

Tissue Mitochondria Isolation Kit

Introduction

Tissue Mitochondria Isolation Kit is designed for the rapid and convenient isolation of mitochondria from animal tissues. 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.

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

Size		
Components	1 Kit (50-100 Tests)	Storage
Mitochondria Isolation Reagent A	60 mL	-20°C
Mitochondria Isolation Reagent B	60 mL	-20°C
Trypsin-EDTA Solution	50 mL	-20°C
Mitochondria Storage Solution	3 mL	-20°C
Mitochondria Lysis Solution	15 mL	-20°C
Shipping: Blue ice		Shelf life: 1 year

Protocol

1. Preparation Before Experiment: Allow all reagents to thaw at room temperature, and then immediately place them on ice and mix well.

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

2. Isolation of Mitochondria from Soft Tissues (such as liver and brain):

- 1) **Tissue Collection:** Take 50-100 mg of fresh tissue in an EP tube and wash once with PBS. Then put the tissue into a pre-chilled EP tube on ice and cut the tissue into small pieces with scissors.

***Note:** Must use Fresh animal tissue, generally no more than 1 h after sacrifice and kept on ice. Do not use cryopreserved tissue for experiments.

- 2) **Addition of Mitochondria Isolation Reagent A:** Add 10-fold the volume of pre-chilled Mitochondria Isolation Reagent A or Mitochondria Isolation Reagent A containing PMSF just before use, and homogenize on ice for about 10 times. If the tissue weight is 50 mg, it can be roughly considered that the tissue volume is 50 μ L, at this time 500 μ L Mitochondria Isolation Reagent A is needed.

***Note:** For preparing mitochondrial protein samples, it is need to use Mitochondria Isolation Reagent A containing PMSF at this step. This kit does not provide PMSF, you will need to purchase PMSF separately (Catalog Number: A2587). Just before use, add PMSF solution to an appropriate amount of Mitochondria Isolation Reagent to prepare a Mitochondria Isolation Reagent containing 1 mM PMSF.

- 3) **Centrifugation:** Centrifuge the tissue homogenate at 600 g for 5 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.

- 4) **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.

- 5) **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. If the cytosolic proteins need to be determined for concentration, they must be diluted at least 2 times with PBS or saline, and then the protein concentration can be determined by the BCA method or Bradford method. At the same time, the standards must also be prepared in PBS or saline containing the corresponding proportion of Mitochondria Isolation Reagent A.

3. Isolation of Mitochondria from Hard Tissues (such as heart muscle or skeletal muscle):

- 1) **Tissue Collection:** Take 50-100 mg of fresh tissue in an EP tube and wash once with PBS. Then put the tissue into a pre-chilled EP tube on ice and cut the tissue into small pieces with scissors.

***Note:** Must use Fresh animal tissue, generally no more than 1 h after sacrifice and kept on ice. Do not use cryopreserved tissue for experiments.

- 2) **Addition of Pre-chilled PBS:** Add 10-fold the volume of pre-chilled PBS and place on ice for 3 min. If the tissue weight is 50 mg, it can be roughly considered that the tissue volume is 50 μ L, at this time 500 μ L pre-chilled PBS is needed.
- 3) **Centrifugation:** Centrifuge at 600 g for 10-20 s at 4°C, and discard the supernatant.
- 4) **Addition of Trypsin-EDTA Solution:** Add 8-fold the volume of pre-chilled Trypsin-EDTA Solution and

place on ice for 20 min. If the tissue weight is 50 mg, it can be roughly considered that the tissue volume is 50 μ L, at this time 400 μ L Trypsin-EDTA Solution is needed.

- 5) **Centrifugation:** Centrifuge at 600 g for 10-20 s at 4°C, and discard the supernatant.
- 6) **Resuspension with Mitochondria Isolation Reagent:** Add 2-fold the volume of the corresponding Mitochondria Isolation Reagent to resuspend the pellet, used to wash off excess trypsin. If the tissue weight is 50 mg, at this time 100 μ L Mitochondria Isolation Reagent is needed.

***Note:** If the sample is cardiac muscle tissue, it is recommended to use Mitochondria Isolation Reagent A; if the sample is skeletal muscle tissue, it is recommended to use Mitochondria Isolation Reagent B. For other similar tissues, Mitochondria Isolation Reagent A can be considered as a priority, and if the results are not satisfactory, Mitochondria Isolation Reagent B can be tried.

- 7) **Centrifugation:** Centrifuge at 600 g for 10-20 s at 4°C, and discard the supernatant.
- 8) **Addition of Mitochondria Isolation Reagent:** Add 8-fold the volume of the corresponding pre-chilled Mitochondria Isolation Reagent or Mitochondria Isolation Reagent added with PMSF just before use, and homogenize on ice for about 20-30 times. If the tissue weight is 50 mg, it can be roughly considered that the tissue volume is 50 μ L, at this time 400 μ L Mitochondria Isolation Reagent is needed.

***Note:** For preparing mitochondrial protein samples, it is need to use Mitochondria Isolation Reagent A containing PMSF at this step. This kit does not provide PMSF, you will need to purchase PMSF separately (Catalog Number: A2587). Just before use, add PMSF solution to an appropriate amount of Mitochondria Isolation Reagent to prepare a Mitochondria Isolation Reagent containing 1 mM PMSF.

- 9) **Centrifugation:** Centrifuge at 600 g for 5 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.

- 10) **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.

- 11) **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. If the cytosolic proteins need to be determined for concentration, they must be diluted at least 2 times with PBS or physiological saline, and then the protein concentration can be determined by the BCA method or Bradford method. At the same time, the standards must also be prepared in PBS or physiological saline containing the corresponding proportion of Mitochondria Isolation Reagent A.

4. Use of Mitochondria

- 1) If used for functional studies of intact mitochondria, the mitochondrial sample isolated from 100 mg of tissue can be resuspended in 40 μ 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.

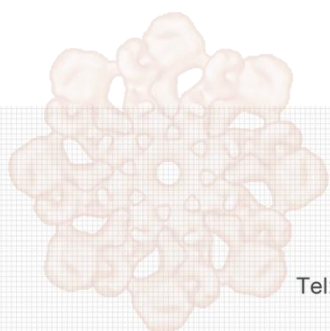
- 2) If used for protein analysis of mitochondria, the mitochondrial sample isolated from 50-100 mg of tissue can be lysed in 150-200 μ L Mitochondria Lysis Solution containing PMSF. The lysed sample can be determined for protein concentration using the BCA method or Bradford method. The lysed sample 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. This kit does not provide PMSF, you will need to purchase PMSF separately (Catalog Number: A2587). 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. 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.



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