Whole blood DNA extract Kit

The kit uses magnetic beads to adsorb DNA to achieve the goal of rapidly purifying whole blood genomic DNA. Suitable for the extraction of genomic DNA with high purity from 150-200 l anticoagulated whole blood sample, The lower reaches can be used in molecular biology experiments such as PCR/RT-PCR, Real-time, PCR/Real-time, RT-PCR, two generation sequencing and gene chip. suitable to Nucleic acid extraction and purification system of ascend biology.

I. Composition,storage and stability

Items	Amount
Kit size	50 preps
Lysate AQ	15 ml
Lysate B	1 tube
Dissolved liquid A	1 ml
Magnetic bead A	500 µl
Washing liquid A	20 ml
Washing liquid S	19 ml
Eluent A (nuclease-free)	5 ml

Lysate AQ and Magnetic beads A Seal and storage at room temperature.
Lysate B：Storage of freeze-dried protease K, placed at -20℃; after using dissolved liquid A dissolved, can be stored for 1 months at 4℃, for a long time, please placed at -20 ℃， repeated freezing and thawing not more than 3 times.Dissloved liquid A：The protease K solution is stored at 4℃.
Washing liquid A ： mixing evenly，seal and storage in room temperature.
Washing liquid S ： mixing evenly，seal and storage in room temperature.
Eluent A（nuclease-free）：Eluent. No DNA/RNA enzyme activity, storage at room temperature.

Ⅱ. Notices

Anticoagulant whole blood has potential infection ability. All kinds of defense measures must be prepared before operation.

In strict accordance with the operating procedures, waste must be put into the waste tank containing disinfectant, so as not to pollute the laboratory and staff.
use the centrifuge tubes, straws, Tip heads, reagents and gloves that do not contain DNA and RNA enzymes, and the staff wear masks.

If the lysate AQ is cloudy or precipitated, it is incubated at 37-56 ℃ until it is clarified.

The lysate AQ and the washing liquid A contain irritating compounds,

operation should wear latex gloves and glasses, avoid contamination of the skin, eyes and clothes, beware of inhalation nose and mouth. If infected with skin or eyes, rinse immediately with plenty of water or saline, if necessary, seek medical advice.

Ⅲ. Preparation

used for the first time, the lysate of B with 550 L A solution fully dissolved.
prepare isopropanol and absolute ethanol (pure, sterile, no RNase pollution).
magnetic rack.
Washing liquid A and washing liquid S, after Kaifeng, immediately label the label to add isopropanol and anhydrous ethanol, and identify on the reagent bottle.

Ⅳ.Operation

The following steps are from 200 L in whole blood extraction DNA, extraction of DNA from other volume of the sample can be added according to the proportion of AQ lysate and isopropanol, the same reagent in the later steps.
1. Add lysis B 10 ul to the EP with 1.5ml
2. Add 200 L anticoagulant blood samples.
If the sample is less than 200 uL, adding sterile saline to 200u L.
3. Add 300 lysate of L AQ, vortex mixing, 30 minutes to water bath at 70 ℃.
4. EP tube from the incubation equipment removed, adding mixed beads oscillation A 10 L, mixing the isopropyl alcohol 300 L (self); room temperature reversed mix for 5 minutes. Then, the EP tube is placed on the magnetic shelf for magnetic separation, and the static magnetic beads are placed on the wall of the tube, and the waste liquid is sucked (the suction pipe cover and the bottom of the pipe).
5. Remove the magnetic frame, adding 800 ul washing liquid A, upside down EP shock tube for 2 minutes.
6. The centrifugal tube is placed on the magnetic frame, and the magnetic beads are fixed to the tube wall, and the liquid in the suction tube is absorbed.
7. Remove the magnetic frame, adding 500 l washing liquid S, upside down EP shock tube for 2 minutes
8. The centrifugal tube is placed on the magnetic frame, and the magnetic beads are fixed to the tube wall, and the liquid in the suction tube is absorbed.
9. Repeat the seventh, eighth steps for one time.
10. Open the tube cover and let the magnetic beads dry for 2 minutes at room temperature.

Please pay attention to the operation in the magnetic frame, it is removed from the magnetic frame on the centrifuge tube.

11. Remove the magnetic frame, adding 50-100 ul eluent A (nuclease-free) or deionized water, flick EP tube beads all infiltration in the liquid, 70℃ water bath for 5-10 minutes.

70 ℃water bath can improve the efficiency of nucleic acid elution

12. The centrifugal tube is placed on the magnetic shelf, and the magnetic beads are placed on the tube wall, and the supernatant is sucked into a new centrifuge tube (sterile, no RNase pollution) to obtain genomic DNA.

Ⅴ. Usual problem and suggestions

Common problem	**Reason**	**Suggestion**
Yield is low	Samples are not adequately mixed before sampling	taken the sample before mixing evenly so that the white blood cells are suspended evenly in the sample
	Incomplete lysis	Reduce sample dosage or extend cracking time
	Inadequate combination	The bead has been suspended during bonding
	The white blood cells in the sample were too low	The sample should be centrifuged at low speed and the white cell layer 50ul should be taken. The dosage of other reagents should be adjusted according to the experimental conditions
	The amount of sample is larger than the quantity given in the instruction	Operate in strict accordance with the instructions
No amplification bands or amplified bands are not bright	With double distilled water instead of eluent A, but there is no regulation of pH	With Tris double distilled water pH to 8, instead of the eluent A
	High blood viscosity and short drying time	Extended drying time