Product Information

Mitochondrial DNA Isolation Kit

I. Kit Contents:

Components	1 Kit	Identifier
5X Cytosol Extraction Buffer	20 ml	
Mitochondrial Lysis Buffer	1.8 ml	Purple
Enzyme Mix (lyophilized)	1 vial	Red
TE Buffer	1.5 ml	

II. Introduction:

The high mutation rate of Mitochondrial DNA (mtDNA) has been linked to some diseases such as Alzheimer's diseases, diabetes and muscle disorders. Isolation and quantification of mtDNA is an important method for studying the links between mtDNA and diseases. This kit offers a simple and effective tool for isolating mtDNA from various cells and tissues with high without genomic DNA contaminations. The purified mtDNA can be applied into enzyme manipulations, cloning and PCR etc.

III. General Consideration and Reagent Preparation:

Read the entire protocol before beginning the procedure.

After opening the kit, store Enzyme B Mix at –70°C, Store all other Buffers at 4°C.

Make 1X Cytosolic Extraction Buffer by mixing 1 ml of the 5X buffer with 4 ml ddH_2O.

Add 275 µl of TE buffer to Enzyme B Mix, mix well, aliquot and refreeze immediately at –70°C, Stable for up to 3 months at –70°C.

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

IV. Mitochondrial DNA Isolation Protocol:

1. Collect cells (5 x 10^7) by centrifugation at 600 x g for 5 min at 4°C.
2. Wash cells with 5 - 10 ml of ice-cold PBS (not provided). Centrifuge at 600 x g for 5 min at 4°C Remove supernatant.
3. Resuspend cells in 1.0 ml of 1X Cytosol Extraction Buffer.
4. Incubate on ice for 10 min.
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 min 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 min at 4°C.
8. Remove supernatant.

9. Resuspend the pellet in 1 ml 1X Cytosol Extraction Buffer and centrifuge at 10000 x g for 30 min at 4°C.

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

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

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

13. Add 100 µl absolute ethanol then mix and keep at –20°C for 10 min.

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 –20°C for future use. (Note: Generally, 5 - 20 µg mtDNA can be generated with each isolation.)

Frequently Asked Questions:

1. 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.

2. I got a smear pattern, begin nearly 22 KDa. Why?

It might 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 µl 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 contains Proteinase K which will kill all endonucleases.

3. Does this kit work with tissues?

Tissues are much more dirtier than cultured cells, and very sticky after homogenization. There will be genomic DNA contamination in the mDNA prep. Also mDNA may get partially degraded by DNases. You need to do some modifications to isolate mDNA from tissue: 1. Use 3 fold more 1x cytosol Extraction buffer, so that the homogenized tissue will not be too sticky to remove the insoluble materials at low spin step. 2. Do step 11 without keep on ice for 10 min, and directly go to step 12, add 10 or 15 ul Enzyme B Mix, then put in 50 degree C overnight. Enzyme B mix will degraded all proteins and DNases.

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

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

5. What is the mass above 16 Kb on the Gel?

It is genomic DNA.

6. What is the Kb for Mitochondrial DNA?

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

7. 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.

8. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

9. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

10. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

11. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

12. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

13. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

14. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

15. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

16. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, It is recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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