ab178779

Yeast Mitochondria Isolation Kit

Instructions for Use

For the isolation of mitochondria from *P. pastoris* and other species of yeast

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 2 Last Updated 30 October 2023

Table of Contents

1. Overview

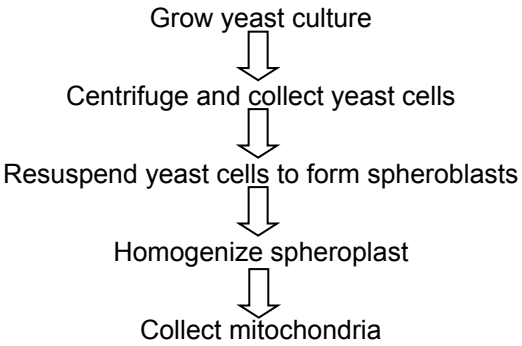
Mitochondria are semi-autonomous organelles which functions in aging process, apoptosis, anti-HIV drugs, and cancers. Mitochondrial DNA (mtDNA) has a very high mutation rate and the mutations on mtDNA appear to be related to certain diseases such as diabetes, Alzheimer's disease, and muscle disorders. Isolation and quantification of mtDNA are often required to study the relationships between the diseases and mtDNA.

Abcam's Yeast Mitochondria Isolation Kit (ab178779) provides a convenient tool for isolating mitochondria from a variety of yeast species, and it has been specifically tested on *Pichia pastoris* and *Saccharomyces cerevisiae*.

Isolated mitochondria can be used for studying mitochondrial respiratory chain, assembly of complexes, apoptosis, mtDNA and mtRNA, and for proein profiling. Isolated mitochondria can be used directly in Western blot and ELISA.

ab178779 is only sufficient for 25 samples.

2. Protocol Summary



3. Kits Components

Item	Quantity
Buffer A	50 mL
Buffer B	50 mL
DTT IV/1 M DTT	1 mL
Yeast Homogenization Buffer/Homogenization Buffer	50 mL
Lysis Enzyme Mix	200 µL
Dilution Buffer I/Storage Buffer	10 mL
Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail (lyophilized)	1 vial

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Ultrapure water or MilliQ
- DMSO
- Appropriate media to grow yeast cells
- Glass douncer
- Spectrophotometer capable of reading absorbance
- Centrifuge with cooling option

6. Reagents Preparation

1. **Dilution Buffer I/Storage Buffer:**
   Ready to use as supplied. Keep on ice while in ice. Store at -20°C or 4°C.

2. **Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail (PIC)**:
   Resuspend Protease Inhibitor Cocktail I/protease inhibitor cocktail in 250 µL of DMSO. Store at -20°C.

3. **Lysis Enzyme Mix**:
   Aliquot Lysis Enzyme Mix and store at -20°C. Avoid repeated freeze/thaw.

4. **DTT IV/DTT**:
   Ready to use as supplied. Store at -20°C.

5. **Buffer A:**
   Store at -20°C or 4°C. Warm to room temperature before use. Prior to use, add DTT IV/DTT to a final concentration of 10mM to buffer A. Buffer A + DTT IV/DTT solution has to be used fresh and cannot be stored for future uses.

6. **Buffer B**:
   Store at -20°C or 4°C. Warm to room temperature before use.

Prior to use, add Lysis Enzyme 5µL/mL of buffer B. Buffer + Lysis Enzyme solution has to be used fresh and cannot be stored for future uses.

7. **Yeast Homogenization Buffer/Homogenization Buffer:**
   Store at -20°C or 4°C. Prior to use, add Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail to 1:1000 final dilution. Yeast Homogenization Buffer/Homogenization Buffer + Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail solution has to be used fresh and cannot be stored for future uses.

7. Assay Protocol

This 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:**
   a) Grow yeast cells in appropriate media overnight at 30°C, shaking at 200 rpm. For temperature sensitive mutants, use the desired temperature.
   b) 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:

a) Centrifuge the yeast culture at 3,000 rpm for 5 min and discard the supernatant. Wash the cells by resuspending in 2 volumes of ultrapure water.

b) Resuspend the cell pellet in 1 mL of Buffer A containing 10 mM fresh DTT IV/DTT and incubate for 10 min at 30°C with gentle shaking. Centrifuge at 1,500 g for 5 min and discard the supernatant.

c) Resuspend the cell pellet in 1 mL of Buffer B containing Lysis Enzyme Mix. Aliquot 10 µL suspension in a separate glass tube (Control).

d) Add 2.5 µL Lysis Enzyme Mix to the remaining cell suspension and incubate for 10 – 15 min at 30°C in a shaking incubator. Aliquot 10 µL of suspension again in another glass tube.

NOTE: TO check the formation of efficient spheroplast (cell from which the cell wall has been almost completely removed), add 900 µL of water to 10 µL aliquots of step 2d (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.

e) After efficient spheroplast formation, centrifuge at 1,500 g for 5 min and discard the supernatant. From this step onwards, keep tubes on ice.

f) Resuspend the cell pellet in 1 mL of Yeast Homogenization Buffer/Homogenization Buffer + Protease Inhibitor Cocktail I/PIC. Transfer the suspension to a glass douncer and stroke 10 – 15 times on ice. Centrifuge at 600 g for 5 min at 4°C and collect the supernatant in a separate tube. The supernatant contains the mitochondria.

g) Centrifuge the supernatant containing mitochondria again at 600 g for 5 min at 4°C and collect the supernatant.

h) Centrifuge the supernatant at 12,000 g for 10 min at 4°C. Carefully discard the supernatant without touching the pellet.

i) Resuspend the pellet in ~ 50 µL of Dilution Buffer I/Storage Buffer. Determine the protein concentration and adjust the desired protein concentration by Dilution Buffer I/Storage Buffer accordingly.

3. Isolated Mitochondria Storage:

The storage conditions will vary based on the application the samples will be used for:

- Intact mitochondria: resuspend mitochondria in Dilution Buffer I/Storage Buffer and snap freeze in liquid nitrogen. Transfer frozen mitochondria to storage at -80°C.
- Mitochondria for Gel loading: mitochondria can be stored in Lysis Buffer with detergent or SDS-PAGE loading dye.

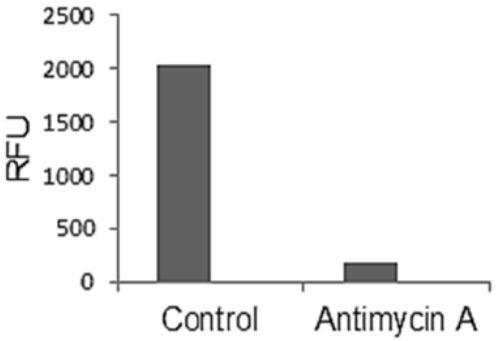


Figure 1: Mitochondrial integrity test – Purified mitochondria were analyzed for intactness by using JC-1 dye, which tests the electrochemical proton gradient ($\Delta\Psi$) of the inner mitochondrial membrane. The intact purified mitochondria show aggregation of JC-1 dye whose signal can be measured at Ex/Em = 530/590 nm. Treatment with antimycin A (100 µM), an inhibitor of the electron transport chain, dissipates the mitochondrial membrane potential resulting in reduced fluorescence signal.

For all technical and commercial enquires please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)