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Blood Mitochondrial DNA Extraction Kit

Product Number: DNK49

Shipping and Storage

Transport and store at room temperature, with a shelf life of one year.

Components

Component	50T	Package
10×Blood Lysis Buffer	15mL	15mL natural color bottle
Frozen Blood Lysis Buffer	250mL×2	250mL natural color bottle
White Cell Wash Buffer	100mL	125mLnatural color bottle
Buffer A	7.5mL	10mLnatural color bottle
Buffer B	15mL	15mLBrown glass bottle
Buffer C	12mL	15mLnatural color bottle
Buffer P	50mL	60mLBrown glass bottle
BufferCarrier (IPA type)	50mL	60mL natural color bottle
Buffer EB	1mL	10mL natural color bottle

Description

This product is developed based on our company's blood DNA extraction product and is specifically used as a reagent for extracting mitochondrial DNA (mtDNA) from fresh or frozen human anticoagulant whole blood. The classification of cells in human blood is as follows: the ratio of red blood cells to white blood cells in human blood is 1000-6000:1, but there is no DNA in red blood cells, so the mitochondrial DNA mainly comes from white blood cells. White blood cells are divided into two types: polymorphonuclear leukocytes (PMNs) and peripheral blood mononuclear cells (PBMCs). The former includes neutrophils, which account for 50-70% of the total white blood cells, eosinophils, which account for 0.5-5%, and basophils, which account for 0-1%. The latter includes lymphocytes (T cells, B cells, and NK cells) that account for 20-40% of the total white blood cell count, monocytes (1-8%), and dendritic cells (0.3-0.9%).

Features

1. High DNA yield. If the sample is fresh blood, the highest mitochondrial DNA yield can reach 1μg/mL fresh blood. If the sample is frozen blood, the difference in DNA yield is significant.
2. The DNA is pure, with OD260/OD280 between 1.8-2.0.
3. It can be directly used for PCR, enzyme digestion, hybridization and other experiments.
4. Applicable to mammalian blood, not applicable to blood of other animals.
5. This product is sufficient for 50 uses and can handle 3mL of anticoagulant blood each time.
6. Each 1E7 white blood cell can be purified to 40-80ng mtDNA.
7. This product can only be used for scientific research.

Self provided reagents

1.5mL plastic centrifuge tube, 75% ethanol.

Protocol

1. Separation of White Blood Cells from Fresh Blood

This kit only provides white blood cell preparation reagents based on whole blood red blood cell lysis method. Due to the many methods available (such as natural sedimentation, differential sedimentation, Ficoll separation, ammonium chloride separation,

etc.) for preparing white blood cells from fresh anticoagulant blood, users can choose their own method for preparing white blood cells. If you choose to prepare white blood cells using self prepared reagents, this step can be skipped.

1.1. Transfer 3mL of fresh human anticoagulant blood into a clean 15 mL plastic centrifuge tube.

1.2. Add 0.3mL of 10×Blood Lysis Buffer in a ratio of 10:1 to achieve a final concentration of 1 ×; Gently invert and mix several times, let it stand for 10 minutes (during this period, gently invert and mix several times). Attention: Once the Blood Lysis Buffer is opened, it is very easy to contaminate bacteria. It is best to store the unused part at -20℃. Before the next use, dissolve it thoroughly and shake well.

1.3. Centrifuge at 500 × g for 8 minutes at room temperature to precipitate white blood cell nuclei. The supernatant will appear red, which is the color of hemoglobin released after red blood cell lysis. Discard the supernatant.

1.4. Add 1 mL of White Cell Wash Buffer to the precipitate, gently resuspend the white blood cell precipitate to obtain a white blood cell resuspension.

1.5. Centrifuge at 500 × g for 8 minutes and discard the supernatant. Add 1mL of White Cell Wash Buffer to the precipitate, gently resuspend the white blood cell precipitate to obtain a white blood cell resuspension.

