CREATIVE BIOMART®
Assay Kit

Yeast Mitochondria Isolation Kit

Product Information

Cat

Kit-0556

Common Name

Mitochondria

Cat.No.

Kit-0556

Product Overview

Yeast Mitochondria Isolation Kit is used for isolating yeast mitochondria from yeast cell cultures.

Description

Mitochondria are the power house of the cells because they generate most of the supply of energy in the form of adenosine tri-phosphate (ATP). Mitochondria are double membrane organelles: an outer membrane and a folded inner membrane called cristae. Isolated mitochondria is a useful tool to study mitochondrial respiration, assembly of the respiratory complexes, apoptosis, mtDNA and mtRNA, and for protein profiling. Yeast Mitochondria Isolation kit will enable fast and easy purification of mitochondria from yeast cells, utilizing yeast cell wall lysis and homogenization.

Applications

Isolation of highly pure mitochondria from yeast cells.
Mitochondrial respiration studies, assembly of the complexes, apoptosis, mtDNA and mtRNA, and for protein profiling.
Western blot

Usage

For research use only (RUO)

Storage

Store kit at -20°C, protected from light. Warm Buffer A and B to room temperature before use.

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Kit Components

Buffer A 50 ml
Buffer B 50 ml
1 M DTT 1 ml
Homogenization Buffer 50 ml
Lysis Enzyme Mix 200 μl
Storage Buffer 10 ml
Protease Inhibitor Cocktail (Lyophilized) 1 vial

Materials Required but Not Supplied

Media to grow yeast cells.
Glass douncer
Spectrophotometer capable of reading Absorbance.
Centrifuge with cooling option.

Target Species

Yeast

Compatible Sample Types

Cell culture

Preparation

Buffer A: Store at -20°C or 4°C. Warm at RT and add DTT to final conc. of 10 mM freshly before use or as needed.
Buffer B: Store at -20°C or 4°C. Warm at RT before use. Add lysis enzyme 5 µl/ml of Buffer before use.
Homogenization Buffer: Store at -20°C or 4°C. Add Protease Inhibitor Cocktail (1:1000) before use or as needed. Keep on ice while in use.
Lysis Enzyme Mix: Aliquot and store at -20°C.
Storage Buffer: Store at -20°C or 4°C. Keep on ice while in use.
Protease Inhibitor Cocktail: Resuspend protease inhibitor cocktail in 250 µl of DMSO. Store at -20°C.

Assay Protocol

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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 30°C, 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:

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 30°C with gentle shaking. Centrifuge at 1,500 g for 5 min. and discard the supernatant.

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

Note: To check the formation of efficient spheroplast, add 990 µl of water to 10 µl 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.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 4°C and collect the supernatant in separate tube. Supernatant contains mitochondria. Centrifuge the supernatant containing mitochondria again at 600 g for 5 min. at 4°C and collect the supernatant. Centrifuge the supernatant at 12,000 g for 10 min. at 4°C. Carefully discard the supernatant without touching the pellet. Resuspend the pellet in Storage Buffer (~50 µl). Determine the protein concentration and adjust the desired protein concentration by Storage Buffer accordingly.

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Note: Storage Conditions based on Application - For intact mitochondria, resuspend in Storage Buffer and snap freeze in liquid nitrogen. Transfer frozen mitochondria to -80°C. For the gel loading purpose, mitochondria can be stored in Lysis Buffer with detergent or SDS PAGE loading dye (Not provided).

Analysis

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) dissipates the mitochondrial membrane potential resulting in reduced florescence signal.

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