Mammalian Mitochondria Isolation Kit for Tissue

Catalog No.: abx098857

Size: 50 rxns

Storage: Store the 100X EDTA-free Protease Inhibitor Cocktail at -20 °C and the Mitochondria Isolation Buffers at 2-8 °C for up to one year.

Introduction: Abbexa's Mammalian Mitochondria Isolation Kit for Tissue is a simple, fast and efficient solution for isolation of mitochondria from tissues. This kit provides two options for the separation of mitochondria from cytosolic components: a reagent-based method or homogenization. Compared with homogenization, the reagent-based method allows multiple samples to be processed concurrently under mild conditions, minimizing damage to mitochondria. The isolated mitochondria are suitable for a variety of downstream applications, including protein analysis, apoptosis, signal transduction and metabolic studies.

Kit components

1. Mitochondria Isolation Buffer 1 (MIB1): 50 ml
2. Mitochondria Isolation Buffer 2 (MIB2): 500 µl
3. Mitochondria Isolation Buffer 3 (MIB3): 65 ml
4. Mitochondria Storage Buffer (MSB): 4 ml
5. Bovine Serum Albumin (BSA): 500 mg
6. 100X EDTA-free Protease Inhibitor Cocktail: 1.2 ml

Materials Required But Not Provided

1. Phosphate-Buffered Saline (PBS)
2. Phenylmethanesulfonyl Fluoride (PMSF)
3. Distilled water
4. High-precision pipette and sterile pipette tips
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice
8. Vortexer
9. Homogenizer

Notes

- Before use, add Protease Inhibitor Cocktail and PMSF (not provided in this kit) to Mitochondria Isolation Buffer 1, Mitochondria Isolation Buffer 3, and Mitochondria Storage Buffer.
- All steps should be carried out on ice or at 2-8 °C.
- Fresh tissues are recommended if the isolated mitochondria will be used for functional assays.

Procedure

A. Isolation from Soft Tissues – Reagent Method

1. Wash 50-200 mg of tissue with 1 ml of pre-chilled PBS. Cut the tissue into small pieces.
2. Transfer the minced tissue to a glass homogenizer and homogenize the tissues (3-5 strokes are recommended to avoid over-homogenization).
3. Resuspend in 2 ml of PBS. Centrifuge at 1000 × g for 3 minutes. Carefully discard the supernatant.
4. Prepare a Mitochondria Isolation Buffer 1 solution containing 4 mg/ml BSA. Add 800 µl of this solution to the mixture, vortex for 5 seconds, then allow to stand on ice for 2 minutes.

5. Add 10 µl of Mitochondria Isolation Buffer 2 to the pellet. Vortex for 5 seconds.
6. Allow to stand on ice for 5 minutes, briefly vortexing after each minute.
7. Add 800 µl of Mitochondria Isolation Buffer 3 to the mixture. Invert the tube 5-6 times to mix. Do **not** vortex.
8. Centrifuge at 700 × g at 2-8 °C for 10 minutes.
9. Carefully transfer the supernatant to a new 2 ml microcentrifuge tube, then centrifuge at 12,000 × g at 2-8 °C for 15 minutes. For higher purity, the tube can instead be centrifuged at 3,000 × g at 2-8 °C for 15 minutes, though this may result in a lower yield.
10. Carefully collect the supernatant (which contains cytoplasmic proteins) to a new tube. The isolated cytoplasmic proteins can be used immediately in downstream applications or stored at -80 °C.
11. Add 500 µl of Mitochondria Isolation Buffer 3 to the pellet and resuspend by vortexing.
12. Centrifuge at 12,00 × g at 2-8 °C for 15 minutes.
13. Carefully discard the supernatant. The remaining pellet contains mitochondria, which can be stored at -80 °C or immediately processed:

    - Protein analysis: The pellet can be dissolved and lysed with protein lysis buffer. Our Mammalian Total Protein Extraction Kit (abx098853) can be used for protein extraction. Mitochondria or mitochondrial lysate can be stored at -80 °C for future use.
    - Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 µl/10^7 cells. Analyze within one hour after resuspension.

B. Isolation from Soft Tissues – Homogenization Method

1. Wash 50-200 mg of tissue with 1 ml of pre-chilled PBS. Cut the tissue into small pieces.
2. Transfer the minced tissue to a glass homogenizer and homogenize the tissues (3-5 strokes are recommended to avoid over-homogenization).
3. Resuspend in 2 ml of PBS. Centrifuge at 1000 × g for 3 minutes. Carefully discard the supernatant.
4. Prepare a Mitochondria Isolation Buffer 1 solution containing 4 mg/ml BSA. Add 1 ml of this solution to the mixture, vortex for 5 seconds, then allow to stand on ice for 2 minutes.
5. Transfer the suspension to a glass homogenizer and homogenize the cells (30-50 strokes).

   Note: To check cell lysis efficiency, stain the cells with Trypan Blue and view under a microscope. If more than 50% cells are stained, the homogenization process can be stopped. Under-homogenization may result in a lower mitochondrial yield, while over-homogenization may damage the mitochondria.

