

## Mitochondria Isolation from Tissues and Cells (Catalog: TBS2016, 50 Assays, Store at -20°C)

### DESCRIPTION

Mitochondria are central organelles controlling the life and death of the cell. They participate in key metabolic reactions and regulate crucial signaling pathways including apoptosis. Mitochondrial dysfunction is involved in many diseases, like cancer, diabetes, cardiac failure, and neurodegenerative disorders. Mitochondria are double membrane organelles: an outer membrane and a folded inner membrane called cristae. Mitochondria isolation is required for studying mitochondrial respiration, assembly of the respiratory complexes, apoptosis, mtDNA, mtRNA, and mitochondrial protein profiling.

Tribioscience Mitochondria Isolation Kit provides an easy, simple approach for the isolation of intact and high quality mitochondria from tissues and cultured cells.

### APPLICATIONS

- 1) Isolation of mitochondria from tissues.
- 2) Isolation of mitochondria from tissues.

### KIT CONTENTS FOR 50 SAMPLE:

Name	Size (50 tests)
Mitochondria Isolation Reagent A (2x)	60 mL
Mitochondria Storage Buffer B	10 mL

**Storage conditions:** Store the Reagent at -20°C protected from light. Shelf life: 12 months.

### PROCEDURES

The whole procedure is operated on ice. Dilute 2x Mitochondria Isolation to 1 x Mitochondria Isolation Reagent.

#### Mitochondria isolation from Cultured Cells

1. Pellet  $2 \times 10^7$  cells by centrifugation in 2mL microcentrifuge tube at 600 x g for 3 min at 4°C.
2. Carefully remove and discard the supernatant.
3. Wash cells with 2mL ice-cold PBS, Centrifuge at 600xg for 3 min at 4°C. Discard the supernatant.
4. Add 1 mL 1x Mitochondria Isolation Reagent into the cell pellet.
5. Homogenize the cells using Polytron tissue disruptor at moderate speed for 20 seconds or Dounce homogenizer Teflon for complete homogenization on ice.
6. Transfer the homogenate to 1.5mL tube and centrifuge the sample at 1000 x g for 5min at 4°C.
7. Transfer the supernatant into a new tube and centrifuge the sample at 8,000 x g for 15 min at 4°C. Discard the supernatant and wash the pellet with 1mL 1x Mitochondria Isolation Reagent again and centrifuge it at 8,000 x g for 15 min at 4°C. Remove the supernatant. Resuspend the pellet (mitochondria) in 50-100 µL Storage Buffer and keep it on ice before proceeding

further.

8. Measure mitochondria concentration with protein quantity kit (TBS2005). Adjust to the desired concentration with Storage Buffer.

#### Mitochondria isolation from Tissues

1. Take 50-100mg tissue and wash it twice with 5mL ice-cold PBS.
2. Add 1 mL 1x Mitochondria Isolation Reagent (ice-cold) and mince the tissue on ice into small pieces using scissors. Then homogenize the tissue using Polytron tissue disruptor at moderate speed for 20 seconds or Dounce homogenizer Teflon for complete homogenization on ice.
3. Transfer the homogenate to 1.5mL tube and centrifuge it at 1000 x g for 5 minutes at 4°C.
4. Transfer the supernatant into a new tube and centrifuge the sample at 8,000 x g for 15 min at 4°C. Discard the supernatant and wash the pellet with 1mL 1x Mitochondria Isolation Reagent again and centrifuge it at 8,000 x g for 15 min at 4°C. Remove the supernatant. Resuspend the pellet (mitochondria) in 50-100 µL Storage Buffer and keep it on ice before proceeding further. The mitochondria is ready for further analysis.
5. Measure mitochondria concentration with protein quantity kit (TBS2005). Adjust to the desired concentration with Storage Buffer.
6. Aliquots of mitochondria should be stored at -80°C.

### RELATIVE PRODUCTS

Mitochondria Complex 1 Activity Assay (TBS2017)  
Mitochondria Oxidase Activity (TBS2105)  
Mitochondrial Membrane Potential Assay (TBS2049)  
Cytochrome C Oxidase (Complex IV) Activity Assay (TBS2115)  
NAD/NADH Colorimetric Assay (TBS2029)  
Resazurin Cell Viability Kit (TBS2001)  
CCK-8 Cell Viability Assay (TBS2022)  
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)  
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)  
Caspase-3 Colorimetric Assay kit (TBS2030)

**This product is for research only.**