Minute™ Yeast Mitochondria Enrichment Kit

Catalog #: YM-017

Description

Traditional protocols for yeast mitochondria isolation typically involve multiple centrifugation-based subcellular fractionation steps. These methods generally include spheroplast preparation, glass-bead lysis with homogenization, differential centrifugation, and various density gradient procedures using ultracentrifugation. These processes can be labor-intensive and time-consuming.

In contrast, we offer a simple and rapid protocol for yeast mitochondria enrichment. This gentle, instrument-free method allows for the isolation of native mitochondria and associated proteins in approximately one hour, without the need for ultracentrifugation. The kit includes optimized, detergent-free protein extraction buffers, yielding 50-100 µg of protein per sample, and is sufficient for 50 extractions.

Applications

Proteins extracted with this kit can be used for many downstream applications, such as SDS-PAGE analysis, Western blotting, IP, ELISA, enzyme activity assays, proteomics and other biochemical analysis.

Kit components(50 Preps):

1. 30 ml buffer A
2. 10 ml buffer B
3. 5 g protein extraction powder
4. 2 pestles for 1.5 ml microcentrifuge tube
5. 1.5 ml microcentrifuge tube X 50

Storage: Store the kit at -20ºC.

Additional Materials Required

Table-Top Microcentrifuge with a maximum speed of >14,000 X g. 1 X PBS.

Important Product Information

Before isolating mitochondria, it is recommended to add a protease inhibitor cocktail to an aliquot of Buffer A. For protein concentration measurement, the BCA kit (Pierce) is suggested. If studying protein

phosphorylation, phosphatase inhibitors, such as PhosStop (Roche), should be added to Buffer A before use.

Protocol

1. Harvest yeast cells during the log growth phase via centrifugation. Collect the cells in the provided 1.5 ml microfuge tube, ensuring the wet pellet volume is between 30-40 µl. You can estimate the volume by comparing it to a 1.5 ml tube containing 30-40 µl of water.
2. Resuspend the pellet in 1 ml of cold water, then add 100 mg of protein extraction powder. Briefly vortex the tube, centrifuge at top speed for 2 minutes, and completely remove the supernatant.
3. Grind the pellet using the provided pestle for about 2-3 minutes with a twisting motion (approximately 300-400 strokes). Add 300 µl of Buffer A and continue grinding for another 30 seconds. (Note: The pestle is reusable; clean it by soaking in bleach, rinsing with water, and drying with a paper towel.) Cap the tube and vortex vigorously for 10 seconds.
4. Centrifuge at 2000 X g for 2 minutes at 4°C. Transfer the supernatant to a pre-chilled 1.5 ml microfuge tube and place it on ice. Repeat step 3 and combine the supernatants (total of 600 µl) in the microfuge tube on ice.
5. Centrifuge at top speed for 20 minutes at 4°C. Transfer the supernatant (cytosolic protein fraction) to a fresh tube. The floating white material is cell wall fragments and can be removed by pipetting. Resuspend the pellet in 200 µl of Buffer B by pipetting up and down 20-30 times, ensuring the tube walls are rinsed with Buffer B. For improved purity, an optional centrifugation step can be performed (see technical notes 4).
6. Add 1.4 ml of cold 1X PBS to the tube containing the 200 µl resuspended pellet. Cap the tube, invert a few times, and centrifuge at top speed for 30 minutes at 4°C. The pellet contains enriched yeast mitochondria, which can be dissolved in 50-150 µl of a detergent-containing buffer of your choice (see technical notes 2). The final preparation typically yields 50-100 µg of protein. For solubilization of the pellet, the reagents in the following table are recommended. For isoelectric focusing (first dimension of 2D gel), we suggest using: 7M urea, 2M thio-urea, 2% CHAPS, and 20 mM DTT (add DTT to the mixture before use).

Tech notes:

1. Protein yield is proportional to the starting cell number and the grinding duration. Grinding can be done using twisting or up-and-down motions. The initial cell pellet volume can be up to 50 µl.
2. Resuspend the mitochondrial pellet in a detergent-containing buffer, ensuring the tube walls are thoroughly rinsed. Incubate the mixture at room temperature for 5 minutes, then centrifuge at 10,000 X g for 3 minutes. The pellet will contain residual intact yeast cells and insoluble materials. Transfer the supernatant to a fresh microfuge tube for further applications.
3. To assess mitochondrial enrichment, we recommend performing Western blotting using yeast mitochondria-specific antibodies, such as Por1. Be sure to compare the total cell lysate and the enriched mitochondrial fraction with equal protein loading.

4. To improve mitochondrial purity, an optional step can be performed: After resuspending the pellet in 200 µl of Buffer B (as in step 5), centrifuge at 4,000 X g for 5 minutes. Transfer the supernatant to a fresh 1.5 ml tube, add 1.4 ml of cold PBS, and perform the centrifugation as described in step 6.

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.