INSTRUCTIONS

Mitochondria Isolation Kit for Cultured Cells

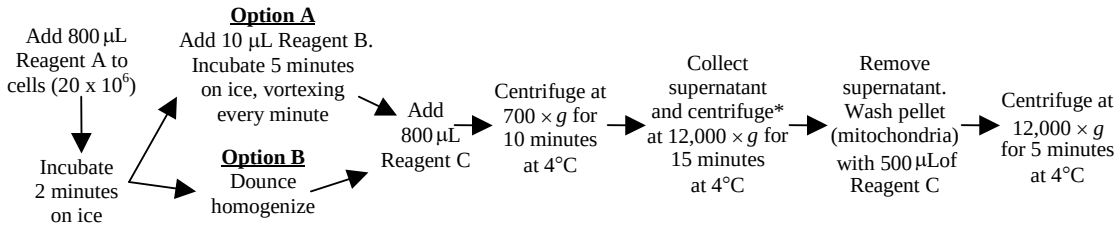
89874

1477.7

Number	Description
89874	**Mitochondria Isolation Kit for Cultured Cells,** contains sufficient reagents to isolate intact mitochondria from 50 pellets of cultured mammalian cells containing 2×10^7 cells each
	Kit Contents:
	Mitochondria Isolation Reagent A, 50mL
	Mitochondria Isolation Reagent B, 500µL
	Mitochondria Isolation Reagent C, 65mL
	Storage: Upon receipt store kit at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Mitochondria Isolation Kit for Cultured Cells enables isolation of intact mitochondria from cultured mammalian cells. The kit offers two options for the separation of mitochondria from cytosolic components (Figure 1). One option is a unique protocol utilizing a reagent-based method allowing multiple samples to be processed concurrently. The second option uses traditional Dounce homogenization and provides approximately two-fold more mitochondria, based on protein analysis. Both protocols rely on differential centrifugation to separate the mitochondrial and cytosolic fractions with a bench-top microcentrifuge and are completed in approximately 40 minutes (post-cell harvest). Each procedure has been optimized for maximum yield of mitochondria with minimal damage to integrity. Once isolated, the mitochondria may be used for a number of downstream applications, including apoptosis, signal transduction and metabolic studies.



*To obtain a more purified preparation of mitochondria, this step may be performed at 3,000 × *g*.

Figure 1. Procedure summary of the Pierce Mitochondria Isolation Kit.

Important Product Information

- This kit allows two options for mitochondria isolation: a reagent-based method or Dounce homogenization. The kit contains sufficient reagents for a **total of 50 applications** using either method exclusively or a combination of both.
- Up to six samples may be processed concurrently using the reagent-based isolation method, whereas only one sample may be processed at a time with the Dounce isolation method. Because of the time delay in processing multiple samples with the Dounce protocol, samples processed initially may yield a higher quantity of mitochondria than cell pellets incubated on ice for subsequent isolation.

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- The protocols presented prepare a crude mitochondrial fraction that includes lysosomal and peroxisomal contaminants. To obtain a more purified fraction of heavy mitochondria, centrifuge the post-nuclear supernatant at 3000 × *g* instead of at 12,000 × *g*. Western blot analysis of purified versus crude mitochondrial fractions prepared with this kit result in a > 50% reduction in cathepsin S (lysosomal protein) and PMP70 (peroxisomal protein) in the purified fraction.

Additional Materials Required

- Variable-speed bench-top microcentrifuge (refrigerated)
- 2.0mL microcentrifuge tubes
- Vortex mixer
- Protease inhibitors, EDTA-free such as Thermo Scientific™ Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X), 1mL (Product No. 87785)
- Dounce tissue grinder, such as 2mL Kontes or Wheaton Dounce Tissue Grinder, if using Option B

Option A: Isolation of Mitochondria using Reagent-based Method

Notes:

- Immediately before use, add protease inhibitors to Reagent A and Reagent C; only add inhibitors to the reagent amount being used for the procedure and not to the stock solutions.
- Process up to six samples concurrently.
- Required speed of vortex changes during the protocol.

1. Pellet 2×10^7 cells by centrifuging harvested cell suspension in a 2.0mL microcentrifuge tube at ~850 × *g* for 2 minutes. Carefully remove and discard the supernatant.
2. Add 800µL of Mitochondria Isolation Reagent A. Vortex at **medium** speed for 5 seconds and incubate tube on ice for exactly 2 minutes.

   Note: Do not exceed the 2 minute incubation.
3. Add 10µL of Mitochondria Isolation Reagent B. Vortex at **maximum** speed for 5 seconds.
4. Incubate tube on ice for 5 minutes, vortexing at **maximum** speed every minute.
5. Add 800µL of Mitochondria Isolation Reagent C. Invert tube several times to mix (**do not vortex**).
6. Centrifuge tube at 700 × *g* for 10 minutes at 4°C.
7. Transfer the supernatant to a new, 2.0mL tube and centrifuge at 12,000 × *g* for 15 minutes at 4°C.

