

# Mitochondria Isolation Kit for Tissue (with Dounce Homogenizer)

**MS851**

Rev.0

## DESCRIPTION

### Mitochondria Isolation Kit for Tissue (with Dounce Homogenizer)

MitoSciences' 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.

#### Kit Contents:

Item	MS851
Wash Buffer	30 mL
Isolation Buffer	100 mL
Dounce Homogenizer (2-mL size) with pestles	1

#### Storage:

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

## INTRODUCTION

### 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
- (ii) Differential centrifugation at low speed to remove debris and extremely large cellular organelles (SPIN 1)
- (iii) Centrifugation at a higher speed to isolate and collect mitochondria (SPIN 2). This crude mitochondrial preparation is often enough for most applications. The procedure detailed in this manual has been designed to provide the highest possible yield of intact and enzymatic active mitochondria.

Suggested amounts of starting material, expected mitochondria yields, and Dounce strokes are shown in the table below:

Table 1.

Sample	Starting Material (wet weight)	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

*\*Hard tissues result in lower yields due to difficult homogenization.*

## ADDITIONAL MATERIALS REQUIRED

### Reagents Needed:

- Double distilled water
- Protease inhibitor cocktail (PI), (Sigma, P8340)
- BCA Protein Assay (Pierce, 23225)

### Equipment Needed:

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

## MITOCHONDRIA ISOLATION PROCEDURE

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.

### RUPTURE:

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  $\mu$ L 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  $\mu$ L 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.

## MITOCHONDRIAL QUALITY ANALYSES

There are several MitoSciences' products that can be used to test mitochondrial quality. Figure 1 demonstrates a typical Western blot using isolated liver mitochondria versus liver homogenate at 2  $\mu$ g and 10  $\mu$ g. Samples were probed with MitoSciences' MS604 Rodent Total OXPHOS Complexes Detection Kit.

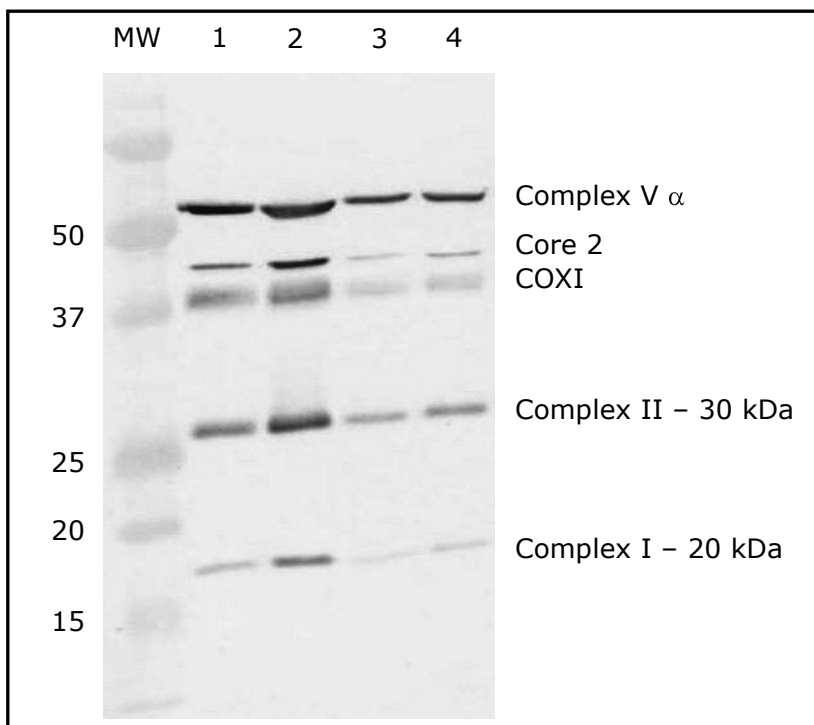


Figure 1. Isolated mitochondria show enriched signal when compared to the crude homogenate. In lanes 1 and 2, liver mitochondria isolated with MitoSciences' Isolation Kit were loaded at 2  $\mu$ g and 10  $\mu$ g. In lanes 3 and 4, liver homogenate was loaded at 2  $\mu$ g and 10  $\mu$ g, respectively.

Mitochondria integrity can also be tested by screening for cytochrome *c*, Porin, or Cyclophilin D in the isolated mitochondria versus in the supernatant fraction using MitoSciences' antibodies MSA06, MSA03 and MSA04. Figure 2 depicts heart mitochondria and supernatant screened with these antibodies.

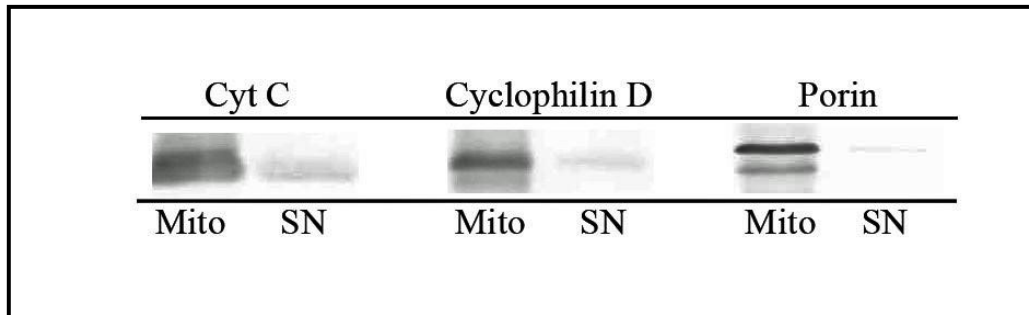


Figure 2. Heart mitochondria were isolated from freshly extracted organs. The supernatant fraction was saved after SPIN 2 (Page 3). 5  $\mu$ g of heart mitochondria and 5  $\mu$ g of supernatant were loaded onto each lane and detected using MSA06 (Cyt *c*), MSA04 (Cyclophilin D), and MSA03 (Porin). Western blots show that minimal loss of cytochrome *c*, Cyclophilin D and Porin occurs during mitochondria isolation.

In addition to MitoSciences Western blotting kits, mitochondria activity can be measured using MitoSciences' Rapid Microplate Assay Kits. See [www.mitosciences.com](http://www.mitosciences.com) for more details.

## OPTIMIZATION STEPS AND GENERAL TIPS

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

### BCA protein assay (Pierce)

Mitochondria protein concentration is determined with the BCA™ Protein Assay kit (Pierce 23225), using bovine serum albumin as a standard according to the manufacturer's instructions.

## FLOW CHART

This guide is for quick reference only. Be completely familiar with the previous details of this document before performing the assay.

