Mitochondrial DNA Isolation Kit
(Catalog #K280-50; 50 assays)

Store at –20°C

I. Introduction:

Mitochondria are semiautonomous organelles which functions in aging process, apoptosis, anti-HIV drugs, and cancers. Mitochondrial DNA (mtDNA) has a very high mutation rate and the mutations on mtDNA appear to be related to certain diseases such as diabetes, Alzheimer's disease, and muscle disorders. Isolation and quantification of mtDNA are often required to study the relationships between the diseases and mtDNA. The Mitochondrial DNA Extraction Kit provides convenient tools for isolating mtDNA from a variety of cells and tissues in high yield and purity, without contaminations from genomic DNA. The purified mtDNA can be used for a variety of studies such as enzyme manipulations, Southern blotting, cloning, PCR analysis, and amplifications.

II. Kit Contents:

Component	K280-50	Color code	Part no.
	50 assays	Cap color	Component
5X Cytosol Extraction Buffer	20 ml	WM	K280-50-1
Mitochondrial Lysis Buffer	1.8 ml	Purple	K280-50-2
Enzyme B Mix (lyophilized)	1 vial	Red	K280-50-3
TE Buffer	1.5 ml	Green	K280-50-4

III. Mitochondrial DNA Isolation Protocol:

A. General Consideration and Reagent Preparation:

• Read the entire protocol before beginning the procedure.
• After opening the kit, store Enzyme B Mix at –70oC. Store all other Buffers at 4oC.
• Make 1X Cytosolic Extraction Buffer by mixing 1 ml of the 5X buffer with 4 ml ddH2O.
• Add 275 µl of TE buffer to Enzyme B Mix, mix well, aliquot and refreeze immediately at –70oC. Stable for up to 3 months at –70oC.
• Be sure to keep all buffers on ice at all times during the experiment.

B. Mitochondrial DNA Isolation Protocol:

1. Collect cells (5 x 107) by centrifugation at 600 x g for 5 minutes at 4oC.

2. Wash cells with 5-10 ml of ice-cold PBS (not provided). Centrifuge at 600 x g for 5 minutes at 4oC. Remove supernatant.
3. Resuspend cells in 1.0 ml of 1X Cytosol Extraction Buffer.
4. Incubate on ice for 10 minutes.
5. Homogenize cells in an ice-cold dounce tissue grinder. Perform the task with the grinder on ice. We recommend 50-100 passes with the grinder; however, efficient homogenization may depend on the cell type.

Note: To check the efficiency of homogenization, pipette 2-3 µl of the homogenized suspension onto a coverslip and observe under a microscope. A shiny ring around the nuclei indicates that cells are still intact. If 70-80% of the nuclei do not have the shiny ring, proceed to step 6. Otherwise, perform 30-50 additional passes using the dounce tissue grinder. Excessive homogenization should also be avoided, as it can cause damage to the mitochondrial membrane which triggers release of mitochondrial components.
6. Transfer homogenate to a 1.5-ml microcentrifuge tube, and centrifuge at 700 x g for 10 minutes at 4oC. The step removes nuclei and intact cells (in pellet).
7. Transfer supernatant to a fresh 1.5-ml tube, and centrifuge at 10,000 x g for 30 minutes at 4oC.
8. Remove supernatant.
9. Resuspend the pellet in 1 ml 1X Cytosol Extraction Buffer and centrifuge at 10000 x g for 30 minutes at 4oC again.
10. Remove the supernatant. The pellet is isolated mitochondria.
11. Lyse the mitochondria in 30 µl of the Mitochondrial Lysis Buffer, keep on ice for 10 minutes.
12. Add 5 µl Enzyme B Mix and incubate at 50oC water bath for 60 min or longer until the solution becomes clear.
13. Add 100 µl absolute ethanol, mix and keep at –20oC for 10 minutes.
14. Centrifuge in microcentrifuge at top speed for 5 min at room temperature.
15. Remove the supernatant. The pellet is mitochondrial DNA.
16. Wash the DNA pellet 2 times with 1 ml of 70% ethanol. Remove the trace amount ethanol using pipet tip. Air dry for 5 min. (Note: Do not completely dry the DNA. It may be difficult to dissolve if it is completely dried.)
17. Resuspend the DNA in 20 µl TE buffer or water. Store the extracted DNA at –20oC for future use. (Note: Generally, 5-20 µg mtDNA can be generated for each isolation.)

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