Fractionation of Light and Heavy Mitochondria by Gradient Cushion 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 Fractionation of Light and Heavy Mitochondria by Gradient Cushion using the FOCUS™ Mitochondria Kit. **protocols.io**
dx.doi.org/10.17504/protocols.io.e88bhzw
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.

Solubilization of Mitochondrial Fractions:

The fractionated mitochondria may be solubilized in any suitable buffer consistent with downstream procedures. For IEF/2D gel electrophoresis, the mitochondria fractions may be solubilized in a chaotropic extraction buffers. G-Biosciences offers a wide selection of buffers and reagents for IEF/2D gel electrophoresis. FOCUS/Extraction Buffer-VI (Cat. # 786-233) is suitable for solubilization of mitochondria fractions.

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.

Suspend the mitochondrial pellet in 100µl 1X SubCell Buffer-II.

Step 2.

Make a step gradient by adding 200µl SubCell Buffer-V to a centrifuge tube and then overlaying with 200µl SubCell Buffer-IV.

Step 3.

Gently float the mitochondrial suspension on the surface of the step gradient.

Step 4.

Centrifuge the gradient at 20,000x g for 20 minutes. Observe the two white bands.

DURATION
00:20:00

NOTES
Colin Heath 29 Jun 2016

The band at the interface of SubCell Buffer-IV and V is the heavy mitochondria fraction, whereas the band above the heavy mitochondria band is the light mitochondria fraction.

Step 5.

Carefully remove each band to a separate tube.

Step 6.

Dilute the mitochondrial suspensions with equal volume of 1X SubCell Buffer-II.

Step 7.

Centrifuge the tubes at 12,000x g for 15 minutes and discard the supernatant.

DURATION
00:15:00

Step 8.

Suspend the mitochondrial pellets in 30-50µl Working Mitochondria Storage Buffer and keep the suspensions on ice before downstream processing. The suspensions may be stored on ice up to 48 hours.

NOTES

Colin Heath 29 Jun 2016

Freezing and thawing may compromise mitochondrial integrity.