Minute™ Mitochondria Isolation Kit for Muscle Tissues/Cultured Muscle Cells
Catalog number: MM-038

Description

Muscle cells have large number of mitochondria because they are continually used to move the body. Another mitochondrion-rich organ is the heart where mitochondria make up about 40% of the heart cells. However, isolation of mitochondria from muscle tissues can be difficult due to that fact that interfibrillar mitochondria are hidden deep among muscle fibrils. Traditional methods usually require polytron tissue disintegrator and ultracentrifuge for purification with high density medium such as Percoll gradient. This kit was developed using our proprietary technologies, making mitochondria isolation from muscles simple, rapid and user friendly. Intact mitochondria can be isolated from fresh/frozen muscle tissues in about 1h with higher yield.

Applications

The kit is designed to rapidly isolate mitochondria from muscle tissues/cultured muscle cells for applications such as SDS-PAGE, immunoblottings, ELISA, IP, 2-D gels, enzyme activity assays, mitochondrial membrane potential assay and other applications.

Kit components (50 preps)

1. 25 ml Buffer A
2. 10 ml Buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5 Plastic rods (2)
6. Tissue dissociation beads (5g)

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

Additional Materials Required

1 X Ca-free phosphate buffered saline (PBS)
Vortexer
Table-Top Microcentrifuge that can reach 16,000 X g in 10 seconds

Important Information:

1. Read the entire protocol carefully. Thaw buffer A and B completely. 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 using a refrigerated microcentrifuge.
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 recommended.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).
5. The plastic rods are reusable. Clean with water and dry with paper towels.

Mitochondria Isolation Procedures

1. **Sample Preparations:** Thaw buffers completely, invert the bottles a few times and place on ice. Place filter cartridges in collection tubs and incubate on ice.
   For Tissue samples: Place a piece of fresh/frozen tissue (30-40 mg) on the surface of a clean glass or plastic plate. Mince the tissue with a sharp blade into small pieces until the tissue is converted into pasty mass (this will take about 3-4 min)). Transfer the minced tissue to the filter cartridge. Add 80 mg tissue dissociation beads to the filter cartridge followed by addition of 200 µl buffer A. Grind the tissue with the plastic rod provided for 2-3 min by pushing the tissue against the surface of the filter repeatedly with twisting force. Add 300 µl buffer A to the filter. Go to step 2.
   For Cultured Muscle Cells: Harvest 20-30 million cells by low speed centrifugation (600 X g for 5 min). Wash once with 1 ml cold PBS and remove PBS completely. Resuspend the pellet in 50 µl buffer A and transfer to a filter cartridge. Add 80 mg tissue dissociation bead to the filter and grind the cells with the plastic rod provided for 2-3 min by pushing the tissue against the surface of the filter repeatedly with twisting force. Add 300 µl buffer A to the filter. Go to step 2.
2. Cap the filter and invert the tube a few times then centrifuge at 16,000 X g for 30 seconds. Discard the filter and vortex the tube briefly to resuspend the pellet.
3. Centrifuge the tube at 1,000 X g for 5 min (the pellet contains nuclei, cell debris and some un-rupture cells). Transfer the supernatant to a fresh microfuge tube and centrifuge at 11,000 X g for 20 min.
4. Remove supernatant completely save it if desired (this is cytosolic fraction). The supernatant may contain some broken mitochondria especially when frozen tissue is used. Resuspend the pellet in 200 µl buffer B by pipetting up and down for 30-40 time then vortex vigorously for 20 seconds.
5. Centrifuge the tube at 11,000 X g for 10 min (the centrifugation time can range from 5-15 min, for a particular sample it needs to be optimized to obtain best results. Generally, shorter centrifugation time has higher final yield, longer centrifugation time can increase the purity but may have lower final yield). After centrifugation, transfer the supernatant to a fresh 1.5 ml microfuge tube and add 0.3 ml cold PBS to the tube, mix well by vortexing briefly.
6. Centrifuge at 16,000 X g for 20 min. The pellet is isolated intact mitochondrial fraction. Typically, 10-100 µg proteins can be obtained. Pellet of mitochondria can be dissolved in 20-100 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 mitochondria yield	Increase starting material, increase in-filter grinding time or increase centrifugation time in step 6.
Significant cross contamination	Wash final mitochondrial pellet with 0.5 ml Ca-free PBS containing 0.3 M NaCl
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