Isolation of Mitochondria from Hard Tissues (Skeletal or Heart Muscle) 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.

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.

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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.

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.

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.

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

NOTES
Colin Heath 29 Jun 2016

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.

Step 2.

Weigh approximately 50-100mg tissues. 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 BufferII 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 in the homogenizer to be discarded.

Step 16.

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

DURATION
00:05:00

Step 17.

Transfer the supernatant to a new tube.

Step 18.

Centrifuge it at 12,000xg for 10 minutes and remove the supernatant. **The pellet contains mitochondria.**

DURATION
00:10:00

NOTES
Colin Heath 29 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 using the FOCUS™ Mitochondria Kit protocol.

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.

NOTES
Colin Heath 29 Jun 2016

Freezing and thawing may compromise mitochondrial integrity.