE SHOOTING

Problem	Probable Cause	Suggestion
Low protein concentration in cytosolic fraction.	Cell lysis was not efficient.	Increase the number of strokes performed with homogenizer.
Mitchondrial protein leakage.	Cell lysis was too vigorous.	Reduce the number of strokes with the homogenizer to 10 strokes.
Low protein concentration in mitochondrial fraction.	Complete mitochondrial lysis buffer was not made properly.	Add more PIC and DTT to mitochondrial lysis buffer.

If you require additional assistance, please contact Imgenex Technical Service:

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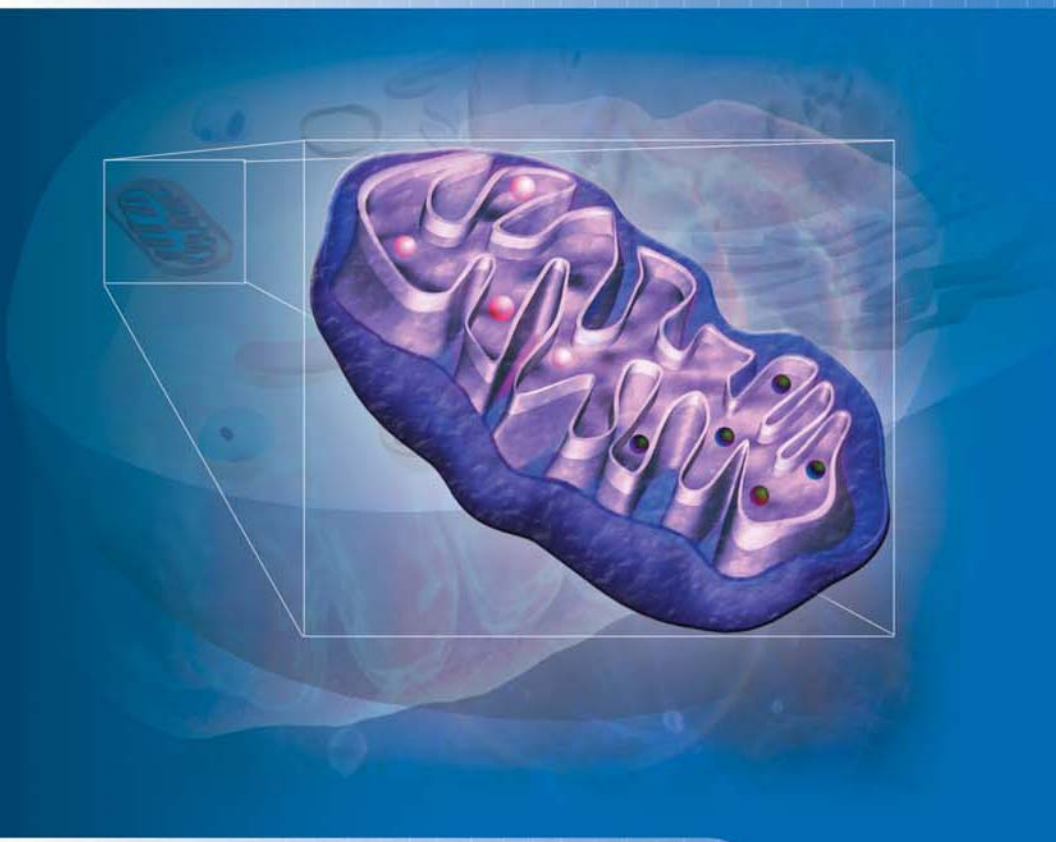

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