ab65320

Mitochondria/Cytosol Fractionation Kit

Instructions for Use

For the rapid, sensitive and accurate isolation of Mitochondrial and Cytosolic fractions from living cells.

View kit datasheet: www.abcam.com/ab65320
(use www.abcam.cn/ab65320 for China, or www.abcam.co.jp/ab65320 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

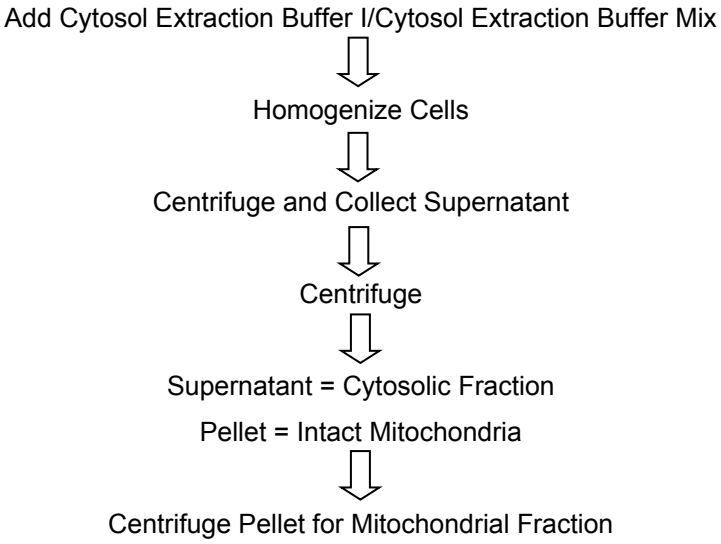
Version 2a, Last updated 6 March 2024

Table of Contents

1. Overview

Abcam's Mitochondria/Cytosol Fractionation Kit provides unique formulations of reagents for effective isolation of a highly enriched mitochondrial fraction from cytosolic fraction of mammalian cells including both apoptotic and non-apoptotic cells. The enriched mitochondrial and cytosolic fractions can be used for studying apoptotic and signal transduction pathways to detect translocation of factors interested between the two fractions by Western blotting, ELISA, or other assays. Procedures are simple and easy to perform; no ultra-centrifugations and toxic chemicals are involved.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity (25 assays)	Quantity (100 assays)
Mitochondria Extraction Buffer I/Mitochondria Extraction Buffer	2.5 mL	10 mL
5X Cytosol Extraction Buffer I/5 X Cytosol Extraction Buffer	5.0 mL	20 mL
DTT II/DTT (1M)	100 µL	100 µL
Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail	1 vial	1 vial

* Store kit at -20°C, protect from light. After opening the kit, store buffers at +4°C.

Store Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail and DTT II/DTT at -20°C.

Be sure to keep all buffers on ice at all times during the experiment.

Read the entire protocol before beginning the procedure.

Protease Inhibitor Cocktail I/PROTEASE INHIBITOR COCKTAIL: Add 250 µl of DMSO, and mix well before use, store at -20°C.

Cytosol Extraction Buffer I/CYTOSOLIC EXTRACTION BUFFER MIX: Make 1X Cytosol Extraction Buffer I/Cytosolic Extraction Buffer by mixing the 5 ml/20 ml of 5X buffer with 20 ml/80 ml ddH_2O.
Add 2 µl Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail + 1 µl DTT II/DTT to 1 ml of 1X Cytosol Extraction Buffer I/Cytosol Extraction Buffer to make 1X Cytosol Extraction Buffer I/Cytosol Extraction Buffer I/Cytosol Extraction Buffer Mix.

Mitochondria Extraction Buffer I/MITOCHONDRIA EXTRACTION BUFFER MIX: Add 2 µl Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail + 1 µl DTT II/DTT to 1 ml of Mitochondria Extraction Buffer I/Mitochondria Extraction Buffer before use.

Prepare enough Mitochondria Extraction Buffer I/Mitochondria Extraction Buffer Mix and Cytosol Extraction Buffer I/Cytosol Extraction Buffer Mix for the number of assays to be performed:

B. Additional Materials Required

- Microcentrifuget
- Pipettes and pipette tips
- Microscope
- Orbital shaker

- Dounce tissue grinder
- 1X PBS

4. Assay Protocol

1. Collect cells (5 x 10^7) by centrifugation at 600 x g for 5 minutes at 4°C.

2. Wash cells with 10 ml of ice-cold PBS. Centrifuge at 600 x g for 5 minutes at 4°C. Remove supernatant.

3. Re-suspend cells with 1.0 ml of 1X Cytosol Extraction Buffer I/Cytosol Extraction Buffer Mix containing DTT II/DTT and Protease Inhibitors (prepared as on Page 6).

4. Incubate on ice for 10 minutes.

5. Homogenize cells in an ice-cold Dounce tissue grinder. Perform the task with the grinder on ice. We recommend 30-50 passes with the grinder; however, efficient homogenization may depend on the cell type.

Notes:

a) To check the efficiency of homogenization, pipette 2-3 µl of the homogenized suspension onto a coverslip and observe under a microscope. A shiny ring around the cells indicates that cells are still intact. If 70-80% of the cells do not have the shiny ring, proceed to step 7. Otherwise, perform 10-20 additional passes using the Dounce tissue grinder.

b) Excessive homogenization should also be avoided, as it can cause damage to the mitochondrial membrane which triggers release of mitochondrial components.

6. Transfer homogenate to a 1.5-ml microcentrifuge tube, and centrifuge at 1000 x g (~3000 rpm) in a microcentrifuge for 10 minutes at +4°C. Collect the supernatant carefully and discard the pellet.

7. Transfer the supernatant to a fresh 1.5 ml tube, and centrifuge at 10,000 x g (~13000 rpm) in a microcentrifuge for 30 minutes at +4°C. Collect Supernatant and save the pellet.

8. Collect the supernatant from Step 7 as **Cytosolic Fraction** (Store at -80°C).

a. If intact mitochondria are desired, re-suspend the pellet from Step 8 in 0.1 ml 1X PBS (Not provided). These are the intact mitochondria.

b. If mitochondrial protein lysate is desired, re-suspend the pellet from Step 8 with 100 µl of the Mitochondria Extraction Buffer I/Mitochondrial Extraction Buffer Mix containing DTT II/DTT and protease inhibitors (as prepared on Page 6), vortex for 10 seconds and save as **Mitochondrial Fraction** (Store at -80°C).

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)