

## Cell Mitochondria Isolation Kit I

### Introduction

Cell Mitochondria Isolation Kit I is designed for the rapid and convenient isolation of mitochondria from cultured cells. The mitochondria isolated using this kit are of high purity and mostly maintain the integrity of their inner and outer membrane structures, retaining the physiological functions of mitochondria. This makes them suitable for studies on mitochondrial physiology and protein analysis. The kit also provides Mitochondria Lysis Solution, which can be used to lyse the isolated mitochondria for subsequent experiments such as SDS-PAGE, Western Blot, and two-dimensional electrophoresis. Additionally, to ensure the quality of the isolated mitochondria, the kit includes Trypan Blue Staining Solution, which can be used to assess the degree of homogenization during the mitochondrial preparation process.

This kit can also obtain cytosolic proteins devoid of mitochondria, which can be collected for studying the release of mitochondrial proteins such as cytochrome c into the cytoplasm. Typically, this kit can be used for the extraction of 50-100 samples.

### Components and Storage

Components	Size	1 Kit (50-100 Tests)	Storage
Mitochondria Isolation Reagent		125 mL	-20°C
Trypan Blue Staining Solution		10 mL	-20°C
Mitochondria Storage Solution		15 mL	-20°C
Mitochondria Lysis Solution		15 mL	-20°C
PMSF		1 vial	-20°C
PMSF Solvent		1.5 mL	Room Temperature
Shipping: Blue ice		Shelf life: 1 year	

### Protocol

- 1. Preparation Before Experiment:** Allow all solutions in the kit to thaw at room temperature, and then immediately place them on ice and mix well. For the first use, dissolve a vial of PMSF with 1.5 mL PMSF Solvent and mix well to make 1.5 mL PMSF (100 mM). Prepared PMSF (100 mM) should be stored at -20°C.
- 2. Cell Collection:** For adherent cells, wash once with PBS, then use trypsin to digest, and collect cells by centrifugation at 100-200 g for 5-10 min. For suspension cells, collect directly by centrifugation.

**\*Note:** This kit does not provide Trypsin. You can purchase 0.25% Trypsin-EDTA Solution with Phenol Red (Catalog Number: K2839).

3. **Washing Cells:** Resuspend cell pellets with prechilled PBS. Then take a small amount of cells for counting. The remaining cells are centrifuged at 600 g for 5 min at 4°C, and the supernatant is discarded.

**All subsequent steps for mitochondrial isolation must be performed on ice.**

4. **Pre-treatment:** For  $2 \times 10^7$ - $5 \times 10^7$  cells, add 1-2.5 mL Mitochondria Isolation Reagent or Mitochondria Isolation Reagent containing PMSF to resuspend the cells, and place on ice for 10-15 min.

**\*Note:** For preparing mitochondrial protein samples, it is need to use Mitochondria Isolation Reagent containing PMSF. Just before use, add PMSF (100 mM) to an appropriate amount of Mitochondria Isolation Reagent to prepare a Mitochondria Isolation Reagent containing 1 mM PMSF.

5. **Homogenization:** Transfer the cell suspension to a glass homogenizer, and homogenize on ice 10-30 times. The number of homogenizations required varies with different cells and homogenizers, and optimization is needed.
6. **Assessment of Homogenization Efficiency:** Typically, after 10 homogenizations, take about 2  $\mu$ L of cell homogenate and add 30-50  $\mu$ L Trypan Blue Staining Solution. After mixing, observe the proportion of Trypan Blue positive (blue) cells under a microscope. If the positive cells are less than 50%, increase the homogenization by 5 times. Then take a sample again for Trypan Blue staining assessment, and continue until the proportion of positive cells exceeds 50% before stopping homogenization.

**\*Note:** Do not over-homogenize, as it can cause mechanical damage to the mitochondria.

7. **Centrifugation:** Centrifuge the cell homogenate at 600 g for 10 min at 4°C.

