Cell Mitochondrial Extraction Assay Kit

Catalog No: E-BC-E006 **Specification:** 50 Assays/100 Assays

Note: ① All the steps for isolating mitochondria need to be carried out on ice or at 4°C, and the solutions and instruments should be pre-cooled to 4°C.

② Adjust the speed of the centrifuge to 1 gear.

③ Increasing the concentration of the cell homogenate will not proportionally increase the amount of mitochondrial extraction. It is recommended to process multiple 1×10^7 cell samples at once instead of n×10^7 cell samples when extracting mitochondria.

④ Perform the operation quickly and use the isolated mitochondrial samples in time.

General information

Intended use This kit can be used to extract mitochondrial from cells.

Detection principle The differential centrifugation method is the most commonly used method for separating cell mitochondria. Usually, the first step is to prepare the cell homogenate, then perform low-speed centrifugation to remove the cell nucleus, cell debris, and undamaged cells, and finally perform high-speed centrifugation to precipitate the mitochondria.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	55 mL × 2 vials	-20°C, 12 months
Reagent 2	Wash Buffer	28 mL × 1 vial	55 mL × 1 vial	-20°C, 12 months
Reagent 3	Preservation Solution	6 mL × 1 vial	12 mL × 1 vial	-20°C, 12 months
Reagent 4	Trypan Blue Staining Solution	5 mL × 1 vial	5 mL × 1 vial	-20°C, 12 months, shading light

Note: The pilot-scale dosage specification of the kit is calculated based on the reagent usage for each processing of 1×10^7 cell samples. The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

Materials prepared by users

Instruments:

High-speed freezing centrifuge, 5 mL Glass homogenizer

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

Before use, place all reagents in ice water at 2-8℃ to restore them to a solution state.

Operation table

① **Cell collection:** Wash with PBS (0.01 M, pH 7.4) for adherent cells, use trypsin digestion, then terminate the digestion, and resuspend gently with a pipette. Then, centrifuge for collection. For suspended cells, centrifuge collection of precipitate.

Note: A small amount of cell suspension should be taken for counting at this step or the next step.

② **Cell washing:** Add 1 mL of pre-cooled PBS (0.01 M, pH 7.4) per 10^7 cells. Use a pipette to slowly resuspend, then centrifuge at 600 × g for 5 min at 4°C, and discard the supernatant.

③ **Pre-treatment:** Add 0.5 mL of pre-cooled extraction solution per 10^7 cells. Use a pipette to slowly resuspend and keep it on ice for 10 min.

④ **Cell homogenization:** Mix gently the cell suspension with a pipette. Transfer 0.5 mL of the cell suspension to a pre-cooled glass homogenizer and homogenize 10-30 times.

Note: If there are a large number of cells to be processed, it is recommended to perform multiple operations. A

5 mL glass homogenizer can handle a maximum of 10^7 cells.

Note: The number of homogenization cycles required when using glass homogenizers of different specifications varies and needs to be verified by the user. It is recommended to homogenize 10 times and then take 10 μL of the cell homogenate for staining with 10 μL of trypan blue staining solution. Observe under a microscope or analyze with a cell counter within 3 min. When the proportion of blue cells exceeds 50%, stop the homogenization and proceed to the next step. If the proportion of blue cells is less than 50%, increase the homogenization by 5 times and then follow the same procedure for staining and identification. At the same time, record the number of homogenization times for this cell type. In subsequent extractions, there is no need to explore the homogenization times again.

⑤ **Low-speed centrifugation:** Transfer the cell homogenate to a pre-cooled EP tube. Use 0.5 mL of pre-cooled extraction solution to rinse the glass homogenizer and add the rinsing solution together to the EP tube. Centrifuge at 600 × g for 10 min at 4°C.

Note: If want to obtain mitochondria of higher purity, increase the centrifugation speed to 3500×g. However, the drawback is that the quantity of extracted mitochondria will decrease.

⑥ **High-speed centrifugation:** Gently transfer the supernatant to a new pre-cooled EP tube. Centrifuge at 12000 × g for 10 min at 4°C.

Note: To obtain higher purity of mitochondria, you can change the centrifugation speed to 3500 × g. However, the amount of mitochondria extracted will decrease.

⑦ **Mitochondrial washing:** Gently remove the supernatant, add 0.5 mL of pre-cooled wash buffer, gently resuspend by pipette, centrifuge at 10000 × g for 10 min at 4°C.

⑧ **Mitochondria collection:** Gently remove the supernatant, and the precipitate material is the extracted cell mitochondria.

⑨ **Mitochondrial use:** If used for the study of the function or activity of complete mitochondria, please use immediately. If it is used for mitochondrial protein analysis, please treat the mitochondrial sample with an appropriate lysis buffer. If it cannot be used in time, the mitochondria can be suspended by adding 50 μL of

stock solution and can be stored for 1 month at -80°C after flash freezing in liquid nitrogen.

Note: Frozen mitochondrial samples are not recommended for membrane potential detection, but they can be used for the detection of mitochondrial proteins or nucleic acids.