Cell Mitochondria Isolation Kit

Catalog# BWR1010

Size: 50-100 assays

Lot # Check on the product label

Introduction

1. Besides the mitochondria, this kit can obtain the mitochondria-free cytoplasmic protein which can be used for studying the release of mitochondrial protein and cytoplasm.
2. This kit enables the fast and easy isolation of an enriched and pure mitochondrial fraction from cells. And most of the isolated mitochondria will contain intact inner and outer membranes.
3. Lysed by the Mitochondria Lysis Buffer (kit component), the isolated mitochondria can be used for SDS-PAGE, Western blot and Di-electrophoresis assays.
4. This kit provides the Trypan Blue Staining Buffer (optional kit component) for controlling homogenization, this can help to obtain more intact mitochondria.
5. The kit component-PMSF as protease inhibitor can quench the protease activity properly during homogenization.
6. This kit is sufficient for 50-100 applications (2-5 × 10^7), isolation of enriched mitochondrial fraction from cells.

Kit Components

Components	Size	Storage Instruction
Mitochondria Isolation Reagent	60 ml	Store at -20°C for one year
Mitochondria Lysis Buffer	20 ml	Store at -20°C for one year
100mM PMSF	1 ml	Store at -20°C for one year

Protocol

1. Defrost the kit components at 37°C, shake thoroughly and then put them on ice.
2. Preparation of reagents:

a. Mitochondria Isolation Reagent: According to the quantity of samples, pipette proper volume of Mitochondria Isolation Reagent. 2-3 minutes before adding to cells, add PMSF (Dilution: 1:100) into this reagent and make PMSF final concentration to 1mM. (i.e. Add 1 μl of PMSF into 99 μl of Mitochondria Isolation Reagent).

b. Mitochondria Lysis Buffer: Pipette proper volume of Mitochondria Lysis Buffer. 2-3 minutes before adding to mitochondria, add PMSF (Dilution: 1:100) into this reagent and

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923

7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China

www.biospes.com

make PMSF final concentration to 1mM. (i.e. Add 1 μl of PMSF into 99 μl of Mitochondria Lysis Buffer).

3. Collect cells:

For **adherent cells**: Wash with PBS. Trypsinize the cells, and collect by centrifugation.

For **suspending cells**：Centrifuge to collect cells directly.

4. Wash the cells-resuspend the cells in ice cold PBS, count the cells, and centrifuge them at 600×g for 5 minutes at 4°C. Discard the supernatant.

5. Add 1-2.5 ml of Mitochondria Isolation Reagent or the prepared PMSF including Mitochondria Isolation Reagent (Step 2) per 2-5 × 10^7 cells. Incubate on ice for 10-15 minutes.

6. Homogenize the cells on ice using a Dounce homogenizer, 10-30 strokes. Each cell type requires an optimization of the number of strokes.

7. Perform the homogenization gradually and follow it by staining with Trypan Blue Staining Buffer (optional). Usually after 10 strokes, pipette about 2μl of cell homogenate, add 30-50μl of Trypan Blue Staining Buffer (optional) and counting the cells under a microscope. If there are less than 50% damaged cells (blue cells), perform additional sequential homogenizations (5 additional strokes each time) until there is at least 50% damaged cells (blue cells). Avoid over homogenization of the cells. This can result in mitochondria breakage.

8. Centrifuge the homogenate at 600×g for 10 minutes at 4°C. (Note: if need to obtain the higher purified mitochondria, change this step to centrifuge the homogenate at 1000×g for 10 minutes at 4°C, but the yield of mitochondria of the same cells will be decreased accordingly.)

9. Carefully transfer the supernatant liquid to a fresh tube. Centrifuge at 11,000×g for 10 minutes at 4°C. (Note: if need to obtain the higher purified mitochondria, change this step to centrifuge the homogenate at 3500×g for 10 minutes at 4°C, but the yield of mitochondria of the same cells will be decreased accordingly.)

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com

10. Carefully remove the supernatant, and pellet is the mitochondria. (Note: if want to get the mitochondria-free cytoplasmic protein, collect the supernatant without touching pellet at this step. Then, centrifuge the supernatant at 12000×g for 10 minutes at 4°C. The obtained supernatant is the mitochondria-free cytoplasmic protein.)

11. Usage of the mitochondria:

A. For applications requiring intact mitochondria (measurement citrate synthase activity or cytochrome c oxidase activity), add 150-200 µl of PBS (0.01M, pH7.4), and resuspend the mitochondria.

B. For mitochondria protein characterization or for performing functional assays, add 150-200 µl of prepared PMSF including Mitochondria Lysis Buffer (Step 2). And the lysed mitochondria can be for SDS-PAGE, Western blot and Immunoprecipitation assays.

C. For Di-electrophoresis assay, lyse the mitochondria with the lysis buffer which is specially for Di-electrophoresis use.

Notes

1. Use the kit components according to different assays, it is not necessary to use all of them.
2. If this kit is used to prepare mitochondria protein, PMSF should be added into Mitochondria Isolation Reagent and Mitochondria Lysis Buffer. And the PMSF has to be added 2-3 minutes before the above two solutions were added to mitochondria sample.
3. If this kit is NOT used to prepare mitochondria protein, PMSF should NOT be added into Mitochondria Isolation Reagent and Mitochondria Lysis Buffer.
4. All the isolation procedures should be performed on ice or at 4℃ with ice cold solutions.
5. For the toxicity, carefully operate the Trypan Blue Staining Buffer (optional) and PMSF.
6. Please wear the lab coat and disposable gloves to operate.

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com