Mitochondrial Isolation Kit

Dyes and Stains

Isolation kit to enrich the mitochondrial subcellular fraction from mammalian cells or tissue

Catalog #100-0990

50 Tests

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Product Description

The Mitochondrial Isolation Kit isolates the mitochondrial and cytosolic subcellular fractions from mammalian cells or tissues. This kit offers two options to isolate the mitochondrial fraction through either reagent-based separation or Dounce homogenization. Reagent-based separation allows for processing up to six samples concurrently, while Dounce homogenization provides a two-fold increase in isolated mitochondria from a single sample. This kit uses a microcentrifuge-based method to enrich the mitochondrial and cytosolic fractions. Isolated mitochondria preserve their biological activity and are compatible with downstream applications, including the study of mitochondrial respiration, apoptosis, membrane potential, and protein profiling (Itahana & Zhang).

Product Information

The following components are sold as a complete kit (Catalog #100-0990) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Isolation Buffer	300-0572	100 mL	Store at 2 - 8°C.*	Product stable until expiry date (EXP) on label. Protect from prolonged exposure to light.
Homogenization Reagent	300-0573	1 mL	Store at -20°C.	Product stable until expiry date (EXP) on label. Protect from prolonged exposure to light.

* For long-term storage, Isolation Buffer can be stored at -20°C upon receipt.

Directions for Use

Please read the entire protocol before proceeding.

Component Preparation

1. Place the Isolation Buffer on ice and keep cold for the duration of the protocol.

   NOTE: If Isolation Buffer was stored at -20°C, thaw at room temperature (15 - 25°C) before placing on ice.
2. Thaw the Homogenization Reagent at room temperature.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. Avoid repeated freeze-thaw cycles.
3. Prepare cold (2 - 8°C) phosphate-buffered saline (PBS) (e.g. Catalog #37350).

Cell Preparation

1. Wash cells with cold PBS and centrifuge at 100 - 300 x *g* for 5 minutes.
2. Resuspend cells in 2 mL of Isolation Buffer and incubate on ice for 10 minutes.

Fractionation

1. Enrich the mitochondrial fraction through either reagent-based separation or Dounce homogenization as follows:
   - Reagent-based separation: Add 40 µL of the Homogenization Reagent to the cells. Vortex briefly and incubate on ice for 10 minutes.
   - Dounce homogenization: Homogenize the cells with a Dounce tissue grinder (not provided) by stroking 4 - 5 times.
2. Centrifuge the homogenized cells at 600 x *g* for 10 minutes at 4°C.
3. Collect the supernatant and centrifuge at 7000 x *g* for 10 minutes at 4°C.
4. Collect the supernatant and set aside. This is the cytoplasmic fraction.
5. Resuspend the pellet in 1 mL of cold Isolation Buffer and centrifuge at 7000 x *g* for 10 minutes at 4°C.
6. Discard the supernatant and resuspend the pellet (the mitochondrial fraction) in a buffer of choice suitable for downstream applications.

Protein Quanfification

Protein concentration can be measured using Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit (AAT Bioquest Catalog #11100).

References

Itahana K & Zhang Y. (2008) Mitochondrial p32 is a critical mediator of ARF-induced apoptosis. Cancer Cell 13(6): 542–53.