Mitochondria Isolation Kit (Cultured Cells)

Product description

Mitochondria are organelles with a bilayer membrane structure that exist in most eukaryotic cells, and are the power plant of cells, the main place where cells breathe aerobically. It is of great significance to isolate mitochondria for mitochondrial physiological function and other mitochondrial protein performance analysis. Ensuring the integrity and purity of mitochondria is critical when isolating, and the Mitochondria Isolation Kit (Cultured Cells) can quickly and easily isolate mitochondria from cells, and the vast majority of the resulting mitochondria have complete inner and outer membranes. The isolated mitochondria can be used for subsequent studies of apoptosis, signaling, metabolism, and proteomics.

Composition and storage conditions

Size / Components	10rxns	20rxns	30rxns
Buffer A	150ml	150ml*2	150ml*3
Store at 4°C.			

Usage

Materials & Reagents not supplied : Cell grinding pestle

1. Take 5x cell volume of Buffer A to resuspend the cells and let them be on ice for 15 min.
2. Transfer the standing cell suspension to a cell grinder, perform grinding strokes with a cell grinding pestle for 25 times and then transfer it to an EP tube.
3. Take a small amount of grinding cell suspension to observe the cell state to ensure that the ratio of ruptured cells reaches 60 %.
4. Collect the cell suspension after grinding and centrifuge at 1,000 g for 10 min to take the supernatant.
5. Take the supernatant after centrifugation of the supernatant at 1,000 g for 10 min.
6. The supernatant obtained in step 5 was centrifuged at 7,000 g for 10 min and then the pellet was taken.
7. Resuspend the pellet obtained in step 6 with 5 ml Buffer A and vortex to mix well.
8. Resuspend centrifugation at 7,000 g for 10 min after taking the pellet.
9. Take 1 ml of Buffer A to resuspend the precipitate obtained in step 8 to finally obtain the full mitochondrial protein.

Stored at -80℃ or performed directly for follow-up experiments.

10. Soluble mitochondrial component acquisition (optional): Take the 9th step resuspending component centrifugation at 100,000 g for 1 h to take the supernatant, that is, the soluble mitochondrial component. Stored at -80℃ or for subsequent experiments.

Instructions for use

1. To ensure the integrity of the extracted mitochondria: fast handling, full cryogenic conditions and cell fragmentation without destroying sub-organelles.
2. Adherent culture cells are more difficult to break the wall when homogenized with a glass homogenizer, so a small-capacity glass homogenizer and a tightly spaced pestle are used to grind the cultured cells up and down.
3. Western Blot and 2D-gel electrophoresis can be performed directly with loading buffer to lyse mitochondria for follow-up experiments.
4. This product is for scientific use only.