YamayBio

UltraPure Mitochondria and Mitochondrial Protein Extraction Kit

QUICK START GUIDE

Research use only.
Not for diagnostic procedures.

Contents and storage

UltraPure Mitochondria and Mitochondrial Protein Extraction Kit

BF7478 30 Samples

Storage: Store at 4°C for 12 months. Product shipped in ice packs.

Component	Cat. No.	Volume	Storage
Mito Buffer A	BF74781	30 mL	4°C Handle Mito Buffer D under **sterile** conditions.
Mito Buffer B	BF74782	30 mL	
Mito Buffer C	BF74783	15 mL	
Mito Buffer D	BF74784	2.5 mL	
Mito Buffer E	BF74785	25 m×2	
Mito Buffer F	BF74786	6 mL	
Mito Buffer G	BF74787	3mL	
Janus Green B solution	BF74788	1 mL	

Introduction

This kit isolates intact mitochondria within 60 minutes using a two-step density gradient centrifugation method. The extracted mitochondria possess complete double-membrane structures and retain high biological activity. They are suitable for downstream applications such as apoptosis analysis, signal transduction, metabolic studies, and mitochondrial proteomics.

Mitochondrial proteins can be obtained by lysing the purified mitochondria with the included lysis buffer, and are compatible with SDS-PAGE, Western blot (WB), and immunoprecipitation (IP) assays.

This kit is suitable for isolating mitochondria from animal tissues and cultured animal cells. Each kit can process up to 30 samples when using 100 mg tissue or 2×10^7 cells per sample.

Protocol

1. Place all kit components on ice.Perform all procedures under aseptic conditions. **Pre-cool the centrifuge** and **glass homogenizer** to 4°C before use.

2. Sample preparation:

   Tissue samples: Wash fresh tissue with an appropriate volume of pre-chilled 1× PBS. Weigh 100 mg of tissue, transfer it into an appropriately sized homogenizer, and add **1 mL of Mito Buffer A**.

   Note: Do not use previously frozen tissue samples.

   Cell samples: Centrifuge the cells at 500 × *g* for 5 min at 4°C and collect the cell pellet. Resuspend the cells in an appropriate volume of pre-chilled 1× PBS. Transfer 2 × 10^7 cells and centrifuge again at 500 × *g* for 5 min at 4°C. Carefully discard the supernatant, resuspend the cell pellet in **1 mL of Mito Buffer A**, and transfer the suspension into an appropriately sized homogenizer.

3. Homogenize the sample repeatedly until approximately 50%–60% of the cells are disrupted.

   Tissue samples: Homogenize approximately 5–10x in short bursts, then mix a small aliquot of the homogenate with Trypan Blue solution to evaluate the extent of cell disruption.

   Cell samples: Use a tightly fitted pestle to ensure effective cell disruption. Homogenize for 5x in short bursts, then mix a small aliquot of the homogenate with Trypan Blue solution. Increase the number of bursts as needed according to the observed extent of cell disruption

   Note: The extent of cell disruption is critical for successful mitochondrial isolation. Insufficient disruption may reduce mitochondrial yield, whereas excessive disruption may compromise mitochondrial integrity. A preliminary experiment is recommended to determine the optimal homogenization conditions. Do not over-homogenize.

4. Add **1 mL of Mito Buffer B** to a 2 mL microcentrifuge tube. Carefully layer the homogenate along the wall of the tube onto the surface of Mito Buffer B. Immediately centrifuge for 10 min at 4°C at:
   - 700 × *g* for tissue samples
   - 600 × *g* for cell samples

   After centrifugation, carefully collect 1 mL of the uppermost layer and transfer it to a 1.5 mL microcentrifuge tube. If no distinct layer separation is observed, collect the entire supernatant.

5. Centrifuge the fraction collected in Step 4 at 10,000 × *g* for 10 min at 4°C. Carefully discard the supernatant. The resulting pellet contains the crude mitochondria. Resuspend the pellet in **0.5 mL of Mito Buffer E**.

6. Add **425 µL of Mito Buffer C** and **75 µL of Mito Buffer D** to a pre-chilled 1.5 mL microcentrifuge tube and mix thoroughly. The volume ratio of C:D is 17:3 (V/V). Prepare the C/D mixture immediately before use.

7. Carefully layer the resuspended crude mitochondrial fraction onto the surface of the C/D mixture prepared in Step 6. Immediately centrifuge at 21,000 × *g* for 10 min at 4°C. Carefully discard the supernatant.

   Note: The centrifuge should reach the specified speed within 15 sec; otherwise, the separation efficiency may be affected.

8. Resuspend the pellet in **1 mL of Mito Buffer E** and centrifuge at 16,000 × *g* for 5 min at 4°C. Carefully discard the supernatant and collect the pellet. The resulting pellet contains high-purity mitochondria.

9. Process the mitochondrial pellet according to the intended downstream application:

   - For studies of intact mitochondrial function or enzyme activity, resuspend the pellet in **200 µL of Mito Buffer F** and mix thoroughly by gentle pipetting.
   - For mitochondrial protein analysis, resuspend the pellet in **100 µL of Mito Buffer G** and mix thoroughly by pipetting. If required, add 100× Protease Inhibitor (Cat. No. BH5481) at a ratio of 1:100 (V/V). The resulting mitochondrial protein sample can be used for SDS-PAGE, Western blotting (WB), immunoprecipitation (IP), Co-immunoprecipitation (Co-IP), and other downstream applications.

10. For mitochondrial staining and identification, mix 5 µL of the mitochondrial suspension prepared in **Mito Buffer F** with 5 µL of Janus Green B Solution. Place the mixture onto a microscope slide, cover with a coverslip, and observe under a microscope. Mitochondria should appear as blue-green rod-shaped or dumbbell-shaped structures.

Notes

1. Perform the entire mitochondrial isolation procedure under cold and aseptic conditions. Keep sample tubes on ice throughout the procedure.

2. Efficient cell disruption while maintaining the integrity of subcellular organelles is critical for successful mitochondrial isolation. The efficiency of cell disruption may vary depending on the tissue or cell type and the homogenizer used. It is recommended to optimize the disruption conditions for each sample type.

3. Cell disruption is particularly critical when processing cell samples. The following methods may be used:
   - Use a tightly fitted pestle. When the pestle is inserted into the homogenizer tube, the tube should remain attached when the pestle is lifted, indicating an appropriately tight clearance.
   - Alternatively, cells may be disrupted using a sonicator. Start with the lowest power setting and optimize the sonication conditions in a preliminary experiment to avoid excessive disruption.

4. Mitochondria resuspended in Mito Buffer F should be used as soon as possible. If immediate use is not possible, store the samples at -80°C. Frozen mitochondrial samples are not recommended for studies of intact mitochondrial function, but may be used for mitochondrial protein analysis, Western blotting (WB), immunoprecipitation (IP), and related applications.