Subcellular Fractionation from Animal Cells (FOCUS™ SubCell Kit)

G-Biosciences

Abstract

This protocol is for processing $20x10^6$ cells (or ~100µl wet cell pellet). It can be scaled up and down accordingly.

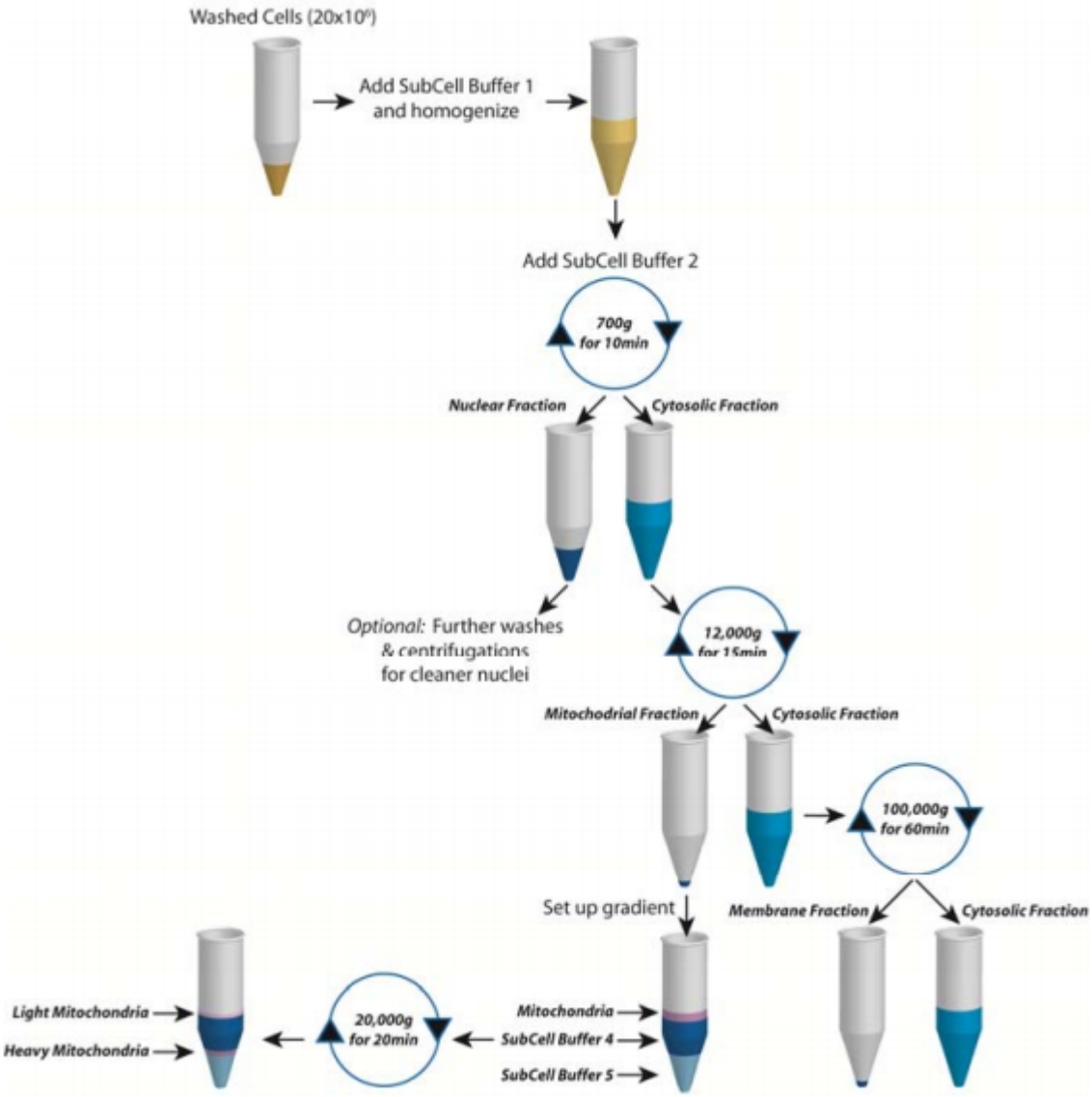
This is part of the collection of FOCUS™ SubCell protocols for the enrichment of subcellular fractions. Please refer to the appropriate protocol depending on your application.

