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ISO 13485:2016 cGMP

Mitochondrial DNA isolation Kit

Catalog #: MT-ISO

User Manual
Last Revised: September 12th, 2024

Introduction

The mitochondrion is a double membraned cytoplasmic organelle that produces over 90% of the cell's chemical energy via respiration. The mitochondrial genome (mtDNA) is a circular, intron-free, double-stranded, haploid DNA strand 16.5 kb in length that encodes 37 genes. mtDNA has a very high rate of mutation, damage, and copy number. The variation in mtDNA significantly relates to certain diseases such as diabetes, Alzheimer's disease, and muscle disorders. Therefore, isolation of high quality mtDNA is required to study the relationship between these diseases and mitochondrial function.

The RayBio® Mitochondrial DNA Isolation Kit offers a simple and effective tool for isolating mtDNA (1.5 hours for 12 samples) from a variety of cells and tissues in high yield and purity, without contaminations from nuclear genomic DNA. Purified mtDNA can be used for a variety of studies such as enzyme manipulations, southern blotting, cloning, PCR analysis, and amplifications.

Storage / Stability

The Lysis bufer-1 and Lysis bufer-2 can be stored at 4°C for a period of 12 months. Store other components in a dry environment at 2 – 30ºC for up to 12 months. Kit must be used within the validity period to ensure correct performance.

Product Use

MT-ISO is for research use only. It is not approved for application in clinical or in vitro diagnostic procedures.

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Kit Components

Component	Catalogue #	Size	Quantity	Storage
Cell Lysis Bufer-1	MT-CLB1	30 mL/bottle	1 bottle	4 °C
Cell Lysis Bufer-2	MT-CLB2	3 mL/bottle	1 bottle	4 °C
mtDNA Lysis Buffer	MT-DLB	30 mL/bottle	1 bottle	RT
Protease K	MT-PK	0.4 mL/vial	1 vial	RT
Magnetic Bead Solution	MT-MAG	0.3 mL/vial	1 vial	RT
Binding Buffer	MT-BB	30 mL/bottle	1 bottle	RT
Wash Buffer A	MT-WB-A	30 mL/bottle	1 bottle	RT
Wash Buffer B	MT-WB-B	30 mL/bottle	1 bottle	RT
Wash Buffer C	MT-WB-C	30 mL/bottle	1 bottle	RT
Elution Buffer	MT-EB	3 mL/bottle	1 bottle	RT

Note: Do not mix reagents from different lots.

Additional Materials Required

- Sterile nuclease-free pipette tips (barrier tips recommended) and microfuge tubes
- 1X PBS buffer
- Microcentrifuge
- Magnetic Separator for Microcentrifuge Tubes (2 mL)
- Vortex Mixer
- Water bath or dry heat block
- 100% ethanol (30 mL)
- Precision pipettes to deliver 2 μL to 1 mL volumes
- Tissue homogenizer (Dounce tissue grinder) for tissue samples
- Refrigerated centrifuge with swing out rotor and refrigerated microcentrifuge

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Sample Preparation

1. Cell culture preparation:
    1. Collect 10^5-10^7 suspension cells or attached cells by trypsin digestion or cell scraper. Centrifuge at 4°C, 800 x g for 5 minutes in 2 mL, 15 mL or 50 mL prechilled tubes.
    2. Add ice cold 1mL 1X PBS and transfer cells to a 2 mL microcentrifuge tube. Wash the cells twice by centrifugation at 4°C, 800 x g for 5 minutes. Remove any remaining PBS carefully.
2. Tissue preparation:
    1. Cut tissue (10 – 100 mg) into small pieces, homogenize tissue with the Dounce tissue grinder on ice, and filter the single cells to remove the insoluble materials.

        Note: a) 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. b) Excessive homogenization should also be avoided, as it can cause damage to the mitochondrial membrane which triggers release of mitochondrial components.

    2. Add 1 mL ice cold 1X PBS and transfer cells to a 2 mL microcentrifuge tube. Wash the cells twice by centrifugation at 4°C, 800 x g for 5 minutes. Remove any remaining PBS carefully.