   Note: To obtain a more purified fraction of mitochondria, with > 50% reduction of lysosomal and peroxisomal contaminants, centrifuge at 3000 × *g* for 15 minutes.
8. Transfer the supernatant (cytosol fraction) to a new tube. The pellet contains the isolated mitochondria.
9. Add 500µL Mitochondria Isolation Reagent C to the pellet, and centrifuge at 12,000 × *g* for 5 minutes. Discard the supernatant.
10. Maintain the mitochondrial pellet on ice before downstream processing. Freezing and thawing may compromise mitochondria integrity.

Option B: Isolation of Mitochondria using Dounce Homogenization

Notes:

- Immediately before use, add protease inhibitors to Reagent A and Reagent C; only add inhibitors to the reagent amount being used for the procedure and not to the stock solutions.
- Process one sample at a time.
- Pre-chill Dounce Tissue Grinder on ice before use.

1. Pellet 2×10^7 cells by centrifuging harvested cell suspension in a 2.0mL microcentrifuge tube at ~850 × *g* for 2 minutes. Carefully remove and discard the supernatant.
2. Add 800µL of Mitochondria Isolation Reagent A. Vortex at **medium** speed for 5 seconds and incubate tube on ice for exactly 2 minutes.

   Note: Do not exceed the 2 minute incubation.
3. Transfer cell suspension to Dounce Tissue Grinder.
4. Homogenize cells on ice. Perform enough strokes to effectively lyse the cells.

   Note: See Additional Information Section for the number of strokes required for > 80% lysis of a select group of cell types.

   Note: To check the cell lysis efficiency, spot 5µL of cell lysate onto a glass slide, add coverslip and view under a microscope. Compare with 5µL of the non-lysed cells.
5. Return lysed cells to original tube and add 800µL of Mitochondria Isolation Reagent C.
6. Rinse Dounce Tissue Grinder with 200µL of Mitochondria Isolation Reagent A and add to tube containing the sample in step B.5.
7. Invert tube several times to mix (**do not vortex**).
8. Centrifuge tube at 700 × *g* for 10 minutes at 4°C.
9. Transfer the supernatant to a new, 2.0mL tube and centrifuge at 12,000 × *g* for 15 minutes at 4°C.

   Note: To obtain a more purified fraction of mitochondria, with > 50% reduction of lysosomal and peroxisomal contaminants, centrifuge at 3000 × *g* for 15 minutes.
10. Transfer the supernatant (cytosol fraction) to a new tube. The pellet contains the isolated mitochondria.
11. Add 500µL Mitochondria Isolation Reagent C to the pellet, and centrifuge at 12,000 × *g* for 5 minutes. Discard the supernatant.
12. Maintain the mitochondrial pellet on ice before downstream processing. Freezing and thawing may compromise mitochondria integrity.

Additional Information

A. Cell Lysis

The number of Dounce homogenization strokes necessary for optimal cell lysis will vary depending upon cell line. The number of strokes required for > 80% lysis of a select group of cell types is indicated in Table 1, which may be used as a guide for other cell lines.

Table 1. Number of strokes required to achieve ~80% lysis efficiency using Dounce homogenization.*

Cell Line	Number of strokes
C6	40
HeLa	60
NIH 3T3	80

* Lysis efficiency was determined by visual estimation using a microscope.

B. Mitochondria Lysis

For analysis by Western blotting or gel electrophoresis, boil mitochondrial pellet with SDS-PAGE sample buffer and apply to the gel. For protein analysis using the Thermo Scientific™ BCA Protein Assay Kit (Product No. 23225), mitochondria may be lysed with 2% CHAPS in Tris-buffered saline (TBS; e.g., 25mM Tris, 0.15M NaCl; pH 7.2; Product No. 28379) as described below:

1. Add 100µL of 2% CHAPS in TBS to the mitochondrial pellet and vortex for 1 minute.
2. Centrifuge mitochondria at high speed for 2 minutes. The supernatant contains soluble mitochondrial protein that can be analyzed by BCA Protein Assay.

Related Thermo Scientific Products

89801	**Mitochondria Isolation Kit for Tissue**
23225	**BCA Protein Assay Kit**
34080	**SuperSignal™ West Pico Chemiluminescent Substrate**, 500mL
87785	**Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X),** 1mL
78833	**NE-PER™ Nuclear and Cytoplasmic Extraction Kit**
89842	**Mem-PER™ Plus Membrane Protein Extraction Kit**

References for Apoptosis

Green, D.R. and Reed, J.C. (1998). Mitochondria and apoptosis. *Science* **281:**1309-12.

Newmeyer, D.D. and Ferguson-Miller, S. (2003). Mitochondria: Releasing power for life and unleashing the machineries of death. *Cell* **112:**481-90.

References for Mitochondrial Proteome

Lescuyer, P., *et al.* (2003). Progress in the definition of a reference human mitochondrial proteome. *Proteomics* **3:**157-67.

Taylor, S.W., *et al.* (2003). Characterization of the human heart mitochondrial proteome. *Nat Biotechnol* **21:**281-5.

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