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Blood Mitochondrial DNA Extraction Kit(Enzyme)

Product Number: DNK51

Shipping and Storage

2-8℃, among which DNase I and RNase A need to be stored at -20℃ with a shelf life of one year.

Components

Component	50T	100T
DNase I	12mg	2×12mg
RNase A	500μL	2×500μL
10×Blood lysis buffer	100mL	2×100mL
Cell lysis buffer	50mL	100mL
Mt Wash buffer	25mL	50mL
DNAase reaction buffer	6mL	12mL
Mt lysis buffer	10mL	20mL
Buffer PP	7.5mL	15mL
Poly Carrier	0.5mL	1mL
Buffer TE	25mL	50mL

Description

This kit can be used to isolate complete and purified mitochondrial DNA from blood. The key to extracting mitochondrial DNA 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.

Note

1. Before use, add 600μL of DNAse reaction solution to DNase I, dissolve and package it, and store it at -20℃ for three months. If the dissolved DNASE I exceeds three months, its activity may decrease. Please order DNase I yourself.
2. Mt lysis buffer should be stored at room temperature before use. If there is precipitation, it can be dissolved in a 37℃ water bath without affecting its use.
3. To ensure the acquisition of complete and as many mitochondria as possible, the homogenization conditions should be as follows: low-temperature operation throughout the process; Quickly; If possible, the homogenized fragments can be observed under a microscope.
4. 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 the precipitate, or it can be directly detected by PCR.
5. Calculate the correct centrifugal speed using the centrifugal force g, and different centrifuges can accurately calculate the centrifugal speed based on this. Generally, low-temperature centrifuges have centrifugal force display. If not, the following formula can be used for simple conversion.

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

   G is 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.

Preparation

1. Add 600μL of DNA enzyme reaction solution to 12mg DNase I, appropriately package and store at -20℃.
2. Red blood cell lysis buffer (10X) is diluted 10 times with double distilled water to form the working solution (if the specimen is

nucleated red blood cell blood, this reagent is not needed and physiological saline or PBS should be prepared).

3. Mt lysis buffer is stored at room temperature and dissolved in a 37℃ water bath if there is precipitation.
4. The temperature of the centrifuge drops to 4℃ (2-8℃). If there is no low-temperature centrifuge, it can also be centrifuged at room temperature, and all centrifugation times of 10 minutes can be changed to 5 minutes. However, the quality and yield of the resulting DNA will be affected to some extent.

Protocol

1. Blood processing:

Blood requirements: Non heparin sodium anticoagulant, preferably using fresh blood. For old blood, the yield of mitochondrial DNA is greatly reduced due to the damage caused by ice crystals to the cell nuclear and mitochondrial membranes during the freezing process.

1.1. For whole blood without nucleated red blood cells (such as mammalian peripheral blood), take about 3-5mL of blood (divided into several tubes appropriately), add 3 times the volume of blood cell lysis buffer (diluted working solution, the same below), mix well, centrifuge at 800 × g for 5 minutes to collect white blood cells. Add 1-2mL of blood cell lysis buffer (diluted working solution), blow and resuspend white blood cells, combine in one tube, centrifuge at 800 × g for 5-10 minutes to collect white blood cells. If white blood cells have visible red color at this time, they can be washed again with a small amount (0.5mL) of red blood cell lysis buffer.

1.2. For whole blood with nucleated red blood cells (such as poultry whole blood), take about 300-500μL of whole blood, centrifuge at 800 × g for 5-10 minutes to collect the whole cells, wash twice with physiological saline or PBS, and leave a precipitate to remove the supernatant. Please use a pointed suction head to blow during cleaning.

2. Add 1.0mL of ice pre cooled Cell lysis buffer to the collected cells and resuspend them. Transfer the cell suspension to a small capacity glass homogenizer and grind it 20-40 times in a 0℃ ice bath.
3. Transfer the homogenate to a centrifuge tube, centrifuge at 4℃ and 1000 × g for 5 minutes.
4. Take the supernatant, add it to a new centrifuge tube, centrifuge again at 4 ℃ and 1000 × g for 5 minutes (a total of two centrifugation cycles, complete removal of nuclei).
5. Take the supernatant, 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 allow mitochondria to settle at the bottom of the tube.
6. Add 0.5mL of Mt Wash buffer to the mitochondrial precipitate and resuspend it. Centrifuge at 4℃ and 1000 × g for 5 minutes.
7. Take the supernatant, add it to a new centrifuge tube, centrifuge at 4℃ and 12000 × g for 10 minutes. Discard the supernatant and deposit high-purity mitochondria at the bottom of the tube.
8. Add 100μL of DNAase reaction buffer to resuspend mitochondria, blow and beat evenly, then add 10μL of DNASE I solution (see preparation work), mix well, and water bath at 37℃ for 10 minutes. This step involves digesting the nuclear DNA adsorbed onto the surface of mitochondria. Centrifuge at 12000 × g for 5 minutes at 4℃. Discard the supernatant as much as possible, add 200μL of TE to resuspend mitochondrial precipitate, centrifuge at 4℃ and 12000 × g for 5 minutes, and wash away residual DNA enzymes.
9. The obtained precipitate was resuspended in 200μL Buffer TE for mitochondrial precipitation, and 10μL RNase A was added. Add 200μL of Mt lysis buffer, gently mix (without blowing), let it sit for 1-2 minutes, then add 150μL of Buffer PP and mix quickly. Centrifuge at 12000 × g for 5 minutes at 4℃. This step can further remove nuclear DNA.
10. Take the supernatant and add it to a new centrifuge tube (if used for enzyme digestion analysis, this step can be added: extract once with equal volume of phenol chloroform isoamyl alcohol 25:24:1, then extract once with chloroform, or directly extract twice with chloroform. Generally speaking, this step can remove some trace proteins and sugars, but it will cause loss of mitochondrial DNA, so it can be saved for PCR and does not affect subsequent experiments). Add 0.6 times the volume of isopropanol (if there is no isopropanol, 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 poly carrier, mix well, precipitate at -20℃ for about half an hour (this step can be saved), 4℃. Centrifuge at 12000 × g for 10 minutes.

11. Discard the supernatant, add 1mL of 70% ethanol for cleaning, centrifuge at 4℃ and 12000 × g for 5 minutes. Repeat washing with 70% ethanol once.
12. Discard the supernatant, centrifuge again for 1 minute and aspirate the supernatant without touching the bottom of the tube. Open the lid and let it dry for about 5-10 minutes.
13. Add 10-20μL of Buffer TE, gently tap the bottom of the tube, and immerse in a 37℃ water bath for 5 minutes to dissolve mitochondrial DNA.
14. Perform DNA testing and store at -20℃ for the next experiment.

Special note

The above methods can remove nuclear DNA relatively completely (to a level that cannot be detected by PCR), but the process is complex and there is a lot of mitochondrial DNA loss. The following are simple methods, but mitochondrial DNA can also be well mentioned. With appropriate lysis time, it can also remove nuclear DNA (to a level that cannot be detected by electrophoresis).

1. Routine blood processing (red blood cell lysis or whole blood washing)
2. Resuspend whole cells in 200μL Buffer TE, add 10μL RNase A, add 200 μ L Mt lysis buffer, gently mix (without blowing), let it sit for 2-3 minutes, then add 150μL Buffer PP and mix quickly. Centrifuge at 12000 × g for 5 minutes at 4℃.
3. Take the supernatant, proceed to step 10 above, extract with chloroform, and precipitate with ethanol.