Assay Procedure

1. Re-suspend cells in 400 µL Cell Lysis Bufer-1 by pipetting up and down gently to disperse the cells. Then, incubate on ice for 15 minutes.
2. Add 50 µL Cell Lysis Bufer-2 to tubes containing cell suspension and mix gently. Incubate cells on ice for 2 minutes.
3. Centrifuge tubes at 4°C, 2000 x g for 5 minutes in microcentrifuge. Carefully transfer supernatant into new tubes and label it as mitochondrial fraction.
4. Add 5 µL Protease K and 400 µL mtDNA Lysis Buffer to the tube with mitochondrial fraction. Vortex to mix well, and heat to 56ºC for 10 minutes in a preheated water bath or dry heat block.
5. Add 550 µL Binding Buffer and then 500 µL 100% ethanol to the lysate. Tightly seal tubes and then vortex well to mix.
6. Add 5 µL Magnetic Bead Solution. Incubate at room temperature for 10 minutes. Vortex for 5 seconds every 2 minutes.

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7. Place the tube onto a magnetic separator for 2 minutes and discard the clarified liquid.
8. Add 0.5 mL Wash Buffer A to the beads, vortex well, and transfer bead solution to new 1.5 mL centrifuge tube. Place on magnetic stand for 2 minutes and discard the clarified liquid.
9. Add 0.5 mL Wash Buffer B, vortex well, briefly centrifuge at low speed. Place on magnetic stand for 2 minutes and discard the clarified liquid.
10. Add 0.5 mL Wash Buffer C, vortex well, briefly centrifuge at low speed. Place on magnetic stand for 2 minutes and discard the clarified liquid.
11. Open tube in clean environment (sterilized PCR hood) and dry for 2 minutes.
12. Once dry, add 20 – 50 µL Elution Buffer to the tube. Tightly seal tube and then vortex thoroughly.
13. Incubate sample at 56^{o}C for 3 minutes in water bath or dry heat block. Vortex for 5 seconds every minute.
14. Place the tube in magnetic stand for 2 minutes. Transfer clarified liquid containing extracted mtDNA to a new 1.5 mL centrifuge tube.
15. Extracted mtDNA can be used immediately or stored below -20^{o}C for 1-2 years.

Assay Procedure Summary

1. Prepare cell or tissue samples as instructed.
2. Cell lysis with Cell Lysis Bufer-1 for 15 min and Cell Lysis Bufer-2 for another 2 minutes.
3. mtDNA lysis with Protease K and mtDNA Lysis Buffer at 56^{o}C for 10 minutes.
4. mtDNA binding with the magnetic beads at RT for 10 minutes.
5. mtDNA washing with Wash Buffer A, B and C.
6. mtDNA elution with elution buffer.
7. mtDNA can be used or stored.

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Typical Data

mtDNA purification kits ensure high yields of high-quality DNA (up to 10 µg) free of contaminants of nuclear DNA and RNAs. Purified mtDNA has high quality metrics, including A260/A280=1.8~2.0 and A260/A230 > 2.0. The purified mtDNA is suitable for downstream applications such as end-point PCR, qPCR and library prep for NGS sequencing. It typically has a peak size of 16.6 kb, making this kit an excellent choice upstream of long-read sequencing platforms. Intra-assay coefficient of variation (CV) is less than 5%.

