Subcellular Fractionation from Skeletal or Heart Muscle Hard Tissues (FOCUS™ SubCell Kit)

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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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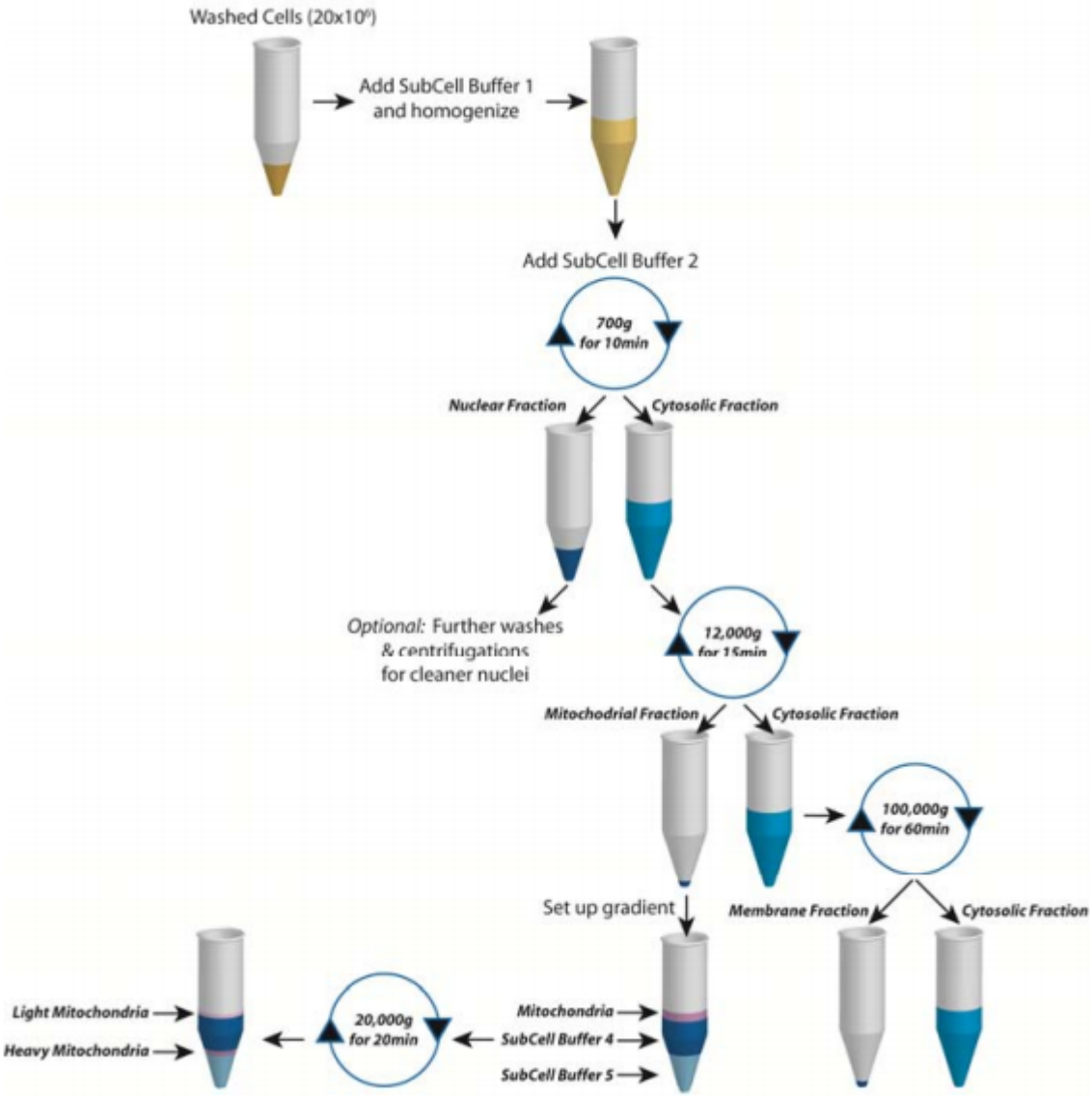
Guidelines

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.

NOTE: For facilitating homogenization of the hard tissue, 0.25mg/ml Trypsin should be added to 1X SubCell Buffer-II. A concentrated BSA solution is needed to quench the proteolytic reaction after Trypsin treatment.

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

Before start

For facilitating homogenization of the hard tissue, 0.25mg/ml Trypsin should be added to 1X SubCell Buffer-II. A concentrated BSA solution is needed to quench the proteolytic reaction after Trypsin treatment.

Materials

FOCUS™ SubCell Kit 786-260 by G-Biosciences

Protocol

Step 1.

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

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For facilitating homogenization of the hard tissue, 0.25mg/ml Trypsin should be added to 1X SubCell Buffer-II. A concentrated BSA solution is needed to quench the proteolytic reaction after Trypsin treatment.

Step 2.

Weigh approximately 50-100mg tissue. On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.

Step 3.

Suspend the sample with 8 volumes of 1X SubCell Buffer-II containing 0.25mg/ml trypsin in a 2ml centrifuge tube.

Step 4.

Incubate on ice for 3 minutes and then spin down the tissue for a few seconds in the centrifuge.

DURATION
00:03:00

Step 5.

Remove the supernatant by aspiration and add 8 volumes of 1X SubCell Buffer-II containing 0.25mg/ml Trypsin.

Step 6.

Incubate on ice for 20 minutes.

DURATION
00:20:00
Step 7.

Add BSA Solution to a final concentration of 10mg/ml and mix.

Step 8.

Spin down the tissue at 1,000 x g for 5-10 seconds in the centrifuge.

DURATION
00:00:05
Step 9.

Remove the supernatant by aspiration.

Step 10.

Wash the pellet with 8 volumes of 1X SubCell Buffer-II without Trypsin, and spin down the tissue for a few seconds in the centrifuge.

Step 11.

Remove the supernatant by aspiration and add 8 volumes of the 1X SubCell Buffer- II without Trypsin.

Step 12.

Transfer the suspension to an ice-cold Dounce tissue homogenizer and using a loose-fitting pestle, disaggregate the tissue with 5-15 strokes or until the tissue sample is completely homogenized.

Step 13.

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

Step 14.

Stand on ice for 2 minutes.

DURATION
00:02:00
Step 15.

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

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

Step 16.

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

DURATION
00:05:00

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

Step 17.

Transfer the supernatant to a new tube.

Step 18.

Centrifuge it at 12,000xg for 10 minutes. Transfer the supernatant (post mitochondria) to a new tube. The pellet contains mitochondria.

DURATION
00:10:00

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

For a crude mitochondrial fraction, continue with step 19.

Step 19.

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.

DURATION
48:00:00

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Freezing and thawing may compromise mitochondria integrity.

Step 20.

Enrichment of other cell organelles: The post mitochondria supernatant from step 17-18 can be further fractionated using a variety of gradient and differential centrifugations.

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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.