Plant Mitochondrial Extraction Assay Kit (Enzyme Method)

Catalog No: E-BC-E005 **Specification:** 50 Assays/100 Assays

Note: ① The centrifuge was shifted to I gear to reduce mitochondrial loss.
② Increasing the sample quality will not increase the yield of mitochondria in equal proportion, and it is recommended to process several small quality plant samples at a time during extraction.
③ Operate quickly and use purified mitochondrial samples in a timely manner.

General information

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

Detection principle In the first step, protoplasts were prepared by enzyme. In the second step, mitochondrial organelles were released by membrane breaking solution. In the third step, purified mitochondria were obtained by differential centrifugation.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Extraction Solution A	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months
Reagent 2	Extraction Solution B	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Extraction Solution C	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months
Reagent 4	Stock Solution	5 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months

Note: The pilot-scale dosage specification of the kit is calculated based on the reagent usage for a single extraction of 200-500 mg of plant 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:

Homogenizer, High-speed freezing centrifuge, 37°C constant temperature shaker

Reagents:

PBS(0.01 M，pH 7.4)

Materials:

Cell Strainer (100 μm)

Reagent preparation

Place all reagents in ice box before use.

Operation steps

① Take 200-500 mg of fresh tissue, wash and dry and remove leaf stems and veins. Cut the tissue into pieces with scissors and place them into a pre-cooled 2 mL EP tube.

② Add 1 mL of extraction solution A and homogenize at 50Hz for 1min at 4°C with a dounce homogenizer.

Note: The main purpose is to crush the plant tissue as finely as possible and maintain the integrity of the plant cell structure.

③ Centrifuge at 10000 × g for 10 min at 4℃ to remove supernatant.

④ Collect precipitate from step ③ and add 0.5 mL of extraction solution B. Incubate in a 37°C constant temperature shaker for 12~72 h to obtain the reaction solution.

Note: The incubation time is determined by the type of sample, growth condition and freshness. Fresh and tender leaves are easier to handle. For example, fresh and tender epipremnum aureum leaves can be treated for 15 hours.

⑤ The reaction solution from step ④ was filtered through a 100 μm cell strainer. The filter residue was rinsed with 1 mL of PBS (0.01 M, pH 7.4) and then filtered. The filtrate was centrifuge at 10000 × g for 10 min at 4℃ to remove supernatant.

⑥ Collect precipitate from step ⑤ and add 1 mL of extraction solution C. Use a pipetting gun to slowly blow the suspension and incubate in a 37°C constant temperature shaker for 20 min to obtain the reaction solution.

⑦ **Low-speed centrifugation:** Take the reaction solution from step ⑥ and centrifuge at 300 × g for 3 min at 4℃. Collect supernatant into a pre-cooled EP tube.

⑧ **Low-speed centrifugation:** Take the supernatant from step ⑦ and centrifuge at 1000 × g for 3 min at 4℃. Collect supernatant into a pre-cooled EP tube.

Note: To obtain higher purity of mitochondria, you can take the supernatant and centrifuge at 1000 × g for 3 min at 4 ℃. The supernatant can be used for the next operation. However, the amount of mitochondria extracted will decrease.

⑨ **High-speed centrifugation:** Take the supernatant from step ⑧ and centrifuge at 10000 × g for 15 min at 4℃ to remove supernatant.

⑩ **Mitochondrial washing:** Collect precipitate from step ⑨ and add 0.5 mL of pre-cooled PBS(0.01 M，pH

7.4). Use a pipetting gun to slowly blow the suspension and centrifuge at 10000 × g for 15 min at 4℃.

⑪ Mitochondrial collection: Remove supernatant carefully and the precipitate is the plant mitochondrial.

⑫ **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 precipitate. If it cannot be used in time, the mitochondria can be suspended by adding 50-100 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.