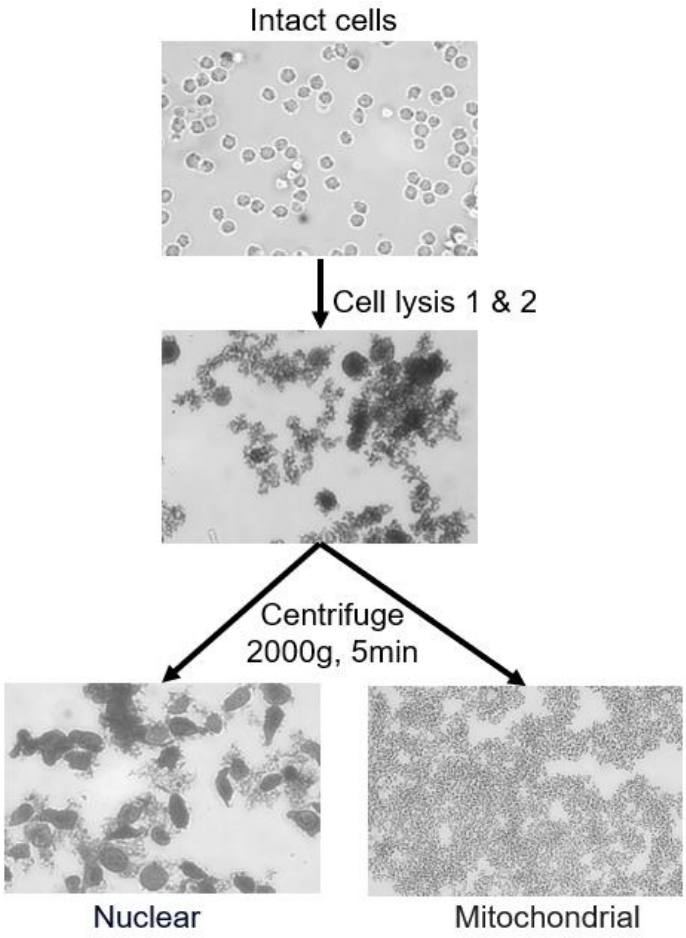

Figure 1. Workflow of mitochondrial extraction from intact cells. Mitochondria was stained with Janus Green B. There were no contaminants of nuclear DNA in the mitochondrial fraction.

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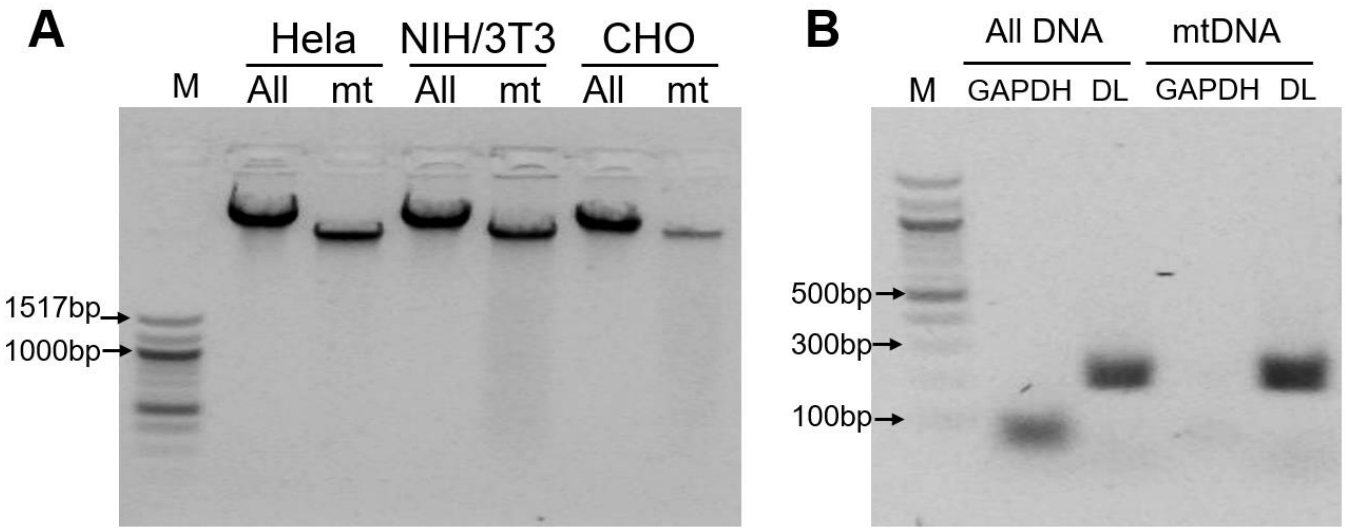

Figure 2. High-quality mtDNA isolation from Hela, NIH/3T3 and CHO cells. All genomic DNA were isolated using a commercial genomic DNA isolation kit and all mtDNA were isolated using the RayBio® Mitochondrial DNA Extraction Kit. **A**, All isolated DNA samples were loaded and run on a 1.0% agarose gel, using the 100bp DNA Ladder as a marker (M). **B**, Agarose gel run for gene amplifications (30 cycles) of the nuclear gene (GAPDH) and mitochondrial gene (D-loop, DL) in all human Hela genomic DNA (All DNA) and mtDNA samples. Results indicate mtDNA is of high-integrity and high-purity.

mtDNA/nuDNA	Hela	NIH/3T3	CHO
All DNA	1.00±0.37	1.00±0.74	1.00±0.70
mtDNA	1513±423	2400±1264	18174±7841

Table 1. The ratio of mtDNA and nuDNA for all genomic DNA (All DNA) and mtDNA in Hela, NIH/3T3 and CHO cells. qPCR was performed to amplify the nuclear gene (GAPDH) and mitochondrial gene (D-loop) in 10 ng DNA samples. Results indicate mtDNA is of high purity with little nuclear DNA contamination.