

Yeast Mitochondria Isolation Kit

Cat. No. YMI-015K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Yeast Mitochondria Isolation kit will enable fast and easy purification of mitochondria from yeast cells, utilizing yeast cell wall lysis and homogenization. This Mitochondria Isolation Kit is tested for <i>Pichia pastoris</i> & <i>Saccharomyces cerevisiae</i> & can be used to isolate mitochondria from other yeast. Under fermentable media, the yield is ~150-200 g of mitochondria & under non-fermentable media (e.g. Glycerol) ~200-250 g of mitochondria from a culture of OD ~20.
Applications	Isolation of highly pure intact mitochondria from yeast cells. Mitochondrial respiration studies, assembly of the complexes, apoptosis, mtDNA and mtRNA, and for protein profiling. Western blot and ELISA
Storage	Store kit at -20centigrade, protected from light. Warm Buffer A and B to room temperature before use. Read the entire protocol before performing the assay.
Kit Components	• Buffer A • Buffer B • 1 M DTT • Homogenization Buffer • Lysis Enzyme Mix • Storage Buffer • Protease Inhibitor Cocktail
Materials Required but Not Supplied	• Media to grow yeast cells.

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	• Glass douncer • Spectrophotometer capable of reading Absorbance. • Centrifuge with cooling option.
Features & Benefits	• Simple & convenient protocol • The kit provides unique formulations of ready-to-use buffers and reagents to isolate intact mitochondria
Preparation	• Buffer A: Store at -20centigrade or 4centigrade. Warm at RT and add DTT to final conc. of 10 mM freshly before use or as needed. • Buffer B: Store at -20centigrade or 4centigrade. Warm at RT before use. Add lysis enzyme 5 µl of Buffer before use. • Homogenization Buffer: Store at -20centigrade or 4centigrade. Add Protease Inhibitor Cocktail (1:1000) before use or as needed. Keep on ice while in use. • Lysis Enzyme Mix: Aliquot and store at -20centigrade. • Storage Buffer: Store at -20centigrade or 4centigrade. Keep on ice while in use. • Protease Inhibitor Cocktail: Resuspend protease inhibitor cocktail in 250 µl of DMSO. Store at -20centigrade.
Separation Protocol	The described procedure is for small-scale isolation (10-20 ml) for OD ~20. For a large scale preparation (OD~200), calculate the volumes of the reagents accordingly. 1. Yeast Culture: Grow yeast cells in appropriate media overnight at 30centigrade, shaking at 200 rpm. For temperature sensitive mutants use desired temperature. When cells are into log phase, determine the OD of the culture at 600 nm. Multiply the OD with the total volume of the culture (ml) to calculate the total OD. Note: To isolate mitochondria in respiring state, grow yeast cells under aerobic condition using non-fermentable media (e.g. Ethanol or Glycerol as carbon source). However, yeast cells will grow very slowly under these conditions with a thicker cell

wall.

2. Mitochondrial Isolation:

2.1 Centrifuge the yeast culture at 3,000 g for 5 min. and discard the supernatant. Wash the cells by resuspending in 2 volumes of ultrapure water. Resuspend the cell pellet in 1 ml of Buffer A containing 10 mM fresh DTT and incubate for 10 min. at 30centigrade with gentle shaking. Centrifuge at 1,500 g for 5 min. and discard the supernatant.

2.2 Resuspend the cell pellet in 1ml of Buffer B. Aliquot 10 μ suspension in separate glass tube (Control). Add 2.5 μ Lysis Enzyme Mix to the remaining cell suspension and incubate for 10-15 min. at 30centigrade in shaking incubator. Aliquot 10 μ of suspension again in another glass tube.

Note: To check the formation of efficient spheroplast, add 990 μ of water to 10 μ aliquot from step 2.2 (Control & with Lysis Enzyme Mix). Measure OD at 600 nm. Incubation should continue until the OD of the sample is decreased 30-40% after adding Lysis Enzyme Mix compared to Control.

2.3 After efficient spheroplast formation, centrifuge at 1,500 g for 5 min. and discard the supernatant. From this step onwards, keep the tubes on ice. Resuspend the cell pellet in 1ml of Homogenization Buffer with protease inhibitor cocktail. Transfer the suspension to a glass douncer (not provided) and stroke 10-15 times on ice. Centrifuge at 600 g for 5 min. at 4centigrade and collect the supernatant in separate tube. Supernatant contains mitochondria. Centrifuge the supernatant containing mitochondria again at 600 g for 5 min. at 4centigrade and collect the supernatant. Centrifuge the supernatant at 12,000 g for 10 min. at 4centigrade. Carefully discard the supernatant without touching the pellet. Resuspend the pellet in Storage Buffer (~50 μ). Determine the protein concentration and adjust the desired protein concentration by Storage Buffer accordingly.

Note: Storage Conditions based on Application - For intact mitochondria, resuspend in Storage Buffer and snap freeze in liquid nitrogen. Transfer frozen mitochondria to -80centigrade. For the gel loading purpose, mitochondria can be stored in Lysis Buffer with detergent or SDS PAGE loading dye (Not provided). For IP or protein profiling, mitochondria can be lysed in desired detergent using Mitochondrial Protein IP Kit (MPI-012K).

Sample Type	Yeast cells culture.
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