Tissue Mitochondria Isolation Kit

Catalog No.: abx090631

Size: 50-100 assays

Storage: Store all reagents at -20 °C for up to one year.

Introduction: Abbexa's Tissue Mitochondria Isolation Kit is a quick and convenient method for isolation of mitochondria from animal tissues. By isolating the mitochondria, non-mitochondrial cytoplasmic proteins are also obtained and can be used in further downstream applications. The purity of the obtained mitochondria is high, and the mitochondria often has the inner and outer membrane intact and is physiologically functional. After lysing with Mitochondria Lysis Buffer, the mitochondria can be used for SDS-PAGE, Western blot, Dimensional electrophoresis and Immunoprecipitation. One kit is sufficient for 50-100 assays if the weight per tissue is 50-100 mg.

Kit components

1. Reagent A (Mitochondria Isolation Reagent): 60 ml
2. Reagent B (Mitochondria Lysis Buffer): 20 ml
3. 100X Enzyme Inhibitor: 1 ml

Materials Required But Not Provided

1. Phosphate-Buffered Saline (PBS)
2. Distilled water
3. High-precision pipette and sterile pipette tips
4. Centrifuge and centrifuge tubes
5. Timer
6. Ice
7. Vortexer
8. Homogenizer

Notes

- The reagent volumes in the procedure assume a tissue weight of 50-100 mg. The reagent volumes can be adjusted accordingly if a higher tissue weight is used.
- All steps should be carried out on ice or at 2-8 °C.
- Fresh tissues should be used. Do not use frozen tissue samples.

Procedure

1. Put all the kit components on ice and allow to thaw. All reagents should be at 2-8 °C.
2. Calculate the volume of Reagent A and Reagent B required (steps 4 and 9). Before adding the reagents to the sample, dilute the 100X Enzyme Inhibitor 1/100 and add 1X Enzyme Inhibitor to both Reagent A and Reagent B. Mix and allow to stand for several minutes.
3. Weigh 50-100 mg of minced tissue and add to a 1.5 ml microcentrifuge tube. Wash the tissue with PBS.
4. Add Reagent A (containing Enzyme Inhibitor) at a ratio of 10 µl : 1 mg tissue (e.g. add 500 µl of Reagent A to 50 mg tissue). Homogenize on an ice bath 10 times.
5. Centrifuge the homogenate at 600 × g at 4 °C for 5 minutes. For higher purity, the tube can instead be centrifuged at 1,000 × g at 4 °C for 5 minutes, though this may result in a lower yield.

6. Carefully transfer the supernatant to a new microcentrifuge tube, then centrifuge at 11,000 × g at 4 °C for 10 minutes. For higher purity, the tube can instead be centrifuged at 3,500 × g at 2-8 °C for 10 minutes, though this may result in a lower yield.
7. Carefully collect the supernatant (which contains cytoplasmic proteins) to a new tube. The pellet contains the mitochondria.
8. (Optional) To purify the cytoplasmic proteins, centrifuge the collected supernatant at 12,000 × g at 4 °C for 10 minutes, then carefully collect the supernatant, which contains mitochondria-excluded cytoplasmic proteins.
9. For mitochondrial protein analysis, add 150-200 µl of Reagent B (containing Enzyme Inhibitor) to the pellet. After lysis, the mitochondria can be used in downstream applications such as SDS-PAGE, WB, IP and enzymatic activity detection. The protein concentration can be determined using a BCA Protein Assay Kit.
10. For dimensional electrophoresis analysis, the mitochondria should be lysed with an appropriate lysis buffer.