1.6. Perform cell counting on the white blood cell suspension. Due to the significant differences in human leukocyte concentration (15-20 × E9 cells/L for newborns, 11-12 × E9 cells/L for children aged half to two, 8 × E9 cells/L for children aged 4-14, and 4-10 × E9 cells/L for adults, where L refers to blood volume), it is necessary to start using a fixed cell count from the next step.

1.7. Transfer 1E7 white blood cells to a 1.5mL plastic centrifuge tube, centrifuge at 1500 × g for 4 minutes, discard the supernatant, and white blood cells will form a precipitate.

1.8. The obtained leukocyte precipitate can be directly used for DNA extraction or stored at -20 ℃ for later use.

2. Extraction of mitochondrial DNA

2.1. Add 150μL of Buffer A to the white blood cell precipitate, blow and mix several times with a pipette, and then place on ice.

2.2. Add 300μL of Buffer B, gently invert and mix 10 times. This solution is blue, and after mixing, the blue color of the mixture will become uniform. Place accurately on ice for 8 minutes.

2.3. Add 225μL of Buffer C, gently invert and mix 10 times, and the mixture will become colorless. Place accurately on ice for 25 minutes. If the color is abnormal, the experiment needs to be stopped and the cause analyzed.

2.4. Centrifuge at 13000 × g for 20 minutes at 4℃.

2.5. Transfer the supernatant (containing mitochondrial DNA) into a new 1.5mL plastic centrifuge tube.

2.6. Add an equal volume of Buffer P and vortex for 3 minutes to mix well.

2.7. Centrifuge at room temperature of 13000 × g for 5 minutes, transfer the colorless aqueous phase to a new 1.5mL plastic centrifuge tube, do not take the blue liquid.

2.8. Add an equal volume of BufferCarrier (IPA type) to the colorless supernatant, invert 10 times and mix well. Centrifuge at room temperature of 13000 × g for 10 minutes to remove the supernatant, being careful not to touch the trace precipitate at the bottom of the tube.

2.9. Add 1mL of self prepared 75% ethanol, mix well, centrifuge at room temperature of 13000 × g for 3 minutes, remove the supernatant, and be careful not to touch any trace sediment at the bottom of the tube. Repeat the previous step once.

2.10. Centrifuge briefly to remove approximately 50μL of residual solution (this step is crucial and should not be omitted).

2.11. Let it dry briefly for half a minute, then add 50μL of DNA eluent (genome specific) to dissolve the DNA. Generally, 40-80ng/mL of DNA can be obtained.

2.12. The dissolved DNA can be immediately used for subsequent OD detection, enzyme digestion, PCR or other experiments, or it can be stored at -20℃ for a long time. If more mtDNA is needed, the rolling ring amplification method can be used for amplification.

3. Purification of mitochondrial DNA from frozen blood

3.1. Freeze the frozen blood in a 37℃ water bath.

3.2. Transfer 2mL of anticoagulant fresh blood into a clean 15mL plastic centrifuge tube.

3.3. Add 4 times the volume (8mL) of frozen blood lysis buffer , gently shake on a desktop shaker for 10 minutes, and mix well. Attention: Once the frozen blood lysis buffer is opened, it is very easy to contaminate bacteria. The unused part is best stored at -20℃. Before the next use, it should be dissolved and shaken thoroughly.

3.4. Centrifuge at 1000 × g at 4℃ for 4 minutes and transfer the supernatant to a new centrifuge tube.

3.5. Centrifuge the supernatant at 12000 × g at 4℃ for 10 minutes, discard the supernatant, and retain the precipitate.

3.6. Follow steps 1.7-2.11 for mitochondrial DNA purification. Attention: Due to the damage caused by ice crystals on the cell nuclear and mitochondrial membranes during the freezing process of old blood, white blood cells obtained by centrifugation cannot be washed. At the same time, due to many mitochondria being punctured by ice crystals, mitochondrial DNA has been lost, resulting in a lower yield than fresh blood mitochondrial DNA. The specific reduction depends on the length of freezing time.