ab288084 – Mitochondria Isolation Kit For Tissue and Cultured Cells

For the isolation of high purity, intact and functional mitochondria from tissues and cultured cells
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288084

Storage and Stability

On receipt entire assay kit should be stored at -20°C protected from light. Upon opening, use kit within 12 months.

Materials Supplied

Item	Quantity	Storage Condition
Mitochondria Isolation Buffer	2 x 100 ml	-20°C
Permeabilization Reagent	1 ml	-20°C
Storage Buffer II	25 ml	-20°C

PLEASE NOTE: Permeabilization Reagent was previously labelled as Reagent A, and Storage Buffer II as Storage Buffer. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Protease inhibitor cocktail
- Dounce tissue grinder

Sample Preparation

Cultured Cells: Pellet 2 x10^7 cells by centrifugation at 600 x g for 10 min. Carefully remove and discard the supernatant.

Tissues: Isolate the tissue of interest. Immerse the tissue (50-200 mg) in 1 ml of ice-cold Mitochondria Isolation Buffer and rinse it twice to remove the blood. Use 1 ml of ice-cold Mitochondria Isolation Buffer to mince the tissue on ice into small pieces using scissors. Spin the minced tissue in a tabletop centrifuge at 10,000 x g for 2 min. Discard the Buffer and replace it with 1 ml of fresh ice-cold Mitochondria Isolation Buffer.

Procedure for Mitochondria Isolation

- In order to avoid protein degradation, we recommend that you add a protease inhibitor cocktail to Mitochondria Isolation Buffer.
- The number of strokes for homogenization will vary depending on the cells or tissue type.
- For cells, to check the cell lysis efficiency, spot 5 µl of cell lysate into a glass slide, add coverslip and view under a microscope. For tissues, perform sufficient strokes to obtain a homogeneous suspension without lysing the cells. Typically for soft tissues 10- 15 strokes and for hard tissues 5-10 strokes are sufficient.

Isolation of Mitochondria Using Dounce Homogenizer:

1. Homogenize the tissue or cells using a precooled glass homogenizer in Mitochondria Isolation Buffer. Stroke the sample 3-4 times on ice. The optimal ratio between tissue or cells and Mitochondria Isolation Buffer ranges from 1:5 - 1:10 (w/v).
2. Transfer the homogenate to a tube and centrifuge at 600 x g for 10 min. at 4°C.
3. Collect the supernatant in a separate tube and centrifuge at 7,000 x g for 10 min. at 4°C.
4. Discard the supernatant and wash the pellet again with Mitochondria Isolation Buffer.
5. Remove the supernatant and resuspend the mitochondria in Storage Buffer II.
6. Determine the protein concentration and adjust to the desired protein concentration with Storage Buffer II.

Isolation of Mitochondria Using Reagent Based Method:

1. To the cell pellet, add 1 ml of Mitochondria Isolation Buffer and vortex for 5 sec., followed by incubation on ice for 2 min.
2. Add 10 µl of Permeabilization Reagent and vortex for 5 sec. Incubate on ice for 5 min. while vortexing every min. for 5 sec.
3. Centrifuge at 600 x g for 10 min. at 4°C.
4. Collect the supernatant in a separate tube and centrifuge at 7,000 x g for 10 min. at 4°C.
5. Discard the supernatant and wash the pellet with Mitochondria Isolation Buffer.
6. Remove the supernatant and resuspend the mitochondria in Storage Buffer II.
7. Determine the protein concentration and adjust to the desired protein concentration with Storage Buffer II.

Storage Conditions based on Application:

For intact mitochondria, resuspend in Storage Buffer II. Keep on ice for immediate downstream applications or snap freeze in liquid nitrogen and store at -80°C for future use. For the gel loading purpose, mitochondria can be stored in an appropriate sample PAGE buffer (Not provided).

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)