

Tissue Mitochondria Isolation Kit

Catalog# BWR1011

Size: 50 -100 assays

Lot # Check on the product label

Introduction

1. Tissue Mitochondria Isolation Kit is designed for quick and convenient isolating mitochondria of animal tissues.
2. While isolating the mitochondria, this kit obtains the mitochondria excluded cytoplasmic protein, which can be used for researching the release of mitochondria proteins (e.g. cytochrome c, etc.) to cytoplasm.
3. The purity of the obtained mitochondria is high, and the majority has intact inner and outer membrane with physiological function.
4. After lysing with Mitochondria Lysis Buffer, the mitochondria can be used for SDS-PAGE, Western blot, Dimensional electrophoresis and Immunoprecipitation.
5. This kit is enough for 50-100 assays if the weight of per tissue is 50-100 mg.

Kit Components

Components	Size	Storage Instruction
Mitochondria Isolation Reagent A (MIR A)	60 ml	Store at -20°C for one year.
Mitochondria Lysis Buffer B (MLB B)	20 ml	Store at -20°C for one year.
Enzyme Inhibitor	1 ml	Store at -20°C for one year.

Protocol

1. Prepare solutions: thaw three reagents of the kit, put them on ice once completely dissolved, and mix thoroughly. If want to obtain the mitochondria protein, according to the number of sample, take the proper volume of MIR A for use, several minutes before adding to sample, add properly diluted Enzyme Inhibitor (Dilution: 1:100). Take the proper volume of MLB B for use, several minutes before adding to sample, add properly diluted Enzyme Inhibitor (Dilution: 1:100).
2. Isolate mitochondria from tissues (Note: use fresh tissue, usually, obtained from the animal which dies within 1 hour, and store the tissue on ice. **Do not use the frozen tissue sample!**)
3. Weight out appropriate amount of tissue (about 50-100 mg) in 1.5 ml of centrifugal tube, and wash tissue once with PBS.
4. Put the tissue in a centrifugal tube or culture dish, and place the container on ice. Cut the tissue into small slices.
5. Add pre-chilled MIR A (about 10 times than the weight of tissue, i.e. Take 50 mg tissue as an example, can be considered the volume of tissue is 50 μ l, then, 500 μ l of MIR A is needed), homogenize on ice-bath for about 10 times.

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

Note: To obtain the mitochondria with higher purity, centrifuge the homogenate at 1000g at 4°C for 5 min, but in this way, the yield of mitochondria of the same cells will be decreased.

7. Carefully transfer the supernatant into a new centrifugal tube, and centrifuge at 11,000g at 4°C for 10 min.

Note: To obtain the mitochondria with higher purity, centrifuge the homogenate at 3500g at 4°C for 10 min, but in this way, the yield of mitochondria of the same cells will be decreased.

8. Carefully remove the supernatant. The precipitate is the obtained mitochondria.

Note: To obtain mitochondria excluded cytoplasmic protein, collect the supernatant at this step (Do not touch the precipitate). Then, centrifuge the supernatant at 12,000g at 4°C for 10 min. The obtained supernatant is mitochondria excluded cytoplasmic protein.

9. For mitochondria protein analysis, add 150-200 µl of MLB B (containing 1:100 diluted Enzyme Inhibitor) into the obtained mitochondria which isolated from 50-100mg tissues. After lysis, the mitochondria can be used for SDS-PAGE, Western blot, Immunoprecipitation and enzymatic activity detection. And the concentration lysed protein sample can be determined by BCA Protein Assay kit.

10. For dimensional electrophoresis analysis, the mitochondria should be lysed with the appropriate lysis buffer.

Notes

1. The protocol takes 50-100mg tissues as the example to indicate the usage of reagents, if customer uses more tissues, the usage of reagents can be added proportionally.

2. All steps of mitochondria isolation should be operated on ice or at 4°C, and all reagents should be ice bath treated or pre-chilled at 4°C.

3. Generally, the speed of the two centrifuges should be at 600g and 11,000g when isolate the mitochondria, but if want to obtain mitochondria with higher purity, can change the speed at 1000g and 3500g.

4. Please wear the lab coat and disposable gloves to operate.

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