Mitochondrial Extraction Assay Kit

Catalog No: E-BC-E001 **Specification:** 50Assays/100 Assays

Note: ① All steps of mitochondria isolation were performed on ice box or at 4°C, and the solutionshould be place in ice water for pre-cooling before use.

② Use a glass homogenizer with tight clearance.

General information

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

Detection principle The principle of mitochondrial seperation and purification: after rupturing cells mechanically, the debris and huge organelles are removed by low-speed differential centrifugation, then purified mitochondria will be obtained by high-speed differential centrifugation.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 1 (100 Assays)	Storage
Reagent 1	Lysis Buffer	30 mL × 1 vial	60 mL × 1 vial	-20°C, 12 months
Reagent 2	Mito-Wash Buffer	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Store Buffer	7 mL × 1 vial	15 mL × 1 vial	-20°C, 12 months

Note: 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:

Double distilled water, PBS(0.01 M，pH 7.4)

Reagent preparation

Place all reagents in ice water for pre-cooling for at least 15 min after they have returned to the solution state before use.

Operation table

① Weight 0.05 g~0.1 g of fresh tissue and wash with PBS（0.01 M，pH 7.4） at 2-8℃ to remove blood. Blot the water with absorbent papper. Cut the tissue into pieces with scissors and place them into a pre-cooled 5 mL glass homogenizer. Add 0.5 mL of pre-cooled lysis buffer. Grind the tissue up and down in the ice bath for about 30-40 times.

② Transfere tissue homogenate to a 2 mL pre-cooled EP tube, centrifuge at 500×g at 4°C for 5 min.

③ Transferre the supernatant to a new 2 mL pre-cooled EP tube, centrifuge at 500×g at 4°C for 5 min.

④ Transferre the supernatant to a new 2 mL pre-cooled EP tube, centrifuge at 15000×g at 4°C for 15 min.

⑤ Discard the supernatant, resuspend the precipitate with 0.2 mL of pre-cooled mito-wash buffer, then centrifuge with 11000×g at 4°C for 10 min.

⑥ Discard the supernatant, resuspend the precipitate with 50-100 μL of pre-cooled store buffer. The prepared mitochondrial extract can be used immediately or be stored at -70°C, avoiding repeated freeze-thaw.