Cell Mitochondria Isolation Kit

Catalogue No.:abx090630

Abbexa's Cell Mitochondria Isolation kit is a quick, convenient, and sensitive method for the isolation of enriched and pure mitochondrial fractions from cells. The isolated mitochondria will contain intact inner and outer membranes, and can be treated with the provided Mitochondria Lysis Buffer for use in subsequent applications such as SDS-PAGE, Western blot and dimensional electrophoresis assays. This kit can be used to perform up to 100 assays for the isolation of enriched mitochondrial fraction from cells.

Kit Contents

- Mitochondria Isolation Reagent: 100 ml
- Mitochondria Lysis Buffer : 50 ml
- PMSF (100 mM): 1 ml
- Trypan Blue Staining Solution: 10 ml

Tested Applications:	WB
Storage:	Store the Trypan Blue Stain solution at 4 °C and all other reagents at -20 °C.
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use: Before carrying out the procedure, thaw all reagents, then place on ice. Gently shake each reagent bottle to ensure thorough mixing. The working solutions should be prepared 2-3 minutes before the carrying out the protocol below.

Preperation of reagents

1. **Mitochondria Isolation Reagent Working Solution:** Dilute the 100 mM PMSF 1/100 with Mitochondria Isolation Reagent to prepare the Mitochondria Isolation Reagent Working Solution with a final PMSF concentration of 1 mM (e.g. add 1 µl of PMSF to 99 µl of Mitochondria Isolation Reagent to prepare 100 µl of Mitochondria Isolation Reagent Working Solution).
2. **Mitochondria Lysis Buffer Working Solution:** Dilute the 100 mM PMSF 1/100 with Mitochondria Lysis Buffer to prepare the Mitochondria Lysis Buffer Working Solution with a final PMSF concentration of 1 mM (e.g. add 1 µl of PMSF to 99 µl of Mitochondria Lysis Buffer to prepare 100 µl of Mitochondria Lysis Buffer Working Solution).

Sample preparation

• **Tissues:** Rinse 100-200 mg of fresh tissue with pre-chilled PBS to remove blood. Dry the tissue with filter paper, then homogenize mechanically. Add 1-2 ml of pre-chilled Mitochonria Isoluation Reagent Working Solution and homogenize on ice.

• **Cells:**

- **Adherent cells:** Remove cells using a cell scraper, then centrifuge to collect the cells.
- **Suspension cells:** Centrifuge directly to collect the cells.

Pellet 10^7 cells by centrifuging at 600 × g at 4 °C for 5 mins. Carefully discard the supernatant, then add 1 ml of pre-chilled Mitochondria Isolation Reagent Working Solution. Mix thoroughly, then allow to stand on ice for 10-15 mins with occasional vortexing. Aliquot a small volume of the cell suspension into a new tube, then add Trypan Blue Staining Solution at a ratio of 1:1. Observe under a microscope. If 80% or more of the cells are stained blue, homogenization is not necessary, otherwise homogenize the cell suspension. Avoid excessive homogenization to avoid mitochondrial breakage.

Assay procedure

1. Aliquot the tissue or cell homogenate to a new tube, then centrifuge at 600 × g at 4 °C for 10 mins. To obtain a higher purity fraction, centrifuge at 1000 × g instead of 600 × g.
2. Collect the supernatant and aliquot into a new tube. Discard the precipitate.
3. Centrifuge the supernatant at 12,000 × g at 4 °C for 10 mins. To obtain a higher purity fraction, centrifuge at 16,000 × g instead of 12,000 × g.
4. Discard the supernatant. Collect the pellet, which contains the mitochondria.

Protein extraction

1. Add 50-70 µl of Mitochondria Lysis Buffer Working solution to 10 µl of isolated mitochondria.
2. Incubate at 4 °C for 10-15 mins with occasional vortexing.
3. Centrifuge at 10,000 × g at 4 °C for 10 mins. Collect the supernatant, which contains the mitochondrial protein extracts.

Applications

• **Applications requiring intact mitochondria:** Carry out the assay at 0-4 °C throughout and the pH at 7.0. Carry out subsequent assays immediately after isolating the mitochondria.

• **Dimensional electrophoresis assays:** Lyse mitochondria with a dimensional electrophoresis lysis buffer.

Notes

• Determine the mitochondrial protein concentration using a BCA Protein Assay Kit (abx090640).

• All isolation steps should be carried out on ice or at 4 °C.

• Insufficient cell lysis will result in a low yield of mitochondria.

• Personal Protective Equipment (PPE), such as lab coats, disposable gloves and lab glasses, should be worn when using this kit.

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