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ab110169 – Mitochondria Isolation Kit for Tissue (with Dounce Homogenizer)

Instructions for Use

For mitochondria isolations from mammalian tissue samples

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Abcam's benchtop mitochondria isolation kit allows for quick and efficient isolation of intact mitochondria from both soft and hard tissues using differential centrifugation. Sufficient reagents are provided in the kit for 10 isolations, each requiring approximately an hour.

Principles of mitochondria isolation: The key steps when isolating mitochondria from any tissue or cell are always the same: (i) rupturing of cells by mechanical and/or chemical means and (ii) differential centrifugation at low speed to remove debris and extremely large cellular organelles (SPIN 1) followed by centrifugation at a higher speed to isolate mitochondria which are collected (SPIN 2). This crude mitochondrial preparation is often enough for most applications. The procedures detailed in this manual have been designed to provide the highest possible yield of intact and enzymatically active mitochondria.

Suggested amounts of starting material, expected mitochondria yields, and Dounce strokes are shown in Table1.

Sample	Starting Material	Expected Yield	Dounce Strokes
Liver	0.3 – 0.5 g	2 - 4 mg*	20 - 35
Heart	0.2 – 0.4 g	1 - 2 mg*	30 - 40
Brain	0.3 – 0.4 g	4 - 5 mg*	20 -35

Table 1. Suggested starting amounts and expected yields of mitochondria. *Hard tissues result in lower yields due to difficult homogenization.*

2. Assay Summary

Suspend washed and minced tissue in Isolation Buffer.

DISRUPT CELLS: Homogenize tissue following guidelines in Table 1.

SPIN 1: 1,000 g 10 minutes 4°C, collect & save supernatant.

SPIN 2: 12,000 g 15 minutes 4°C, collect & save pellet.

Wash the pellet by resuspending in Isolation Buffer and protease inhibitor (PI), Centrifuge at 12,000 x g for 15 minutes, collect pellet, repeat.

Resuspend the pellet in Isolation Buffer and PI, aliquot and freeze at -80°C.

Assay mitochondria: protein concentration, Western blot, OXPHOS activities.

3. Kit Contents

- Wash Buffer: 30 ml
- Isolation Buffer: 100 ml
- Dounce Homogenizer (2 ml size) with pestles

4. Storage and Handling

Wash Buffer and Isolation Buffer should be stored at 4°C.

5. Additional Materials Required

Reagents:

- Double distilled water
- Protease inhibitor cocktail (PI)
- BCA Protein Assay

Equipment:

- High speed benchtop centrifuge
- 2.0 ml microtubes
- Weighing balance and other standard lab equipment
- Scalpel

6. Protocol

The mitochondria preparation follows three simple steps: cell rupturing, centrifugation to remove large particles and centrifugation to isolate mitochondria. Below are guidelines for the preparation of mitochondria from liver, brain and heart. Buffers and samples should be chilled when possible.

1. Weigh out the appropriate amount of tissue (see Table 1).

2. Wash the sample tissue twice with 1.5 ml of Wash Buffer (provided).

3. Mince the tissue and place in pre-chilled Dounce homogenizer.

4. Add up to 2.0 ml of Isolation Buffer (provided) to the tissue in the homogenizer.

RUPTURING:

5. To rupture the cells, perform the number of Dounce strokes suggested in Table 1. Use pestle A (large clearance) for the initial strokes, then use pestle B (small clearance) for the remaining strokes.

SPIN 1:

6. Transfer the homogenate into a 2.0 ml microtube.

   Note: If 300 mg or more of starting tissue was used, split the homogenate equally into two 2.0 ml microtubes.

7. Fill each tube to 2.0 ml with Isolation Buffer.

8. Centrifuge the homogenate at 1,000 g for 10 minutes at 4°C.

9. Save the supernatant and discard the pellet.

SPIN 2:

10. Transfer the supernatant into two new tubes and fill each tube to 2.0 ml with Isolation Buffer.

11. Centrifuge the supernatant at 12,000 g for 15 minutes at 4°C.

12. Collect the pellet (supernatant can be saved for quality analysis, as described below).

13. Wash each pellet by resuspending in 1.0 ml of Isolation Buffer supplemented with 10 µl protease inhibitor cocktail (stock = 100x).

