ALLELE Reagents

Contents

Mitochondrial Isolation Kit for Cultured Cells

1. Description

1.1 Product Components & Storage

This product is for research use only.

Components	Reagent A, 50mL (DPBS-) Reagent B (10x), 5mL (Mouse Tail) Reagent C, 60mL (Tris-Isolation Buffer)
Storage	Upon receipt store product at 4°C.

1.1 Background Information

The Mitochondrial Isolation Kit for Cultured Cells enables rapid enrichment of intact mitochondria from cultured mammalian cells. The isolated mitochondrial fraction can be separated from cytosolic components and used directly for downstream biochemical and functional analyses. Similar mitochondrial isolation approaches are widely used for studies of mitochondrial metabolism, signaling pathways, and organelle-specific protein composition.

1.2 Applications

Isolated mitochondria are suitable for a variety of downstream applications including mitochondrial protein analysis, enzymatic activity assays, apoptosis studies, and metabolic or signaling pathway analysis. Purified mitochondrial fractions may also be used for proteomic studies or mitochondrial DNA analysis.

2. Protocol

2.1 Additional Materials Required

- Variable-speed bench-top microcentrifuge (refrigerated)
- 2.0mL microcentrifuge tubes
- Vortex Mixer
- Protease inhibitor, EDTA-free such as Thermo Scientific Halt Protease Inhibitor Cocktail, EDTA-free (100X) (Cat# 87785)
- gentleMACS™ C Tubes (Cat#130-093-237**)**
- gentleMACS™ Dissociator

2.2 Method

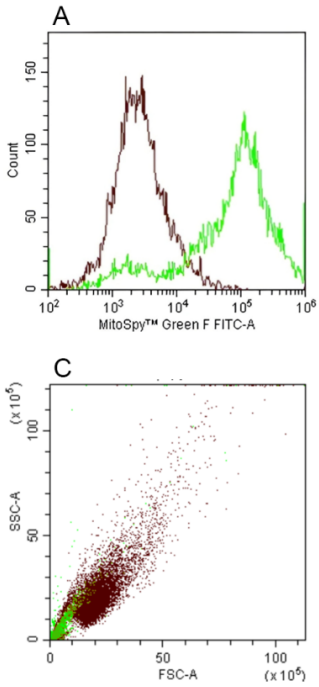
Reagent Preparation

- Add Protease inhibitor to Reagent A and C.
- Make 1x Reagent B

e.g. add 100ul of Reagent B (10x) to 900ul of Reagent A w/ Protease inhibitor, this is for 1 sample.

Note: Don't add Protease inhibitor to the storage buffer.

1. Harvest ~2x10^7 cells, collect cell pellet using centrifugation at 500xg for 3 minutes in a 2.0mL microcentrifuge tube.
2. Add 1ml of 1x Reagent B.
3. Incubate on ice for 1-15 minutes, vertexing at maximum speed every minute.
4. Transfer all components to a C-tube.
5. Dissociate cells with gentleMACS™Dissociator (program name: h-mito-tissue-01 C Tube)
6. Transfer all the components to a 2.0mL microcentrifuge tube.
7. Centrifuge tube at 700xg for 10 minutes at 4°C.
8. Transfer the supernatant to a new 2.0mL tube and centrifuge at 3000xg for 15 minutes at 4°C.
9. Transfer the supernatant (cytosol fraction) to new tube. The pellet contains isolated mitochondria.
10. Resuspend pellet with 500uL of reagent C, and centrifuge at 12,000xg for 5 minutes. Discard the supernatant. Repeat one more time.
11. Keep the mitochondrial pellet on ice before downstream processing. Freezing and thawing may compromise mitochondria integrity.



Flow cytometric analysis of isolated mitochondria stained with MitoSpy™ Green FM.

(**A**) Histogram showing fluorescence in MitoSpy ™-stained mitochondria (**Green**) versus unstained controls (**Brown**).

(**B**) SSC-A vs. FITC-A dot plot distinguishing MitoSpy™-positive isolated mitochondria (**Green**) from background (**Brown**).

(**C**) FSC-A vs. SSC-A plot differentiating intact cells (**Brown**) from isolated mitochondria (**Green**) populations based on size and granularity.