Cleaning of the Nuclear Fraction (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.

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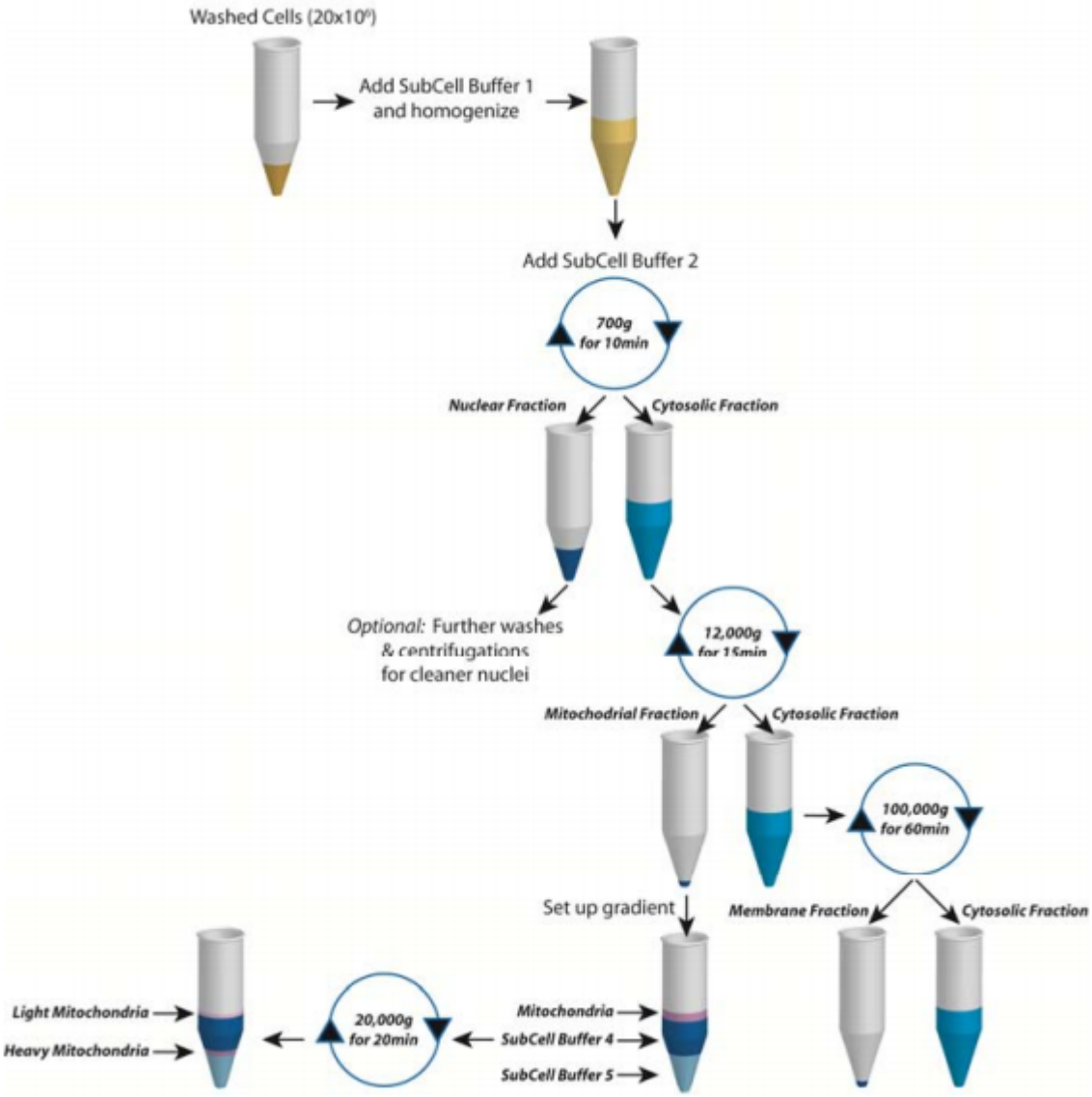
Guidelines

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.

Resuspend the nuclear pellet in 300µl SubCell Buffer-III.

Step 2.

Using a sharp pipette tip, remove the sticky lump if any. The lump is formed from dead cells and some lysed nuclei.

Step 3.

Centrifuge the tube at 700x g for 5 minutes and discard the supernatant. The pellet containing nuclei is clean enough for most purposes. If further cleaning required, go to next step.

DURATION
00:05:00

Step 4.

Pipette 300µl SubCell Buffer-IV to a 1.5ml centrifuge tube.

Step 5.

Resuspend the nuclear pellet in 100µl SubCell Buffer-III.

Step 6.

Carefully overlay the nuclear suspension on the surface of SubCell Buffer-IV.

Step 7.

Centrifuge the tube at 1,000x g for 10 minutes.

DURATION
00:10:00

Step 8.

Remove the supernatant and collect the very clean nuclear pellet in the tube.