For research use only Version Number: 1.0-1404

Blood DNA Mini Kit

For genomic DNA purification from whole blood using <1ml blood

Kit composition	DE-05111 50 T
Buffer BL1	15 ml
Buffer BL2 *	15 ml
Buffer DC	100 ml
Buffer PW *	25 ml
Buffer WB1	15 ml
Buffer EB	10 ml
Foregene Protease Plus	1.25 ml
DNA-Only Column	50
Manual	1

*: Buffer BL2 and Buffer PW contain irritating liquid salt. Please wear gloves and take relevant protective measures when operating.

Introduction

This kit adopts new Foregene Protease Plus and unique BL1 and BL2 buffer systems, which can completely digest anticoagulant blood samples in a short period of time, thus avoiding DNA degradation to the maximum degree and obtaining the maximum amount of genomic DNA.The rapid blood processing system and the simple centrifugal column operation greatly simplify the extraction of blood genome, making the genomic DNA of high quality and high purity can be obtained within 40 minutes.

The DNA-only silica gel membrane used in the centrifuge column of this kit is a new type of material unique to our company, which can adsorb DNA efficiently and specifically, and can remove RNA, impurities, proteins, ions and other organic compounds in cells to the maximum extent.The obtained DNA fragments are large, high purity, stable and reliable quality. The maximum carrying capacity of a centrifugal column is 80μg DNA.The obtained DNA can be used in molecular biology experiments such as enzyme digestion, PCR, Southern hybridization and library construction.

The kit can process up to 1ml of blood at a time to obtain a sufficient amount of high-purity genomic DNA.

Storage and Stability

The kit can be stored for 12 months at normal temperature (15-25 ℃) under dry conditions.If need to store longer time can be placed in 2-8 ℃.Foregene Protease Plus solution has a unique formula and has activity when stored at room temperature for a long time (3 months).Stored at 4℃, its activity and stability will be better, so it is recommended to store it at 4℃, remember not to store it at -20℃.

Note: the solution is easy to precipitate if stored at low temperature.The solution in the kit must be placed at room temperature for a period of time before use. If necessary, it can be preheated in a water bath for 10 minutes to dissolve the precipitation and be mixed before use.37℃

Warnings and Precautions

- EDTA is recommended as a blood anticoagulant.
- Repeated freeze-thaw should be avoided, otherwise the extracted DNA fragments will be smaller and the extracted amount will decrease.
- Before use, carefully check whether there is precipitation in Buffer BL1, Buffer BL2 and Buffer PW. If there is precipitation, please dissolve it at 37℃ and mix well before use.
- Before using the kit, be sure to check whether anhydrous ethanol has been added to Buffer WB1 as instructed.60 mL anhydrous ethanol (DE-05111) was added to Buffer WB1 before use.
- Elution volume: Buffer EB should not be less than 100 μ L, otherwise it will affect DNA production.
- Remember not to add RNA enzymes to any Buffer.
- All centrifuges are centrifuged at room temperature (15-25℃).
- All experimental steps were carried out at room temperature (15-25℃).

Samples

- Non-nuclear anticoagulant blood: single treatment, dosage should not exceed 1 ml.
- Nuclear anticoagulant blood: single treatment, dosage do not exceed 50 μl.

Procedure

Please add anhydrous ethanol to Buffer WB1 before use. Please refer to the label on the bottle for the volume added.

1. The sample processing

1a When the sample volume was less than 200μ L, directly add Buffer BL1 to supplement to 300μ L, and proceed directly to Step 2.When the blood volume was 200-1000μ L (if the blood volume was 500-1000μ L, the blood sample could be evenly divided into two tubes), add twice the volume of Buffer DC, mix upside down, centrifuge at 12,000 RPM (~13,400 ×g) for 1min, discard the supernatant, add 300μ L Buffer BL1, and shake until completely mixed, proceed to Step 2.

Note: Heparin is anticoagulant. It is recommended to use twice the volume of Buffer DC after treatment (same method as above), and then proceed to Step 2.

1b Nucleated red cell anticoagulant blood: Add 5-20 μ L anticoagulant blood and 300 μ L Buffer BL1 into 1.5 ml centrifuge tube, proceed to Step 2.

2. Add 20 μ L Foregene Protease Plus to the mixture, mix upside down, and place at 65℃ for 10 min.
3. Add 300 μ L Buffer BL2 to the mixture, vortex mixing until the stratification disappears, place at 65℃ for 10 min, vortex mixing several times during the period, and the solution strain is clear.

Note: Vortex mixing is carried out several times during incubation to ensure adequate cell lysis.If the solution does not become clear, indicating that the cell lysis is not complete, the time can be appropriately extended.When the blood volume is less than 200 μ L and the Buffer DC treatment is not adopted, or the sample storage conditions are not good, the color of the blood may be dark brown after the treatment of water bath or metal bath, and it should be noted that there should be no clumps and other precipitates in the solution.

4. Add 120 μ L anhydrous ethanol and mix well by eddy shock. Flocculent precipitation may appear at this time.
5. The solution and flocculent precipitate obtained in the previous step were moved to the DNA-only Column, centrifuged at 12,000 RPM (~13,400 ×g) for 30sec-1min, and the waste liquid in the collection tube was discarded.

Note: If flocculent precipitation occurs in the mixture, transfer the precipitation to the centrifugal column.

6. 500 μ L Buffer PW was added into the centrifuge column, centrifuged at 12,000 RPM (~13,400 ×g) for 30 sec-1 min, and the waste liquid in the collection tube was discarded.
7. 700 μ L Buffer WB1 was added into the centrifugal column, centrifuged at 12,000 RPM (~13,400 ×g) for 30 sec-1 min, and the waste liquid in the collection tube was discarded.
8. Repeat Step 7 once.
9. The centrifugal column was put back into the collecting tube, and the 12,000 RPM (~13,400 ×g) empty tube was centrifuged for 1 min.
10. The centrifuge column was moved to a new 2 ml centrifuge tube, and 100 μl Buffer EB preheated at 65℃ was suspended in the center of the membrane (do not add the eluent to the pressure ring, otherwise a large volume of eluent will be lost). The centrifuge was centrifuged at 12,000 RPM (~13,400 ×g) for 1 min at room temperature for 5min.Then, 100μ L of preheated Buffer EB was added to the center of the membrane, centrifuged at 12,000 RPM (~13,400 ×g) for 1 min.The eluents collected from the two collections are combined.

Note: If the concentration of DNA is desired, the solution obtained by the first centrifugation can be added back to the centrifuge column and centrifuged at 12,000 RPM (~13,400 ×g) for 1 min.