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Mammalian Mitochondria Isolation Kit for Tissue and Cultured Cells *rev. 02/17*

(Catalog # K288-50; Store at -20°C)

I. Introduction:

Mitochondria are the powerhouse of the cells because they generate most of the supply of energy in the form of adenosine tri-phosphate (ATP). Mitochondria are double membrane organelles: an outer membrane and a folded inner membrane called cristae. Isolated mitochondria are the sample of choice to study mitochondrial respiration, assembly of the respiratory complexes, apoptosis, mtDNA and mtRNA, and mitochondrial protein profiling. The kit offers two options for the isolation of intact mitochondria. The first option is utilizing a reagent-based method allowing parallel processing of multiple samples. The second option uses traditional dounce homogenization, which provides better mitochondrial yield.

II. Application:

- Isolation of high purity, intact and functional mitochondria from tissues and cultured cells.
- Mitochondrial respiration studies, assembly of the complexes, apoptosis, mtDNA and mtRNA, and protein profiling.
- Western blot and ELISA.

III. Sample Type:

- Mammalian tissues.
- Cultured Cells.

IV. Kit Contents:

Components	K288-50	Cap Code	Part Number
Mitochondria Isolation Buffer	2x100 ml	NM	K288-50-1
Reagent A	1 ml	Blue	K288-50-2
Storage Buffer	25 ml	NM	K288-50-3

V. User Supplied Reagents and Equipment:

- Dounce Tissue Grinder (Cat. # 1998).
- Protease Inhibitor Cocktail (Cat. # K272-1EA).

VI. Storage and Handling:

Store kit at -20°C, protected from light. Thaw the reagents before use. Read the entire protocol before performing the assay.

VII. Mitochondria Isolation Protocol:

1. Sample Preparation:

a. Cultured Cells: Pellet 2 x10^7 cells by centrifugation at 600 x g for 10 min. Carefully remove and discard the supernatant.

b. Tissues: Isolate the tissue of interest. Immerse the tissue (50-200 mg) in 1 ml of ice-cold Mitochondria Isolation Buffer & rinse it twice to remove the blood. Use 1 ml of ice-cold Mitochondria Isolation Buffer to mince the tissue on ice into small pieces using scissors. Spin the minced tissue in a table top centrifuge at 10,000 x g for 2 min. Discard the Buffer and replace it with 1 ml of fresh ice-cold Mitochondria Isolation Buffer.

2. Procedure For Mitochondria Isolation:

a. Isolation of Mitochondria Using Dounce Homogenizer: Homogenize the tissue or cells using a precooled glass homogenizer in Mitochondria Isolation Buffer. Stroke the sample 3-4 times on ice. The optimal ratio between tissue or cells and Mitochondria Isolation Buffer ranges from 1:5 - 1:10 (w/v). Transfer the homogenate to a tube and centrifuge at 600 x g for 10 min. at 4°C. Collect the supernatant in a separate tube and centrifuge at 7,000 x g for 10 min. at 4°C. Discard the supernatant and wash the pellet again with Mitochondria Isolation Buffer. Remove the supernatant and resuspend the mitochondria in Storage Buffer. Determine the protein concentration and adjust to the desired protein concentration with Storage Buffer.

b. Isolation of Mitochondria Using Reagent Based Method: To the cell pellet, add 1 ml of Mitochondria Isolation Buffer and vortex for 5 sec., followed by incubation on ice for 2 min. Add 10 µl of Reagent A and vortex for 5 sec. Incubate on ice for 5 min. while vortexing every min. for 5 sec. Centrifuge at 600 x g for 10 min. at 4°C. Collect the supernatant in a separate tube and centrifuge at 7,000 x g for 10 min. at 4°C. Discard the supernatant and wash the pellet with Mitochondria Isolation Buffer. Remove the supernatant and resuspend the mitochondria in Storage Buffer. Determine the protein concentration and adjust to the desired protein concentration with Storage Buffer.

Notes:

a. In order to avoid protein degradation we recommend that you add protease inhibitor cocktail (Cat # K272-1EA) to Mitochondria Isolation Buffer.

b. The number of strokes for homogenization will vary depending on the cells or tissue type.

c. For cells, to check the cell lysis efficiency, spot 5 µl of cell lysate into a glass slide, add coverslip and view under a microscope. For tissues, perform sufficient strokes to obtain a homogeneous suspension without lysing the cells. Typically for soft tissues 10-15 strokes and for hard tissues 5-10 strokes are sufficient.

3. **Storage Conditions based on Application:** For intact mitochondria, resuspend in Storage Buffer. Keep on ice for immediate downstream applications or snap freeze in liquid nitrogen & store at -80°C for future use. For the gel loading purpose, mitochondria can be stored in an appropriate sample PAGE buffer (Not provided).

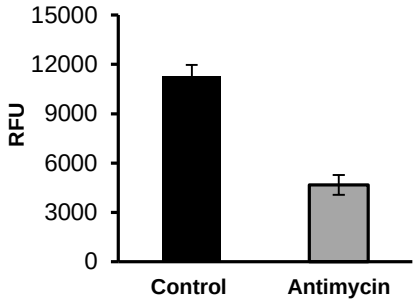

Figure: Mitochondria Integrity Test: Mitochondria (100 µg) were purified from the mouse liver according to the above-mentioned protocol. Purified mitochondria were analyzed for intactness by using JC-1 dye, which tests the electrochemical proton gradient (ΔΨ) of the inner mitochondrial membrane. Purified mitochondria was thawed on ice and incubated with JC-1 dye (1 µg/ml) for 15 min. at 37°C. The intact purified mitochondria show aggregation of JC-1 dye whose signal can be measured at Ex/Em = 530/590 nm. Treatment with Antimycin A (100 µM) dissipates the mitochondrial membrane potential resulting in reduced florescence signal.

VIII. RELATED PRODUCTS:

Mitochondria/Cytosol fraction Kit (K256)
Mitochondrial DNA Isolation Kit (K280)
Cytochrome P450 Antibody (3084R)
VDAC/Porin Antibodies (3594)
Cytochrome c (2120)
Cytochrome c Apoptosis Assay Kit (K257)
Antimycin A (2247)
Cytochrome Oxidase Activity Colorimetric Assay Kit (K287)
Protease Inhibitor Cocktail (Cat. # K272-1EA).
FractionPREP™ Cell Fractionation Kit (K270)
Mitochondrial Protein IP Kit (K285)
MitoCapture™ Mitochondrial Apoptosis Detection Fluorometric Kit (K250)
JC-1 Mitochondrial Membrane Potential Dye (1130)
Cytochrome c Antibody (3025, 3026, 3352, 3353)
Cytochrome c Blocking peptide (3025BP, 3352BP, 3353BP)
Valinomycin (2238)
Dounce Tissue Grinder (Cat. # 1998).

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