Yeast Mitochondria Isolation Kit

(Catalog #BN00527; 50 assays; Store at -20°C)

I. Introduction:

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. Assay Genie's 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 *Pichia pastoris* & *Saccharomyces cerevisiae* & can be used to isolate mitochondria from other yeast. Under fermentable media, the yield is ~150-200 µg of mitochondria & under non-fermentable media (*e.g.* Glycerol) ~200-250 µg of mitochondria from a culture of OD ~20.

II. Application:

- 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 and ELISA

III. Sample Type:

Yeast cells culture.

IV. Kit Contents:

Components	BN00527	Cap Code	Part Number
Buffer A	50 ml	Blue	BN00527-1
Buffer B	50 ml	NM	BN00527-2
1 M DTT	1 ml	Green	BN00527-3
Homogenization Buffer	50 ml	Amber	BN00527-4
Lysis Enzyme Mix	200 µl	Orange	BN00527-5
Storage Buffer	10 ml	NM	BN00527-6
Protease Inhibitor Cocktail	Lyophilized	Red	BN00527-7

V. User Supplied Reagents & Equipments:

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

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm Buffer A and B to room temperature before use. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **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.

VIII. Yeast Mitochondria Isolation 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 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:**

   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 30°C 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 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.

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 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.

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). For IP or protein profiling, mitochondria can be lysed in desired detergent using Assay Genie's Mitochondrial Protein IP Kit (K285-50).

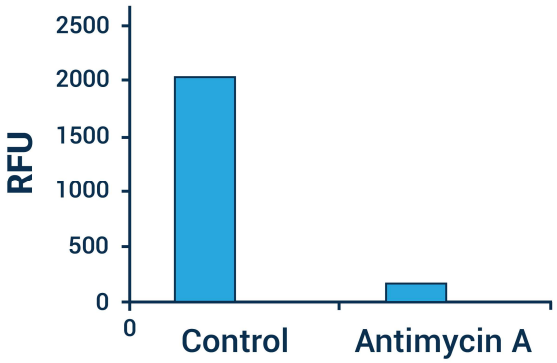

Figure: Mitochondrial integrity test - Purified mitochondria were analyzed for intactness by using JC-1 dye, which tests the electrochemical proton gradient (ΔΨ) of the inner mitochondrial membrane. The intact purified mitochondria show aggregation of JC-1 dye whose signal can be measured at Ex/Em = 530/590 nm. Treatment with Antimycin A (100 µM) dissipates the mitochondrial membrane potential resulting in reduced florescence signal.

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