ab65321

Mitochondrial DNA Isolation Kit

Instructions for Use

For the rapid isolation of Mitochondrial DNA in various cell and tissue samples.

View kit datasheet: www.abcam.com/ab65321
(use www.abcam.cn/ab65321 for China, or www.abcam.co.jp/ab65321 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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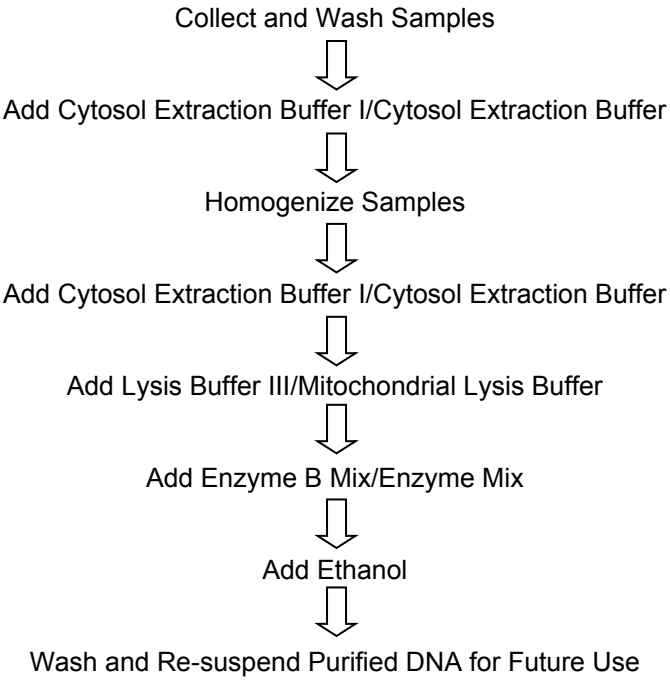
1. Overview

Mitochondria are semi-autonomous 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.

Abcam's Mitochondrial DNA Isolation Kit provides a convenient tool 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
5X Cytosol Extraction Buffer I/5X Cytosol Extraction Buffer	20 mL
Lysis Buffer III/Mitochondrial Lysis Buffer	1.8 mL
Enzyme B Mix/Enzyme Mix (Lyophilized)	1 vial
TE Buffer I/TE Buffer	1.5 mL

* Store kit at -20°C.

After opening the kit, store Enzyme B Mix/Enzyme Mix at -80°C. Store all other Buffers at 4°C.

Be sure to keep all buffers on ice at all times during the experiment.

Read the entire protocol before beginning the procedure.

CYTOSOL EXTRACTION BUFFER I/CYTOSOL EXTRACTION BUFFER: Make up 1X Cytosol Extraction Buffer I/1X Cytosol Extraction Buffer by mixing 1 ml of the 5X Cytosol Extraction Buffer I/5X Cytosol Extraction Buffer with 4 ml ddH_2O.

ENZYME B MIX/ENZYME MIX: Add 275 µl of TE Buffer I/TE buffer to Enzyme B Mix/Enzyme Mix, mix well, aliquot and re-freeze immediately at -80°C. Stable for up to 3 months at -80°C.

B. Additional Materials Required

- Centrifuge and microcentrifuge
- Pipettes and pipette tips
- Absolute ethanol
- Orbital shaker
- Dounce tissue grinder

4. Assay Protocol

1. Collect cells (5 x 10^6) by centrifugation at 600 x g for 5 minutes at 4°C.

2. Wash cells with 5-10 ml of ice-cold PBS (not provided). Centrifuge at 600 x g for 5 minutes at 4°C. Remove supernatant.

3. Re-suspend cells in 1.0 ml of 1X Cytosol Extraction Buffer I/1X Cytosol Extraction Buffer.

Note:

When using tissue samples use 3-fold more 1X Cytosol Extraction Buffer I/1X Cytosol Extraction buffer, so that the homogenized tissue will not be too sticky to remove the insoluble materials at low spin step. Use 10 – 100 mg of tissue

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.

Notes:

a) To check the efficiency of homogenization, pipette 2-3 µl of the homogenized suspension onto a cover-slip 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.

Note: Please pay particular attention to this important homogenization step and only proceed with the protocol once at least 80% of the cells are lysed.

b) 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 1200 x g for 10 minutes at 4**°C**. 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 4°C.

8. Remove supernatant.

9. Re-suspend the pellet in 1 ml 1X Cytosol Extraction Buffer I/1X Cytosol Extraction Buffer and centrifuge at 10000 x g for 30 minutes at 4°C again.

10. Remove the supernatant. The pellet is isolated mitochondria.

11. Lyse the mitochondria in 30 µl of the Lysis Buffer III/Mitochondrial Lysis Buffer, keep on ice for 10 minutes.

Note: When using tissue samples don't keep on ice for 10 minutes and directly go to step 12.

12. Add 5 µl Enzyme B Mix/Enzyme Mix and incubate at 50°C water bath for 60 min or longer until the solution becomes clear.

Note: When using tissue samples add 10 or 15 ul Enzyme B Mix/Enzyme Mix, then put in 50 degree C overnight. Enzyme B Mix/Enzyme mix will degrade all proteins and DNases.

13. Add 100 µl absolute ethanol, mix and keep at -20°C 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 pipette 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. Re-suspend the DNA in 20 µl TE Buffer I/TE buffer or water. Store the extracted DNA at -20**°C** for future use.

Note:

Generally, 5-20 µg mtDNA can be generated during each isolation.

FAQs

What is the temperature needed for the enzyme inactivation of enzyme mix?

Enzymes in the Enzyme B Mix/enzyme mix can be inactivated by heating at 95 °C for 10 min.

Is there a need to degrade the contaminated RNA in mitochondrial DNA with RNase in additional step after using this kit?

The lysis buffer has RNase added to it. Therefore, you don't need to add RNase additionally.

How do you determine the purity of mitochondrial DNA?

The circular mitochondrial DNA runs ~15-20 kDa on agarose gel, whereas genomic DNA runs much big size. The size difference can easily be used to differentiate between the two. You can try different enzymatic digestions also to see the difference.

Is it normal to see a smear on my gel?

Yes it can happen due to the cells containing high levels of endonuclease. You add endonuclease inhibitor into the buffer system at beginning homogenization step, where the DNA degradation may occur. In addition, it may help if you add 5 ul

Enzyme B Mix/Enzyme Mix immediately after lysing the mitochondria without keeping on ice 10 min. Decrease the time to lyse the mitochondria may decrease mtDNA degradation, since Enzyme B Mix/Enzyme Mix contains Proteinase K which will kill all endonucleases.

What is the mass at the bottom of the gel and how to get rid of it?

It is RNA. The Lysis Buffer III/mitochondrial lysis buffer contains Rnase. After step 15, when you collect the pellets of mitochondrial DNA, wash with the Lysis Buffer III/mitochondrial lysis buffer and precipitate again.

What is the Kb for Mitochondrial DNA?

The Kb of mitochondrial DNA on agarose gel runs ~16-20.

Do you have any information about the expected mtDNA yield when 10 mg of tissue are extracted?

~5 - 20 µg mtDNA can be isolated from each mg of tissue.

Technical Support

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