Minute™ Mitochondria Isolation Kit
(For Mammalian Cells and Tissues)

Catalog number: MP-007

Description

Invent Biotechnologies Minute™ mitochondria isolation kit is composed of optimized buffers and protein extraction filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly isolate native mitochondrial proteins from cultured mammalian cells or tissues. Due to the use of protein extraction filter cartridges, the protein isolation procedure is simple, easy and user friendly with high yield. Unlike many commercial mitochondrial preparation kits, this kit offers wide range of starting cells (5-40 millions/sample) and intact mitochondria are isolated. The buffers are detergent and EDTA free. A Dounce homogenizer or a tissue blender is not needed. The procedure can be completed in about 30 min.

Applications

The kit is designed to rapidly isolate mitochondria from cultured cells or tissues for applications such as SDS-PAGE, immunoblottings, ELISA, IP, membrane protein structure analysis, 2-D gels, enzyme activity assays and other applications.

Buffer Formulations: Proprietary

Kit components (50 preps)

1. 15 ml buffer A
2. 30 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5 Plastic rods (4)
6. Tissue dissociation beads

Storage: Store Buffer A and Buffer B at -20^{o}C upon arrival.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge

Important Information:

1. Read the entire procedures carefully. Thaw buffer A and buffer B completely, invert the bottles a few times and place on ice. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated mirocentrifuge.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. The use of protease inhibitor cocktails is optional.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Mitochondrial membrane Isolation Procedures

(The rpm in this protocol is based on Eppendoff 5415C table top microcentrifuge.)

1. Thaw buffers completely, place the buffers and the filter cartridges in collection tubs on ice.
2. Collect 5-40 X 10^6 cells by low speed centrifugation (500-600 X g 5 min).
3. Wash cells once with cold PBS. Remove supernatant completely and resuspend the cell pellet in 250 µl buffer A by vortexing briefly. Incubate the cell suspension on ice for 5-10 min and vortex vigorously for 20-30 seconds. Transfer the cell suspension to a filter cartridge. Go to step 4.

   For tissue samples place a piece of fresh/frozen tissue (20-30 mg) in a filter cartridge. Add 250 µl buffer A to the filter and grind the tissue with a plastic rod for one min by pushing the tissue against the surface of the filter repeatedly with twisting force and incubate the tube on ice with **cap open** for 5 min. go to step 4. If you are working with skeletal muscles add 100-120 mg tissue dissociation beads to the filter before grinding.

   Note: The presence of a small amount of un-homogenized tissue will not affect the quality of the sample. The plastic rod is reusable. For cleaning wipe it with 75% alcohol or rinse it with distilled water.

4. Cap the filter cartridge and centrifuge at 14,000 rpm (16,000 X g) for 30 seconds. Discard the filter and resuspend the pellet by vortexing briefly.
   Optional: For cultured cells it is recommended to resuspend the pellet in collection tube from step 4, transfer the cell suspension to the same filter and spin at 14,000 rpm (16,000 X g) for 30 seconds. Re-passing the cells through the filter can increase the yield by 20-30%.

5. Resuspend the pellet by vortexing and centrifuge at 3,000 rpm for 1 min, carefully transfer the supernatant to fresh 2.0 ml tube and add 400 µl buffer B to the tube. Mix by vortexing for 10 seconds.

6. Centrifuge at 14,000 rpm (16,000 X g) for 10 min. Remove the supernatant completely and resuspend the pellet in 200 µl buffer B by vigorously vortexing for 10 seconds.
   Centrifuge the tube at 10,000 rpm (7,800 X g) for 5 min. Transfer the supernatant to a fresh 2.0 ml tube; add 1.6 ml cold PBS to the tube and centrifuge at minimum 14,000 rpm (16,000 X g) for 15 min. Discard the supernatant and save the pellet (isolated mitochondrial proteins). Typically 5-200 µg proteins can be obtained. Pellet of mitochondrial proteins can be dissolved in 10-200 µl detergent containing buffers of your choice such as 0.5% Triton X-100 in calcium-free PBS.

Troubleshooting

Problem	Solution
Low protein yield	Increase starting cell numbers Increase incubation time to 10 min (step3)
Low protein activity	Keep lysate cold/add proteinase inhibitors
Retention of cell lysate in protein filter cartridge after 30 seconds of centrifugation	Reduce amount of starting material or increase centrifugation time to 2 min