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Mitochondrial DNA Extraction Kit

Product Number: MDE01B

Shipping and Storage

This reagent kit is stored as a whole at 2-8℃ for one year, with DNASE I and RNase A stored at -20℃. Before use, add 600ul of DNA enzyme reaction solution to 12mg DNASE I, and store at -20℃ for three months after packaging. If the dissolved DNASE I is stored for more than three months, its activity may decrease, affecting the removal of nuclear DNA. Please order DNASE I yourself. Buffer ML should be stored at room temperature before use. If there is any sediment, it can be dissolved in a 37℃ water bath without affecting its use.

Components

Components	MDE01B (50T)	MDE01B (100T)
DNase I	12mg	12mg*2
RNase A	0.5ml	0.5ml*2
Buffer LY	50 mL	100 mL
Buffer MC	25 mL	50 mL
DNA enzyme reaction solution	6ml	12ml
Buffer ML	10ml	20ml
Buffer PP	7.5ml	15ml
Buffer NAP	0.5ml	1ml
Buffer TE	25ml	50ml

Description

The key to mitochondrial DNA extraction is to remove nuclear DNA as much as possible. This reagent kit uses differential centrifugation to obtain relatively pure mitochondria, and then uses multiple steps such as DNA enzyme digestion and lysis buffer system to remove nuclear DNA, finally obtaining pure mitochondrial DNA. Can be used for experiments such as PCR that require high purity.

This kit is used to isolate intact and purified mitochondria from animal cells or tissues. Suitable for the extraction and preparation of mitochondrial DNA from animal soft tissues (liver or brain tissue), hard tissues (muscle), and cultured cells. Not suitable for extracting plants and other specimens.

Protocol

Preparation: Add 600μl (50T) or 1100μl (100T) of DNA enzyme reaction solution to DNASE I, divide appropriately, and store at -20℃. Buffer ML should be stored at room temperature. If there is a precipitate dissolved in a 37℃ water bath, the centrifuge temperature should be lowered to 4℃(2-8℃). If there is no low-temperature centrifuge, it can also be centrifuged at room temperature and the centrifugation time should be changed from 10 minutes to 5 minutes. However, the quality and yield of the resulting DNA may be affected to some extent.

1. **Sample processing**

1.1. **Tissue homogenization:** Weigh 100-200 mg of fresh tissue such as liver, brain, myocardium, etc., wash with PBS or physiological saline, wash with blood and water, absorb with filter paper, cut into small pieces with scissors, and place them in a small capacity glass homogenizer. Add 1.0 mL of pre cooled buffer LY and grind the tissue up and down in a 0℃ ice bath 20 times;

1.2. **Cultivate cell homogenate:** digest cells, wash with PBS, collect cells by centrifugation at 800×g for 5-10 minutes. Count. Each extraction requires 5×10^7 cells. Add 1.0 mL of buffer LY pre cooled with ice to resuspend the cells, transfer the cell

suspension to a small volume glass homogenizer, and grind in an ice bath at 0℃ for 30-40 times;

2. Transfer the tissue or cell homogenate to a centrifuge tube, centrifuge at 4℃, 1000×g for 5 minutes;
3. Take the supernatant and add it to a new centrifuge tube. Centrifuge again at 4℃ for 5 minutes at 1000×g.
4. Take the supernatant and add it to a new centrifuge tube. Centrifuge at 4℃ and 12000×g for 10 minutes. The supernatant after centrifugation contains cytoplasmic components, from which cytoplasmic proteins can be extracted. Transfer the supernatant to a new centrifuge tube, and the mitochondria settle at the bottom of the tube;
5. Add 0.5 mL of Buffer MC to resuspend mitochondrial precipitate, centrifuge at 4℃ for 5 minutes at 1000×g;
6. Take the supernatant and add it to a new centrifuge tube. Centrifuge at 4℃ and 12000×g for 10 minutes. Abandon the supernatant, and high-purity mitochondria precipitate at the bottom of the tube;
7. Add 100μl of DNA enzyme reaction solution to resuspend mitochondria, blow well, then add 10μl of DNASE I solution (see preparation work), mix well, and take a water bath at 37℃ for 10 minutes. This step involves digesting the nuclear DNA adsorbed on the surface of mitochondria. Centrifuge at 4℃ and 12000×g for 5 minutes. Discard the supernatant as much as possible, add 200μl of TE resuspended mitochondrial precipitate, centrifuge at 4℃ and 12000×g for 5 minutes, and wash off any remaining DNA enzymes.
8. The obtained precipitate was resuspended with 200μl Buffer TE, and 10μl RNase A was added. 200μl Buffer ML was added, gently mixed (not blown), left to stand for 1-2 minutes, and then 150μl Buffer PP was added, quickly mixed. Centrifuge at 4℃ and 12000×g for 5 minutes. This step can further remove nuclear DNA.
9. Take the supernatant and add it to a new centrifuge tube (if used for enzyme digestion analysis, this step can be added: choose an equal volume of phenol chloroform isoamyl alcohol 25:24:1 to extract once, then extract once with chloroform, or directly extract twice with chloroform. Generally, this step can remove some trace proteins and sugars, but it will cause loss of mitochondrial DNA, so it can be saved when used for PCR and does not affect subsequent experiments). Add 0.6 times the volume of isopropanol (if isopropanol is not available, 2.5 times the volume of ethanol can be added to precipitate DNA, if the centrifuge tube is too full, it can be divided into two tubes) and 5-10μl of nucleic acid buffer NAP, mix well, and precipitate at -20℃. About half an hour (this step can be saved), centrifuge at 4℃ and 12000×g for 10 minutes.
10. Discard the supernatant and add 1ml of 70% ethanol for cleaning. Centrifuge at 4℃ and 12000×g for 5 minutes. Wash again with 70% ethanol.
11. Discard the supernatant and centrifuge for another 1 minute to remove it. Do not touch the bottom of the tube, open the lid and let it dry for about 5-10^5 minutes.
12. Add 20-30μl buffer TE, lightly tap the bottom of the tube, and take a water bath at 37℃ for 5 minutes to dissolve mitochondrial DNA.
13. Perform DNA electrophoresis detection and store at -20℃ for the next experiment.

Note

1. To ensure the acquisition of complete and as many mitochondria as possible, the homogenization conditions should be as follows: firstly, the entire process should be operated at low temperature. The second is speed. Thirdly, if conditions permit, the fragments after homogenization can be observed under a microscope. Compared with tissue blocks, it is difficult to break the walls of cultured cells, especially adherent cells, when using a glass homogenizer for homogenization. Therefore, it is necessary to use a small capacity glass homogenizer and a tightly spaced pestle to grind the cultured cells up and down.
2. The content of mitochondrial DNA itself is very low. If it cannot be detected by electrophoresis, the sample size can be increased, and a smaller amount of TE can be used to dissolve and precipitate, or directly enter PCR detection.
3. Calculate the correct centrifugal speed using centrifugal force g, and different centrifuges can accurately calculate the centrifugal speed based on this.

Calculate

Generally, low-temperature centrifuges have centrifugal force display. If not, the following formula can be used for simple conversion.

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Conversion of rotational speed and centrifugal force

$G = 1.11\times(10^{-5})\times R\times[rpm]^2$

G is the centrifugal force, usually expressed as a multiple of g (gravitational acceleration);

$[rpm]^2$ is the square of the rotational speed; R is the radius, measured in centimeters.