Mitochondria Isolation Kit for Tissue

1 Contents

Components	HY-K1061
Mitochondria Isolation Reagent A	60 mL
Mitochondria Isolation Reagent B	60 mL
Trypsin Buffer	50 mL
Mitochondria Storage Buffer	3 mL
Mitochondria Lysis Buffer	15 mL

2 Introduction

Mitochondria, the site of most energy production in eukaryotic cells, have a double membrane structure: an outer membrane and a folded inner membrane. The key to the preparation of mitochondria is to ensure the integrity and purity of mitochondria.

Mitochondria Isolation Kit for Tissue enables the fast and efficient isolation of mitochondria from tissue using differential centrifugation. Most of the isolated mitochondria have intact inner and outer membranes, as well as physiological functions. In addition, this kit can also be used to extract mitochondrial proteins and mitochondria-free cytoplasmic protein. This kit contains enough reagents for 50-100 isolation procedures from 50-100 mg tissue.

3 General Protocol

Preparation of mitochondria from soft tissues (liver or brain)

1. Use a fresh tissue sample (obtained within 1 h of sacrifice) kept on ice. Do not freeze.
2. Weigh 50-100 mg of tissue and wash with PBS.
3. Cut the tissue to as small portions as possible and suspend the sample with 10 volumes of pre-cooled Mitochondria Isolation Reagent A (containing 1 mM PMSF).

Note: a. If 50 mg of starting tissue was used, add 500 µL of Mitochondria Isolation Reagent A .

b. PMSF is not provided in this kit.

4. Homogenize the tissue on ice with a homogenizer for about 10 strokes.

5. Centrifuge the homogenate at 600 g for 5 minutes at 4°C.

Note: The pellet contains nuclei, cell debris and unbroken cells. For more purified Mitochondria, change the centrifugation to 1,000 g. The drawback of this method is a lower yield of mitochondria.

6. Carefully transfer the supernatant to a fresh tube. Centrifuge at 11,000 g for 10 minutes at 4°C.

Note: For more purified Mitochondria that are less contaminated with lysosomes and peroxisomes, change the centrifugation to 3,500 g. The drawback of this method is a lower yield of mitochondria.

7. Carefully remove the supernatant, and the pellet is mitochondria.

Note: Mitochondria-free cytoplasmic protein can also be obtained in this step. Collect the supernatant and centrifuge at 12,000 g for 10 minutes at 4°C, and the supernatant is Cytoplasmic proteins. Determine the concentration of protein by BCA or Bradford.

Preparation of mitochondria from hard tissues (heart or skeletal muscle)

1. Use a fresh tissue sample (obtained within 1 h of sacrifice) kept on ice. Do not freeze.

2. Weigh 50-100 mg of tissue and wash with PBS.

3. Cut the tissue to as small portions as possible and suspend the sample with 10 volumes of pre-cooled PBS. Incubate on ice for 3 minutes. Centrifuge at 600 g for 10-20 seconds and then discard the supernatant.

4. Suspend the tissue with 8 volumes of pre-cooled Trypsin Buffer and incubate on ice for 20 minutes. Centrifuge at 600 g for 10-20 seconds and then discard the supernatant.

5. Suspend the tissue with 2 volumes of pre-cooled appropriate Mitochondria Isolation Reagent. Centrifuge at 600 g for 10-20 seconds and then discard the supernatant.

Note: For heart muscle, use Mitochondria Isolation Reagent A and for skeletal muscle, use Mitochondria Isolation Reagent B. It is recommended to use Mitochondria Isolation Reagent A for other tissue.

6. Suspend the tissue with 8 volumes of appropriate Mitochondria Isolation Reagent (containing 1 mM PMSF) and homogenize on ice for 20-30 strokes.

7. Centrifuge the homogenate at 600 g for 5 minutes at 4°C.

Note: The pellet contains nuclei, cell debris and unbroken cells. For more purified Mitochondria, change the centrifugation to 1,000 g. The drawback of this method is a lower yield of mitochondria.

8. Carefully transfer the supernatant to a fresh tube. Centrifuge at 11,000 g for 10 minutes at 4°C.

Note: For more purified Mitochondria that are less contaminated with lysosomes and peroxisomes, change the centrifugation to 3,500 g. The drawback of this method is a lower yield of mitochondria.

9. Carefully remove the supernatant, and the pellet is mitochondria.

Note: Mitochondria-free cytoplasmic protein can also be obtained in this step. Collect the supernatant and centrifuge at 12,000 g for 10 minutes at 4°C, and the supernatant is Cytoplasmic proteins. Determine the concentration of protein by BCA or Bradford.

Application of mitochondria

a. For applications requiring intact mitochondria, add 40 μL of Mitochondria Storage Buffer per 100 mg tissue. It is recommended to use JC-1 Mitochondrial Membrane Potential Assay Kit (HY-K0601) for measurement of the mitochondrial membrane potential.

b. For mitochondrial protein characterization or performing functional assays, add 150-200 μL of Mitochondria Lysis Buffer containing 1 mM PMSF per 50-100 mg tissue. The expected protein concentration should be approximately 10–20 mg/mL. The mitochondrial proteins obtained after cleavage can be used for SDS-PAGE, WB, IP and enzyme activity determination, and can also be used for protein concentration determination by BCA method or Bradford method (centrifuge at 12,000 g for 3-5 minutes at 4°C before detecting) .

4 Storage

Store at -20°C for one year.

5 Precautions

1. To obtain mitochondrial protein, add PMSF to Mitochondria Isolation Reagent and Mitochondria Lysis Buffer just before use. Only add PMSF to the reagent amount being used for the procedure and not to the stock solution.
2. All the isolation procedures should be performed at 4°C or on ice. Use pre-cooled buffers.
3. Store intact mitochondria at -80°C if not used immediately. Mitochondria that have been frozen are not recommended for detection of membrane potential.
4. PMSF is harmful, take care during use.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.