6. Carefully transfer the supernatant to a new 2 ml microcentrifuge tube.
7. Add 800 µl of Mitochondria Isolation Buffer 3 to the mixture. Invert the tube 5-6 times to mix. Do **not** vortex.
8. Centrifuge at 700 × g at 2-8 °C for 10 minutes.
9. Carefully transfer the supernatant to a new 2 ml microcentrifuge tube, then centrifuge at 12,000 × g at 2-8 °C for 15 minutes. For higher purity, the tube can instead be centrifuged at 3,000 × g at 2-8 °C for 15 minutes, though this may result in a lower yield.
10. Carefully collect the supernatant (which contains cytoplasmic proteins) to a new tube. The isolated cytoplasmic proteins can be used immediately in downstream applications or stored at -80 °C.
11. Add 500 µl of Mitochondria Isolation Buffer 3 to the pellet and resuspend by vortexing.
12. Centrifuge at 12,00 × g at 2-8 °C for 15 minutes.
13. Carefully discard the supernatant. The remaining pellet contains mitochondria, which can be stored at -80 °C or immediately processed:

- Protein analysis: The pellet can be dissolved and lysed with protein lysis buffer. Our Mammalian Total Protein Extraction Kit (abx098853) can be used for protein extraction. Mitochondria or mitochondrial lysate can be stored at -80 °C for future use.
- Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 µl/10^7 cells. Analyze within one hour after resuspension.

C. Isolation from Hard Tissues – Reagent Method

1. Wash 50-200 mg of tissue with 2-4 ml of pre-chilled PBS. Carefully discard the PBS and cut the tissue into small pieces.
2. (Optional) For trypsin pre-treatment, add 750 µl of trypsin (0.25%) to the tissue and allow to stand on ice for 3 minutes. Centrifuge at 1000 × g for 3 minutes. Carefully discard the supernatant.
3. Transfer the minced tissue to a glass homogenizer and homogenize the tissues (3-5 strokes are recommended to avoid over-homogenization).
4. Prepare a Mitochondria Isolation Buffer 1 solution containing 4 mg/ml BSA. Add 800 µl of this solution to the mixture, vortex for 5 seconds, then allow to stand on ice for 2 minutes.
5. Add 10 µl of Mitochondria Isolation Buffer 2 to the pellet. Vortex for 5 seconds.
6. Allow to stand on ice for 5 minutes, briefly vortexing after each minute.
7. Add 800 µl of Mitochondria Isolation Buffer 3 to the mixture. Invert the tube 5-6 times to mix. Do **not** vortex.
8. Centrifuge at 700 × g at 2-8 °C for 10 minutes.
9. Carefully transfer the supernatant to a new 2 ml microcentrifuge tube, then centrifuge at 12,000 × g at 2-8 °C for 15 minutes. For higher purity, the tube can instead be centrifuged at 3,000 × g at 2-8 °C for 15 minutes, though this may result in a lower yield.
10. Carefully collect the supernatant (which contains cytoplasmic proteins) to a new tube. The isolated cytoplasmic proteins can be used immediately in downstream applications or stored at -80 °C.
11. Add 500 µl of Mitochondria Isolation Buffer 3 to the pellet and resuspend by vortexing.
12. Centrifuge at 12,00 × g at 2-8 °C for 15 minutes.
13. Carefully discard the supernatant. The remaining pellet contains mitochondria, which can be stored at -80 °C or immediately processed:

    - Protein analysis: The pellet can be dissolved and lysed with protein lysis buffer. Our Mammalian Total Protein Extraction Kit (abx098853) can be used for protein extraction. Mitochondria or mitochondrial lysate can be stored at -80 °C for future use.
    - Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 µl/10^7 cells. Analyze within one hour after resuspension.

D. Isolation from Hard Tissues – Homogenization Method

1. Wash 50-200 mg of tissue with 1 ml of pre-chilled PBS. Cut the tissue into small pieces.
2. (Optional) For trypsin pre-treatment, add 750 µl of trypsin (0.25%) to the tissue and allow to stand on ice for 3 minutes. Centrifuge at 1000 × g for 3 minutes. Carefully discard the supernatant.
3. Prepare a PBS solution containing 4 mg/ml BSA. Add 750 µl of this solution to the mixture and mix thoroughly. Centrifuge at 1000 × g for 3 minutes. Carefully discard the supernatant.
4. Prepare a Mitochondria Isolation Buffer 1 solution containing 4 mg/ml BSA. Add 1 ml of this solution to the mixture, vortex for 5 seconds, then allow to stand on ice for 2 minutes.
5. Transfer the suspension to a glass homogenizer and homogenize the cells (30-50 strokes).

Note: To check cell lysis efficiency, stain the cells with Trypan Blue and view under a microscope. If more than 50% cells are stained, the homogenization process can be stopped. Under-homogenization may result in a lower mitochondrial yield, while over-homogenization may damage the mitochondria.

6. Carefully transfer the supernatant to a new 2 ml microcentrifuge tube.
7. Add 800 µl of Mitochondria Isolation Buffer 3 to the mixture. Invert the tube 5-6 times to mix. Do **not** vortex.
8. Centrifuge at 700 × g at 2-8 °C for 10 minutes.
9. Carefully transfer the supernatant to a new 2 ml microcentrifuge tube, then centrifuge at 12,000 × g at 2-8 °C for 15 minutes. For higher purity, the tube can instead be centrifuged at 3,000 × g at 2-8 °C for 15 minutes, though this may result in a lower yield.
10. Carefully collect the supernatant (which contains cytoplasmic proteins) to a new tube. The isolated cytoplasmic proteins can be used immediately in downstream applications or stored at -80 °C.
11. Add 500 µl of Mitochondria Isolation Buffer 3 to the pellet and resuspend by vortexing.
12. Centrifuge at 12,00 × g at 2-8 °C for 15 minutes.
13. Carefully discard the supernatant. The remaining pellet contains mitochondria, which can be stored at -80 °C or immediately processed:

    - Protein analysis: The pellet can be dissolved and lysed with protein lysis buffer. Our Mammalian Total Protein Extraction Kit (abx098853) can be used for protein extraction. Mitochondria or mitochondrial lysate can be stored at -80 °C for future use.
    - Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 µl/10^7 cells. Analyze within one hour after resuspension.