Isolation of Mitochondria from Animal Cells using the FOCUS™ Mitochondria 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.

It is part of the FOCUS™ Mitochondria Kit collection. Please refer to the appropriate protocol depending on your application.

Citation: G-Biosciences Isolation of Mitochondria from Animal Cells using the FOCUS™ Mitochondria Kit. **protocols.io** dx.doi.org/10.17504/protocols.io.e8fbhtn
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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.

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

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.

OPTIONAL: Add appropriate protease inhibitor cocktail (e.g. G-Biosciences' Protease Arrest, Cat# 786-108) to SubCell Buffer-I just before 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
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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. (Alternatively, use Dounce homogenizer as described in the annotation below.)

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NOTE: To check the cell lysis efficiency, spot 5µl of cell lysate onto a glass slide, add coverslip and view under a phase-contrast microscope. Pulling times or strokes in the above lysis step are only guidelines. Mechanical force to lyse cells depends on cell types, the total number of the cells and hands on experience. 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 high yield mitochondria fractions will be obtained with some contamination from nuclei.

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Lysing with Dounce homogenizer: 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 BufferI and pool together. Invert the tube several times to mix.

Step 6.

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

Step 8.

Centrifuge supernatant at 12,000x g for 15 minutes. The pellet contains mitochondria.

DURATION
00:15:00

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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.'

For a crude mitochondrial fraction, continue with step 11.

Step 9.

Transfer the supernatant (cytosol fraction) to a new tube.

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.

Suspend the mitochondrial pellet in 50-100µl Working Mitochondria Storage Buffer 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.