**\*Note:** For higher purity mitochondria, the centrifugation speed can be adjusted to 1000 g, all else being equal. However, this will reduce the number of mitochondria extracted from the same number of cells.

8. **Second Centrifugation:** Carefully transfer the supernatant to another centrifuge tube, and centrifuge at 11000 g for 10 min at 4°C.

**\*Note:** For higher purity mitochondria, the centrifugation speed can be adjusted to 3500 g, all else being equal. However, this will reduce the number of mitochondria extracted from the same number of cells.

9. **Collection of Mitochondria:** Carefully remove the supernatant, and the pellet is the cellular mitochondria. If cytosolic proteins devoid of mitochondria are needed, collect the supernatant at this step, being careful not to touch the pellet. Then centrifuge the supernatant at 12000 g for 10 min at 4°C. The supernatant is the cytosolic protein devoid of mitochondria. The cytosolic proteins can be determined for protein concentration using the BCA method or Bradford method.

10. **Use of Mitochondria:**

- 1) If used for functional studies of intact mitochondria, the mitochondrial sample isolated from  $2 \times 10^7$ - $5 \times 10^7$  cells can be resuspended in 150-200  $\mu$ L Mitochondria Storage Solution.

**\*Note:** Mitochondria stored in the Mitochondria Storage Solution should be used in time. If it cannot be used in time, it is recommended to store at -80°C. Cryopreserved mitochondrial samples are not recommended for mitochondrial membrane potential, but can be used for mitochondrial protein or nucleic acid testing.

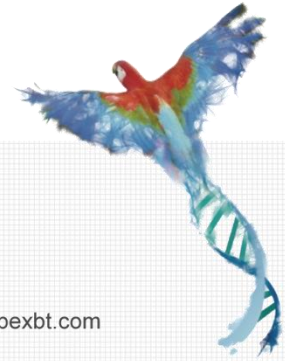
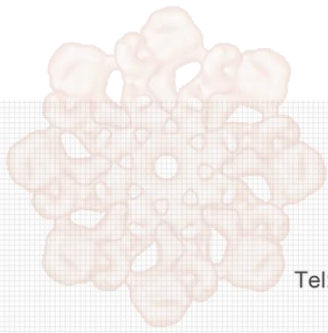
- 2) If used for protein analysis of mitochondria, the mitochondrial sample isolated from  $2 \times 10^7$ - $5 \times 10^7$  cells can be lysed in 150-200  $\mu$ L Mitochondria Lysis Solution containing PMSF. The lysed sample can be determined for protein concentration using the BCA method or Bradford method. The lysed sample can be used for SDS-PAGE, Western Blot, IP, enzyme activity assays in mitochondria, etc.

**\*Note:** It is need to use Mitochondria Lysis Solution containing PMSF in this step. Just before use, add PMSF (100 mM) to an appropriate amount of Mitochondria Lysis Solution to prepare a Mitochondria Lysis Solution containing 1 mM PMSF.

- 3) If used for two-dimensional electrophoresis, please treat the mitochondrial sample with a suitable lysis solution for two-dimensional electrophoresis.

## **Note**

1. If preparing mitochondrial protein samples, PMSF is needed during the experiment. PMSF should be added just before use to prevent it from becoming ineffective in aqueous solutions.
2. All steps for mitochondrial isolation must be performed on ice or at 4°C, and all solutions used must be chilled or pre-cooled.
3. Mitochondria stored in the Mitochondria Storage Solution should be used in time. If it cannot be used in time, it is recommended to store at -80°C. Cryopreserved mitochondrial samples are not recommended for mitochondrial membrane potential, but can be used for mitochondrial protein or nucleic acid testing.
4. Trypan Blue and PMSF are toxic to humans, so handle with care and ensure effective protection.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.



## APEx BIO Technology

[www.apexbt.com](http://www.apexbt.com)

7505 Fannin street, Suite 410, Houston, TX 77054.

Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: [info@apexbt.com](mailto:info@apexbt.com)

