# Isolation of Mitochondria from Soft Tissues (Liver or Brain) using the FOCUS™ Mitochondria Kit

**G-Biosciences**

## Abstract

This protocol is part of the FOCUS™ Mitochondria Kit collection. Please refer to the appropriate protocol depending on your application.

**Citation:** G-Biosciences Isolation of Mitochondria from Soft Tissues (Liver or Brain) using the FOCUS™ Mitochondria Kit. **protocols.io**
dx.doi.org/10.17504/protocols.io.e89bhz6
**Published:** 12 Jul 2016

## Guidelines

### INTRODUCTION FOCUS™

Mitochondria kit enables the fast and easy isolation of enriched mitochondrial fractions from animal cells and tissues. The majority of the isolated mitochondria obtained from this kit contain intact inner and outer membranes. This kit contains reagents for processing 50-80 preparations of 20 million cultured mammalian cells or 20-30 preps of 50-100mg tissue. The number of preparations varies depending on the protocol, preparation size and cell or tissue type.

### ITEM(S) SUPPLIED (Cat. #: 786-022)

| Description | Size |
|---|---|
| SubCell Buffer-I | 60ml |
| SubCell Buffer-II [3X] | 30ml |
| SubCell Buffer-IV | 25ml |
| SubCell Buffer-V | 15ml |
| Mitochondria Storage Buffer | 10ml |
| Mitochondria Storage Component | 1 vial |

### STORAGE CONDITION

The kit is shipped at ambient temperature. After receiving store the kit components at 4°C except Mitochondria Storage Component at -20°C. The kit is stable for one year when stored unopened. Use aseptic techniques when handling the reagent solutions.

### ADDITIONAL ITEMS REQUIRED

Syringes and 20 gauge needles or Wheaton Dounce homogenizer, centrifuge and centrifuge tubes. Optional reagents: Delipidated BSA, Trypsin, PBS and protease inhibitor cocktail.

### PREPARATION BEFORE USE

All buffers should be kept ice cold. Dilute appropriate volume of 3X SubCell Buffer-II to 1X with SubCell Buffer-I as needed (e.g. mix 2ml SubCell Buffer-I with 1ml SubCell Buffer-II). All centrifugation steps should be performed at 4°C.

### Preparation of Working Mitochondria Storage Buffer

Pipette 0.5ml Mitochondria Storage Buffer to Mitochondria Storage Component vial. Pipette up and down a few times to dissolve all components completely. Transfer the solution of Mitochondria Storage Component to Mitochondria Storage Buffer bottle and mix well. The Working Mitochondria Storage Buffer should be kept frozen for long-term use.

## Before start

All buffers should be kept ice cold. Dilute appropriate volume of 3X SubCell Buffer-II to 1X with SubCell Buffer-I as needed (e.g. mix 2ml SubCell Buffer-I with 1ml SubCell Buffer-II). All centrifugation steps should be performed at 4°C.

## Materials

FOCUS™ Mitochondria 786-022 by G-Biosciences

## Protocol

### Step 1.

**OPTIONAL**: Delipidated BSA can be added to 1X SubCell Buffer-II to the concentration of 2mg/ml for removing free fatty acids prior processing. An appropriate amount of protease inhibitor cocktail also can be added to the 1X SubCell Buffer-II just before use.

### Step 2.

Use a fresh tissue sample (obtained within one hour of sacrifice) kept on ice. **Do not freeze.**

**Step 3.**

Weigh approximately 50-100mg tissue.

**Step 4.**

On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.

**Step 5.**

Perform this step on ice. Transfer the minced tissue to an ice-cold Dounce tissue homogenizer.

**Step 6.**

Add 10 volumes of 1X SubCell Buffer-II and using a loose-fitting pestle disaggregate the tissue with 5-10 strokes or until the tissue sample is completely homogenized.

**Step 7.**

Using a tight-fitting pestle, release the nuclei with 8-10 strokes. Do not twist the pestle as nuclei shearing may occur.

**Step 8.**

Stand on ice for 2 minutes.

DURATION
00:02:00

**Step 9.**

Transfer the homogenate to a centrifuge tube and leave large chunks of tissue fragments in the homogenizer to be discarded.

**Step 10.**

Centrifuge the lysate at 700x g for 5 minutes to pellet the nuclei.

DURATION
00:05:00

**Step 11.**

Carefully transfer the supernatant into a new tube. Centrifuge supernatant at 12,000x g for 10 minutes.

DURATION
00:10:00

**Step 12.**

Remove the supernatant and resuspend the pellet in 10 volumes of 1X SubCell Buffer-II without BSA.

**Step 13.**

(Centrifuge as in steps 9-10.) Transfer the homogenate to a centrifuge tube and leave large chunks of tissue fragments in the homogenizer to be discarded.

**Step 14.**

(Centrifuge as in steps 9-10.) Centrifuge the lysate at 700x g for 5 minutes to pellet the nuclei.

DURATION
00:05:00

**Step 15.**

Carefully transfer the supernatant into a new tube. Centrifuge supernatant at 12,000x g for 10 minutes.

DURATION
00:10:00

**Step 16.**

Remove the supernatant. **The pellet contains mitochondria.**

NOTES
**Colin Heath** 08 Jul 2016

NOTE: To fractionate light and heavy mitochondria, and obtain more purified mitochondrial fractions, see '[Fractionation of Light and Heavy Mitochondria by Gradient Cushion using the FOCUS™ Mitochondria Kit]'.

**Step 17.**

Suspend the mitochondrial pellet in Working Mitochondria Storage Buffer (approximately 50µl for pellet from 100mg tissue) and keep the suspension on ice before downstream processing. The suspension may be stored on ice for up to 48 hours.

NOTES
**Colin Heath** 29 Jun 2016

Freezing and thawing may compromise mitochondrial integrity.