PI-0105

Mitochondrial DNA Isolation Kit

(Sufficient for 50 isolations, Store at -20°C)

Introduction:

Mitochondria are membrane-bound organelles found in all eukaryotic cells. They are often referred to as the powerhouses of the cell. Their primary function is to produce ATP necessary for various cellular processes, including metabolism, growth, and movement. Dysfunction in mitochondria is associated with mitochondrial disorders including muscle weakness, fatigue, metabolic strokes, seizures, cardiomyopathy, arrhythmias, developmental or cognitive disabilities, diabetes mellitus etc. The Mitochondrial DNA Extraction Kit provides a simple, fast and reliable tool for isolating intact mitochondrial DNA from a variety of cells and tissue types without contamination from genomic DNA. The recovered mitochondrial DNA is compatible with various downstream applications including PCR, cloning etc.

Kit Components:

Extraction Buffer	100 ml	NM	PI-0105A
Mitochondrial Lysis Buffer	1.8 ml	Purple	PI-0105B
Proteinase	lyoph	Red	PI-0105C
Tris-EDTA Buffer	1.5 ml	Green	PI-0105D

User-Supplied Materials:

Ice-cold PBS Buffer
Absolute ethanol
70% ethanol
50°C water bath

Storage and Handling:

Store unopened kit at -20°C. Bring to room temperature before use. Place all buffers on and keep on ice while in use.
Extraction Buffer: Ready to use as supplied. Store at 4°C.
Mitochondrial Lysis Buffer: Ready to use as supplied. Store at 4°C.
Proteinase K: Reconstitute with 275 µl of Tris-EDTA Buffer. Divide into convenient aliquots and refreeze immediately at -80ºC.
Tris-EDTA Buffer: Ready to use as supplied. Store at 4°C.

Isolation Protocol:

1. Precool all materials and buffers at 0-4°C. Make sure water bath is at 50°C.
2. Centrifuge 5 – 10 x 10^6 cultured cells at 300 x g for 3 min at 4ºC to pellet cells. Remove the media carefully.
3. Wash the cells with ice-cold PBS and centrifuge at 300 x g for 5 min at 4ºC. Remove the supernatant.
4. Resuspend the cells in 1.0 ml of ice-cold Extraction Buffer on ice. Place on ice for 10 minutes.
5. Homogenize the cells on ice using an ice-cold Dounce tissue grinder with a loose pestle, using 50-100 passes with the tissue grinder.

Note: Efficient homogenization depends on the cell type. To check efficiency of homogenization, observe 2-3 µl of the homogenized suspension on a coverslip under a microscope. A shiny ring around the nuclei indicates that cells are still intact. If 75% of the nuclei do not have the shiny ring, homogenization has been sufficient, and you should proceed to the next step. If not, perform 30-50 additional passes using the Dounce homogenizer. Excessive homogenization should also be avoided, as it will damage the mitochondria.

6. Transfer the homogenate to a 1.5 ml microcentrifuge tube. Centrifuge at 1200 x g for 10 min at 4ºC to pellet the nuclei and cells debris.
7. Carefully transfer only the supernatant to a fresh 1.5 ml microcentrifuge tube, and centrifuge at 13,000 x g for 15 min at 4ºC.
8. Transfer the supernatant to a fresh tube. Centrifuge at 10,000 X g for 30 minutes at 4°C. Discard the supernatant.
9. Resuspend the pellet in 1 ml Extraction Buffer. Centrifuge at 10,000 X g for 30 minutes at 4°C. Discard the supernatant.
10. The pellet at this stage is the isolated mitochondria.
11. Add 30 µl of Mitochondrial Lysis Buffer to the pellet on ice, mix and place on ice for 10 minutes to lyse the isolated mitochondria.
12. Add 5 µl of Proteinase K and incubate in a 50ºC water bath for 1 -2 hours, checking every 30 minutes, until the solution becomes clear.
13. Add 100 µl absolute ethanol, vortex and place in a freezer at -20ºC for 10 min.
14. Centrifuge at 16,000 x g for 5 min at room temperature.
15. Remove the supernatant. The pellet is mitochondrial DNA.
16. Wash the mitochondrial DNA pellet two times by adding 1 ml of 70% ethanol, mix and centrifuge at 13,000 x g for 1 min at RT and repeat.
17. Remove the trace amount of ethanol using a pipet tip. Air dry for 5 min.

Note: Do not completely dry the DNA. It will be difficult to dissolve if it is completely dried.

18. Resuspend the mitochondrial DNA in 20 µl Tris-EDTA Buffer or water. Store the extracted mitochondrial DNA at -20ºC.

Note: 5 - 20 µg mtDNA is prepared per each isolation.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic applications.

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