

# 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](http://www.abcam.com/ab65321)

(use [www.abcam.cn/ab65321](http://www.abcam.cn/ab65321) for China, or [www.abcam.co.jp/ab65321](http://www.abcam.co.jp/ab65321) for Japan)

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

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# 1. Overview

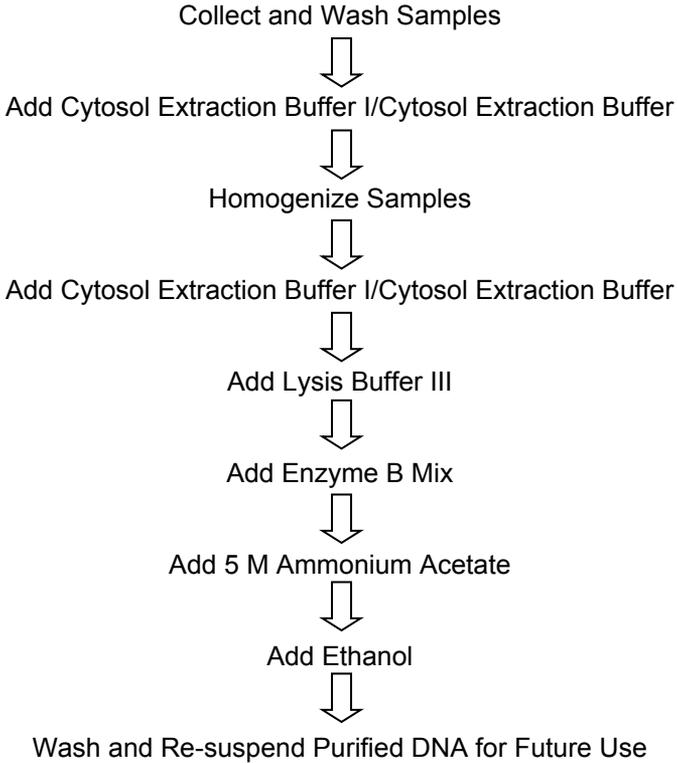
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Mitochondria are semi-autonomous organelles which functions in aging process, apoptosis, anti-HIV drugs, and cancers. Mitochondrial DNA (mtDNA) has a very high mutation rate and the mutations on mtDNA appear to be related to certain diseases such as 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

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### 3. Components and Storage

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#### A. Kit Components

Item	Quantity
5X Cytosol Extraction Buffer I	20 mL
Lysis Buffer III	1.8 mL
Enzyme B Mix	1 vial
TE Buffer I	1.5 mL
5 M Ammonium Acetate	2 x 1.25

PLEASE NOTE: 5X Cytosol Extraction Buffer I was previously labelled as 5X Cytosol Extraction Buffer, and Enzyme B Mix as Enzyme Mix (Lyophilized), and Lysis Buffer III as Mitochondrial Lysis Buffer, and TE Buffer I as TE Buffer. The composition has not changed.

\* Store kit at -20°C.

After opening the kit, store Enzyme B Mix and other buffers at -20°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 with 4 ml ddH<sub>2</sub>O.

ENZYME B MIX: Add 275 µl of TE Buffer I to Enzyme B Mix, mix well, aliquot and re-freeze immediately at -20°C. Stable for up to 3 months at -20°C.

5 M AMMONIUM ACETATE: If precipitate forms, gently heat at 37°C and mix until salt returns into solution.

## **B. Additional Materials Required**

- Centrifuge and microcentrifuge
- Pipettes and pipette tips
- Absolute ethanol
- Orbital shaker
- Dounce tissue grinder
- Older lots of this kit will require a user supplied 5 M Ammonium Acetate Solution

## 4. Assay Protocol

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1. Collect cells ( $5 \times 10^6$ ) 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:**

For tissue, use the recommended optimal ratio of cytosol extraction buffer 1:5-1:10 w/v, 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  $\mu\text{l}$  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  $\mu$ l of the Lysis Buffer III, 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  $\mu$ l Enzyme B 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  $\mu$ l Enzyme B Mix, then put in 50 degree C overnight. Enzyme B Mix will degrade all proteins and DNases.

**13.** Add 35  $\mu$ l of 5 M Ammonium Acetate and mix (1 Volume equivalent)

**14.** Add 140  $\mu$ l absolute ethanol (2 Volumes) mix and keep at -20°C for a minimum of 30 minutes- 2 hours. DNA at this step could be stored overnight with no impact to DNA integrity.

**15.** Centrifuge in microcentrifuge at top speed for 15 min at room temperature.

**16.** Carefully remove the supernatant by pipetting. The pellet is mitochondrial DNA.

**Note:** Do not disturb pellet. A 200  $\mu$ l pipette or less is recommended as a 1000  $\mu$ l tip makes losing the pellet more likely.

17. Wash the DNA pellet 2 times with 200  $\mu$ l of 70% ethanol by adding the ethanol, briefly centrifuging and carefully removing the supernatant. 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.

18. Re-suspend the DNA in 20  $\mu$ l TE Buffer I or water. Store the extracted DNA at -20°C for future use.

**Note:**

Generally, 5-20  $\mu$ g 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 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 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 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 contains RNase. After step 15, when you collect the pellets of mitochondrial DNA, wash with the Lysis Buffer III and precipitate again.

### **What is the Kb for Mitochondrial DNA?**

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



## **Technical Support**

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