14. Centrifuge at 12,000 g for 15 minutes at 4°C.

15. Collect the pellets and repeat this wash performed in Step #14.

16. Combine the pellets and resuspend them in 500 µl of Isolation Buffer supplemented with protease inhibitor cocktail.

17. Freeze the aliquots at -80°C until use. If desired, mitochondrial quality assays described below can be performed immediately.

7. Mitochondrial Quality Analysis

There are several Abcam products that can be used to test mitochondrial quality. Figure 1 demonstrates a typical Western blot using isolated Rat liver mitochondria versus liver homogenate at 2 µg and 10 µg. Samples were probed with Abcam's Rodent MitoProfile® Total OXPHOS Complexes Detection Kit (ab110413/MS604).

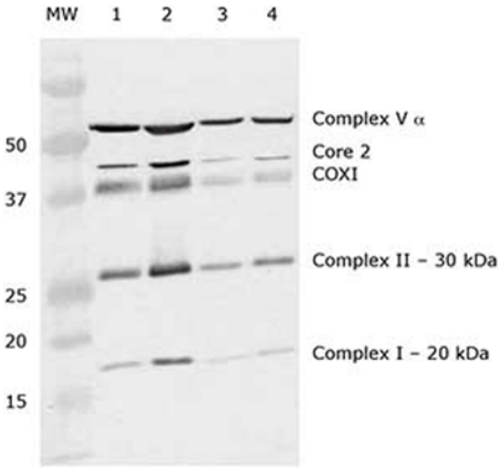

Figure 1. Isolated mitochondria show enriched signal when compared to the crude homogenate. In lanes 1 and 2, liver mitochondria isolated with Abcam's Isolation Kit were loaded at 2 µg and 10 µg. In lanes 3 and 4, liver homogenate was loaded at 2 µg and 10 µg, respectively.

Mitochondria integrity can also be tested by screening for cytochrome *c* (intermembrane space), Porin (outer membrane), Cyclophilin D (matrix), in the isolated mitochondria versus in the supernatant fraction using Abcam's antibodies ab110325(MSA06), ab14734(MSA03), and ab110324(MSA04). Figure 2 depicts heart mitochondria and supernatant screened with these antibodies.

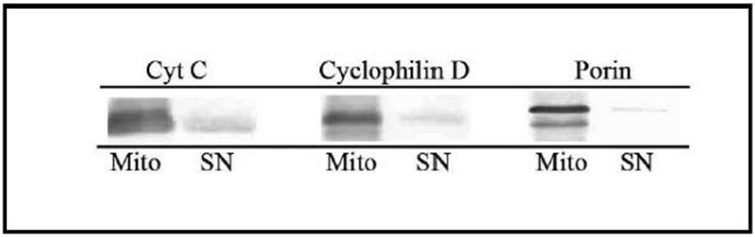

Figure 2. Heart mitochondria were isolated from freshly extracted organs. The supernatant fraction was saved after SPIN 2. 5 µg of heart mitochondria and 5 µg of supernatant were loaded in each lane and detected using ab110325/MSA06 (Cyt *c*), ab110324/MSA04 (Cyclophilin D), and ab14734/MSA03 (Porin). Western blots show that minimal loss of cytochrome *c*, Cyclophilin D and Porin occurs during mitochondria isolation.

8. Optimization Steps and General Tips

Problem	Probable Cause	Solution
Small mitochondrial pellet	Insufficient lysis occurred	Increase Dounce strokes
Large amount of Cytochrome *c* in the cytosol	Cells over-lysed/ Tissues not fresh	Reduce Dounce strokes/Isolate from freshly extracted tissues

UK, EU and ROW
Email:
technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp