Minute™ Yeast Mitochondria Enrichment Kit

Catalog number: YM-017

Description

Traditional protocols for yeast mitochondria isolation/extraction involve a range of centrifugation-based subcellular fractionation procedures. Typically the techniques include spheroplast preparation, glass-bead lysis using a homogenization instrument, differential centrifugation and several density gradient procedures using a variety of gradient media with ultracentrifugation. The procedures are very tedious and time consuming. Here we feature a simple and rapid protocol for yeast mitochondria enrichment. The procedure is gentle and instrument-free. Nativ mitochondrial proteins can be isolated from yeast in about one hour without ultracentrifugation. This kit contains optimized detergent-free protein extraction buffers. The protein yield is in the range of 150-250 µg/sample. The materials provided are 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

1. 30 ml buffer A
2. 10 ml buffer B
3. 5 g protein extraction powder
4. 4 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 rpm of 14,000-16,000. 1 X PBS.

Important Product Information

Prior to plasma membrane isolation addition of protease inhibitor cocktail to buffer A is recommended. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use.

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Protocol

1. Harvest yeast cells (*S. cerevisiae or S. pombe*) in log growth phase by centrifugation. Collect yeast cells in a 1.5 ml microfuge tube provided. Make sure that the wet volume of pellet is between 30-40 µl. The volume can be easily estimated by comparing it to a 1.5 ml tube with 30-40 µl water.

2. Resuspend the pellet in 1 ml cold water and add 100 mg protein extraction powder to the tube. Vortex the tube briefly and centrifuge at top speed in a microcentrfuge for 2 min. **Remove supernatant completely**.

3. Grinding the pellet repeatedly with the pestle provided for about 2 min with twisting force. Add 300 µl buffer A to the tube and continue to grind for about thirty seconds (note: The pestle is reusable, for cleaning simply soak it in bleach, rinse with water and dry it with paper towel). Cap the tube and vortex vigorously for 10 seconds.

4. Centrifuge the tube at 5000 rpm for 2 min at 4°C. Transfer the supernatant to a fresh pre-chilled microfuge tube and place on ice. Repeat step 3 one more time and combine the supernatants (600 µl) in the microfuge tube on ice.

5. Centrifuge the tube at top speed for 20 min at 4°C. Transfer the supernatant to a fresh microfuge tube (this is yeast cytosolic protein fraction). Resuspend the pellet in 200 µl buffer B by vortexing vigorously. Centrifuge the tube at 8000 rpm for 5 min at 4°C.

6. Transfer the supernatant to a pre-chilled 1.5 ml tube used in your Lab and add 1 ml cold 1 X PBS to the tube. Invert the tube a few times and centrifuge at top speed at 4°C for 30 min. The pellet contains enriched yeast mitochondria. It can be dissolved in a detergent of your choice depending upon downstream applications.

Application tips: The final protein yield is proportional to grinding frequency and time in step 3. The pestles fit the best with 1.5 ml tubes provided.

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