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 intact native mitochondria 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 other commercial mitochondrial preparation kits, this kit offers wide range of starting cells (5-40 millions/sample). 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 intact mitochondria from cultured cells or tissues for applications such as SDS-PAGE, immunoblottings, ELISA, IP, 2-D gels, membrane potential assays, enzyme activity assays and other applications.

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 (2)

Storage: Store Buffer A and Buffer B at -20°C upon arrival.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge

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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 micro centrifuge.
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).

Mitochondria Isolation Procedures

1. Thaw buffers completely, place the buffers and the filter cartridges in collection tubs on ice.
2. Collect 10-40 X 10^6 cells by low speed centrifugation (500-600 X g 5 min).
3. Wash cells once with 1 ml 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. For muscle samples, please use **Minute™ Mitochondria Isolation Kit for Muscle Tissues/Cultured Muscle Cells** (Cat# MM-038).

   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 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 16,000 rpm for 30 seconds. Re-passing the cells through the filter can increase the yield.

5. Resuspend the pellet by vortexing and centrifuge at 700 X g for 1 min, carefully transfer the supernatant to fresh 1.5 ml tube and add 300 µl buffer B to the tube (the supernatant to buffer B ratio is 1:1.2 (250/300 µl). Mix by vortexing for 10 seconds. The ratio could be ranging from 1:1 to

1:1.6 (250/400 µl). If final mitochondrial yield is low use a ratio of 1:1. If cross-contamination is a problem use a higher supernatant to buffer B ratio such as 1: 1.5 or 1: 1.6.

6. Centrifuge at 16,000 X g for 10 min (If final mitochondrial yield is low increase centrifugation time to 30 min). Remove the supernatant completely and resuspend the pellet in 200 µl buffer B by repeat pipetting up and down followed by vigorously vortexing for 10-20 seconds.
7. Centrifuge the tube at 8,000 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 16,000 X g for 15-30 min. Discard the supernatant and save the pellet (isolated mitochondria). Typically, 10-200 µg proteins can be obtained. Pellet of mitochondria can be dissolved in 10-200 µl detergent containing buffers of your choice. For isoelectric focusing (First dimension of 2D gel) we recommend to use: 7M urea/2M thio-urea/2% Chaps and 20 mM DTT (add DTT to above mix prior to use). Following reagents are recommended for solubilize isolated mitochondria depending on downstream applications.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.

Troubleshooting

Problem	Solution
Low protein mitochondria yield	Increase starting cell numbers Change supernatant to buffer B ratio (step 5) Increase centrifugation time in step 6.
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