Citation: G-Biosciences Subcellular Fractionation from Animal Cells (FOCUS™ SubCell Kit). **protocols.io** dx.doi.org/10.17504/protocols.io.e9dbh26
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.

OPTIONAL: Add appropriate protease inhibitor cocktail (e.g. G-Biosciences' ProteaseArrest, Cat. # 786-108) to SubCell Buffer-I immediately prior to use.

Step 2.

Use fresh cells only. Pellet the harvested cells by centrifugation at 800 x g for 1 minute. Carefully remove and discard the supernatant.

DURATION
00:01:00

NOTES
Colin Heath 30 Jun 2016

OPTIONAL: Wash the cell pellet with 1ml ice cold PBS, centrifuge it as above and discard the supernatant.

Step 3.

Add 500µl of ice cold SubCell Buffer-I.

Step 4.

Gently vortex to suspend the cells and incubate on ice for 10 minutes.

DURATION
00:10:00

Step 5.

Perform this lysis step on ice. Using a narrow opening (20 gauge) syringe needle, gently pull the suspension up and down 10-30 times.

NOTES
Colin Heath 30 Jun 2016

Alternatively, transfer cell suspension to ice cold Dounce homogenizer. Homogenize the cells on ice using tight pestle. Perform 5 to 20 strokes to lyse the cells effectively. Transfer the lysate to a microcentrifuge tube. Rinse Dounce homogenizer with 200µl of SubCell Buffer- I and pool together. Invert the tube several times to mix.

Colin Heath 30 Jun 2016

NOTE: To check the cell lysis efficiency, spot 5µl of cell lysate onto a glass slide, add cover slip and view under a phase-contrast microscope. Pulling times or strokes in the above lysis step are only guidelines. Mechanical force needed to lyse cells depends on cell types, the total number of the cells and hands on experience. It is also dependent on the ultimate goal of lysis. Insufficient force

will not lyse all the cells, but will achieve cleaner mitochondrial fractions with less nuclear contamination. Excess force may damage some nuclei, but a cleaner nuclear fraction will be obtained.

Step 6.

Add 250µl 3X SubCell Buffer-II (350µl if Dounce homogenizer is used) and mix by inverting.

NOTES
Colin Heath 30 Jun 2016

This generates a 1X final concentration of SubCell Buffer-II.

Step 7.

Centrifuge the tube at 700x g for 10 minutes to pellet the nuclei. Transfer the supernatant to a new tube.

DURATION
00:10:00

NOTES
Colin Heath 30 Jun 2016

NOTE: For further cleaning the nuclear fraction, see 'Cleaning of the Nuclear Fraction (FOCUS™ SubCell Kit)'.

Step 8.

Centrifuge supernatant at 12,000x g for 15 minutes.

DURATION
00:15:00

Step 9.

Transfer the supernatant (post mitochondria) to a new tube for further processing. The pellet contains mitochondria.

NOTES
Colin Heath 30 Jun 2016

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

For a crude mitochondrial fraction, continue with step 10.

Step 10.

Add 500µl 1X SubCell Buffer-II to the pellet, and centrifuge again at 12,000 x g for 5 minutes. Discard the supernatant.

DURATION
00:05:00

Step 11.

Resuspend the mitochondrial pellet with 50-100µl Working Mitochondria Storage Buffer and keep the suspension on ice before downstream processing.

Step 12.

Enrichment of other cell organelles: The post mitochondria supernatant from steps 8-9 can be further fractionated using a variety of gradient and differential centrifugations.

NOTES
Colin Heath 30 Jun 2016

For example, centrifugations of the post mitochondrial supernatant at 100,000x g for 60 minutes will sediment cellular membranes. The resulting pellet is an enriched cytosolic membrane fraction and the supernatant is soluble cytosolic fraction. This cytosolic fraction may be used for further fractionation.