Fractionation of Light and Heavy Mitochondria by Gradient Cushion (FOCUS™ SubCell Kit)

G-Biosciences

Abstract

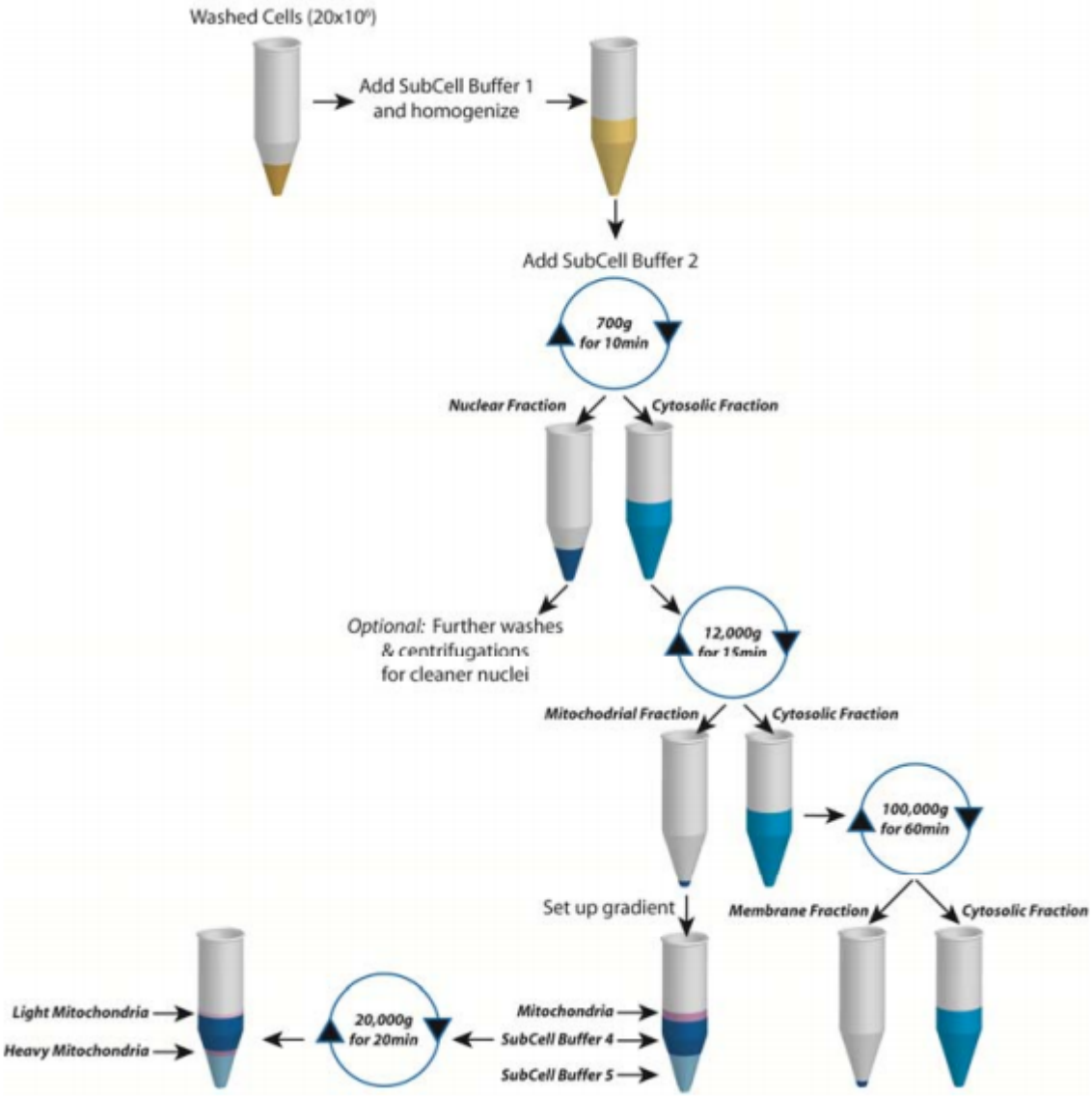
This is part of the collection of FOCUS™ SubCell protocols for the enrichment of subcellular fractions. Please refer to the appropriate protocol depending on your application.

Citation: G-Biosciences Fractionation of Light and Heavy Mitochondria by Gradient Cushion (FOCUS™ SubCell Kit). **protocols.io**
dx.doi.org/10.17504/protocols.io.e9gbh3w
Published: 13 Sep 2016

Guidelines

INTRODUCTION

FOCUS™ SubCell kit enables the fast and easy enrichment of nuclear, mitochondrial, membrane and cytosolic fractions from animal cells. The mitochondrial fraction can be subsequently separated into heavy and light fractions by gradient centrifugation. An additional step is included to minimize contaminations of the nuclear fraction by cytoplasmic elements (see schematic on the right). The majority of mitochondria, isolated with this kit, contain intact inner and outer membranes. FOCUS™ SubCell is suitable for cultured animal cells and can be adapted for animal tissues.



ITEM(S) SUPPLIED (Cat. # 786-260)

Description	Size
SubCell Buffer-I	60ml
SubCell Buffer-II [3X]	30ml
SubCell Buffer-III	25ml
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 all the kit components at 4°C except

store Mitochondria Storage Component at -20°C. The kit is stable for one year when stored unopened. Use aseptic techniques when handling the reagent solutions.

ITEMS NEEDED BUT NOT SUPPLIED

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).

NOTE: *Do not dilute all 3X SubCell Buffer-II as some steps require the 3X concentrated 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 the sub-cell fractions:

The fractionated cell organelles (nuclei or mitochondria) may be solubilized in any suitable buffer consistent with downstream procedures. For IEF/2D gel electrophoresis, the enriched 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 all pellet fractions. The soluble cytosolic fraction can be concentrated using Perfect-FOCUS™ kit (Cat# 786-124). For more information visit our website at www.GBiosciences.com

Materials

FOCUS™ SubCell Kit 786-260 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 30 Jun 2016

The band at the interface of SubCell Buffer-IV and V is the heavy mitochondria fraction. 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 with 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.

DURATION
48:00:00

NOTES

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Freezing and thawing may compromise mitochondria integrity.