

EXOBX® Mitochondria Isolation Kit

Cat. no. R6301

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Catalog Number	Reagent In Kit	Size	Storage
R6301	Reagent MA	50 ml	4 °C
	Reagent MB	1 ml	4 °C
	Reagent MC	80 ml	4 °C
	Manual	1	

1. Transportation and Storage

This product is transported at room temperature, stored at 4 °C and valid for 12 months.

2. Important Product Information

- 1) The whole experimental process should be carried out on ice.
- 2) The membrane potential should be measured by using the freshly isolated mitochondria, but not frozen mitochondria, because cryopreservation could disrupt the integrity of mitochondria.
- 3) To avoid the contamination of lysosomes and peroxisomes, the cytoplasm solution could be purified by centrifugation at 12000 g for 15 min.
- 4) The concentration of proteins in cytoplasm solution should be measured by using Bradford protein concentration assay method, but not BCA protein concentration assay method.
- 5) To inhibit protein degradation during the procession of mitochondria isolation, the protease inhibitors (such as Cocktail) could be added in reagent A and C.
- 6) Please wear lab clothes, gloves and safety glasses during the experiment.

3. Product Introduction

The EXOBOX®™ Mitochondria Isolation Kit for cultured cells enables isolation of intact mitochondria from cultured mammalian cells. The isolated mitochondria can be used for various subsequent experiments, including mitochondria membrane potential measurement, metabolism enzymes activity detection, mitochondria protein level determination, and even mitochondrial DNA purification.

4. Additional Materials Required

- Phosphate Buffered Saline (PBS)
- RIPA Lysis Buffer (without SDS)
- Protease inhibitors, EDTA-free such as Protease Inhibitor Cocktail.
- Variable-speed bench-top microcentrifuge (refrigerated)
- 2.0 mL microcentrifuge tubes
- Vortex mixer
- Dounce tissue grinder, such as 2 mL Wheaton Dounce Tissue Grinder

5. Experimental Procedure

Add protease inhibitors to Reagent A and Reagent C used in the current experiment but not the whole stock solutions.

1) Sample preparation.

- a) Cell samples. Pellet 2×10^7 cells by centrifuging harvested cell suspension in a 2.0 mL microcentrifuge tube at 1000 g for 3 minutes. Carefully remove and discard the supernatant.
- b) Liver tissue samples. Liver tissues (50-200 mg) were cut into pieces, suspended with 1 ml PBS and then homogenized (30-200 times) by using Tissue Grinder. Transfer the homogenates to 2.0 ml microcentrifuge tubes and then precipitate the isolated cells by centrifugation at 1000 g for 3 minutes. Carefully remove and discard the supernatant.
- c) Heart tissue samples. Heart tissues (50-200 mg) were cut into pieces, suspended with 1 ml trypsin solution (0.3 mg/ml), and then stayed on ice for 3 minutes. Centrifuge the solution with heart tissue at 1,000 rpm for 5 min, discard the supernatant, and resuspend the precipitate with 1 ml PBS. Homogenize the resuspended heart tissues with tissue grinder until the heart tissue were grinded into power. Transfer the homogenates to 2.0 ml microcentrifuge tubes and then precipitate the isolated cells by centrifugation at 1000g for 3 minutes. Carefully remove and discard the supernatant.

- 2) Add 800 μ L of Mitochondria Isolation Reagent A. Vortex at medium speed for 5 seconds and incubate tube on ice for exactly 2 minutes.

Note: Do not exceed the 2-minute incubation.

- 3) Add 10 μ L of Mitochondria Isolation Reagent B. Vortex at maximum speed for 5 seconds.
- 4) Incubate tube on ice for 5 minutes, vortexing at maximum speed every minute.
- 5) Add 800 μ L of Mitochondria Isolation Reagent C. Invert tube several times to mix (do not vortex).
- 6) Centrifuge tube at 700 g for 10 minutes at 4 °C.
- 7) Transfer the supernatant to a new 2.0 mL tube and centrifuge at 3 000 g for 15 minutes at 4 °C.

Note: To obtain more mitochondria, centrifuge the supernatant at 12 000 g for 30 minutes, but high centrifugation increases the contamination of lysosome and peroxidase.

- 8) Transfer the supernatant (cytosol fraction) to a new tube. The pellet contains the isolated mitochondria.
- 9) Add 500 μ L Mitochondria Isolation Reagent C to the pellet, vortex at medium speed to resuspend mitochondria pellet, and centrifuge at 12,000 g for 5 minutes. Discard the supernatant.
- 10) Maintain the mitochondrial pellet on ice before downstream processing. Freezing and thawing may compromise mitochondria integrity.
- 11) The isolated mitochondria could be lysed by using three different reagents or methods.
 - a) Lyse the mitochondria by using 2 $\times$ Laemmli Buffer, and the lysates could be used for western blot, but not the enzyme activity assay.

b) Lyse the mitochondria by using RIPA Buffer (without SDS), and the lysates could be used for both western blot and the enzyme activity assay.

c) Resuspend the mitochondria with PBS, and lyse the mitochondria by repeated freezing and thawing, and the lysates could be used for both western blot and the enzyme activity assay.

12) To remove the lysosome, peroxisome and other organelles in cytoplasm solution, centrifuge the cytoplasm solution at 12 000 g for 15 min. Transfer the supernatant into new microcentrifuge tubes and discard the precipitate. Mix the cleared cytoplasm solution with 4 × Laemmli Buffer, boil for 5 min and cool by using ice. The lysate could be used for western blot.

6. Identification of Isolated Mitochondria

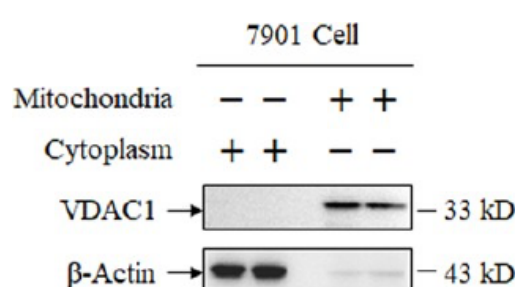


Figure 1. Identification of mitochondria isolation

7. Frequently Asked Questions and Analysis

Questions	Reasons	Solutions
The isolated mitochondria are less.	Less cells or tissues were used to isolate mitochondria.	Increase samples for mitochondria isolation.
	The speed of centrifugation is low during the procession of mitochondria precipitation.	Centrifugate the supernatant at 12 000 g for 15-30 min to precipitate mitochondria
	The tissue homogenization is not enough to separate cells from tissues.	Increase the homogenization times.
	No trypsin was used to digest heart tissue.	Digest the heart tissue with trypsin.
	Reagent failure.	Please use new Reagent.
The isolated mitochondria are broken.	Mitochondria were lysed by Reagent B.	Shorten the time of cell lysis by using Reagent B.
	Repeated freezing and thawing of isolated mitochondria.	Aliquote the isolated mitochondria to avoid repeated freezing and thawing.
The concentration of mitochondria protein is low.	Little nucleus was used for lysing.	Increase nucleus for lysing.
	The lysis buffer is weak for lysing nucleus.	Select the lysis buffer with strong protein denaturant, such as 2 × Laemmli Buffer.