Mitochondria Isolation Kit for Cultured Cells

1 Contents

Components	HY-K1060
Mitochondria Isolation Reagent	125 mL
Trypan Blue	10 mL
Mitochondria Storage Buffer	15 mL
Mitochondria Lysis Buffer	15 mL
PMSF (crystal)	For 1.5 mL 100 mM
PMSF (solvent)	1.5 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 Cultured Cells enables the fast and efficient isolation of mitochondria from cells 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 2-5×10^7 cells.

3 General Protocol

1. Thaw the reagents.
2. Add 1.5 mL of PMSF (solvent) to PMSF (crystal) to obtain 1.5 mL of 100 mM PMSF.
3. Inoculate cells in advance until the density reaches 70-90%.
4. For adherent cells, discard the culture medium and wash the cells with PBS. Trypsinize the cells with Trypsin Buffer and then centrifuge at 4°C for 5-10 minutes at 100-200 g.

Note: Trypsin Buffer is not provided in this kit.

For cells in suspension, perform the centrifugation only.

5. Resuspend the cells in pre-cooled PBS, centrifuge at 600 g at 4°C for 5 minutes, and then discard the supernatant.
6. Add 1-2.5 mL of Mitochondria Isolation Reagent (containing 1 mM PMSF) per 2-5×10^7 cells. Suspend gently and incubate on ice for 10-15 minutes.
7. Homogenize the cells on ice with a homogenizer, 10-30 strokes.

Note: a. Optimize the number of strokes according to cell types.

b. Perform the homogenization gradually and follow it by staining an aliquot with Trypan Blue and counting the cells under a microscope. If there are

less than 80% damaged cells (blue cells), perform additional sequential homogenizations (5 additional strokes each time) until there is at least 80% damaged cells (blue cells).

c. Avoid over homogenization of the cells, which can result in mitochondria breakage.

8. Centrifuge the homogenate at 600 g for 10 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.

9. 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.

10. 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.

11. Application of mitochondria

a. For applications requiring intact mitochondria, add 150-200 μL of Mitochondria Storage Buffer per 2-5×10^7 cells. It is recommended to use MCE 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 2-5×10^7 cells. 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 the intact mitochondria at -80°C if not used immediately. Mitochondria that have been frozen are not recommended for detection of membrane potential.

4. Trypan Blue and PMSF are 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.