

Whole Blood Genome DNA Extraction Kit (Magnetic bead method)

Cat: DM1800

Size: 50T/100T

Storage: 2-8°C (Note: RNase A will be shipped as an accessory, please store at -20°C; Magnetic bead and Protease K, stored at 4°C, Magnetic bead do not freeze)

Kit Components:

Kit Components	50T	100T	Storage
Lysing and binding solution	25mL	50mL	RT
RNase A	100μL	100μL×2	-20°C
Protease K	20mg	20mg×2	2-8°C
Dissolved enzyme liquid	1mL	1mL×2	RT
Magnetic bead	0.5mL	1mL	2-8°C, do not freeze
Washing Buffer I	15mL	30mL	RT
Washing Buffer II	8mL	16mL	RT
Elution Buffer	15mL	30mL	RT

Introduction:

It is suitable for rapid and efficient extraction of genomic DNA from whole blood samples treated with anticoagulation. The extraction process uses superparamagnetic microspheres without centrifugal operation. With prefabricated buffers, extraction can be carried out directly without the need to remove the red blood cells beforehand. This product can be used for both manual extraction of small samples and high throughput operations with automated workstations. The extracted products can be used for enzyme digestion, PCR amplification, detection and other subsequent experiments.

Preparatory work:

Whirlpool oscillator, Rotary mixer, Thermostatic water bath/metal bath: 55°C

Magnetic separation device: Solarbio magnetic separation device is available Cat: YA3100/YA3102

Protocols:

Please add isopropyl alcohol/absolute ethanol to the washing buffer before use. Please refer to the label on the bottle for the added volume (15mL/30mL of isopropyl alcohol should be added to each bottle of 50T/100T washing buffer I, 24mL/48mL of absolute ethanol should be added to each bottle of 50T/100T washing buffer II).

Configuration of protease K solution: 1mL Dissolved enzyme liquid was added into 20mg protease K powder to form 20mg/mL protease K solution, and the prepared protease solution was stored at -20°C.

1. Take a new 1.5mL centrifuge tube, add 200μL anticoagulant blood sample (less than 200μL can be filled with PBS or elution buffer), then add 20μL protease K, 2μL RNase A and 500μL lysis solution, respectively, and swirl with the maximum speed of vortex oscillator for 10s, then put in a water bath at 60°C for 5min.

2. Add 450 μ L isopropyl alcohol and 10 μ L magnetic bead (**the magnetic bead should be completely shaken and mixed before use**), and swirl for 10min at the maximum speed of the vortex oscillator (or react on the Rotary mixer for 10min after the vortex oscillates for 10s). Then the centrifuge tube was placed on the magnetic separation device for 2min, the supernatant was removed with a pipette and the centrifuge tube was removed. Note: The magnetic separation time of this step should not be less than 2min.
3. Add 600 μ L washing buffer I (**check whether isopropyl alcohol has been added**), vortex oscillation for 30s or slowly blow the magnetic bead 20 times with a pipette to make the magnetic bead fully suspended, and then place the centrifuge tube on the magnetic separation device until the solution is clarified, remove the supernatant with a pipette and remove the centrifuge tube. Repeat this step once.
4. Add 600 μ L washing buffer II (**check whether absolute ethanol has been added**), vortex oscillation for 30s or slowly blow the magnetic bead 20 times with a pipette to make the magnetic bead fully suspended, put at room temperature for 1min, and then place the centrifuge tube on the magnetic separation device until the solution is clarified, remove the supernatant with a pipette. Note: In this step, the washing buffer should be removed as much as possible.
5. Keep the centrifuge tube on the magnetic separation device, put at room temperature for 10min, and remove the centrifuge tube. If there is excess liquid at the bottom of the centrifuge tube during the standing period, use a pipette to suck it out.
6. Remove the centrifugal tube from the magnetic separation device, put it on the ordinary centrifugal tube rack, add 100-200 μ L elution buffer, and slowly blow the magnetic bead 50 times with the pipette to make the magnetic bead fully suspended. Then incubated at room temperature for 5min, put the centrifuge tube on a magnetic separation device until the solution is clarified, and transfer the supernatant to the new centrifuge tube, which is the purified genomic DNA and could be stored at -20°C.

Notes:

1. Please read this manual carefully before operation.
2. The quality of blood samples has a great influence on the purification amount of product DNA, so repeated freezing and thawing of blood samples should be avoided.
3. Avoid freezing, centrifuging and other operations on magnetic bead.
4. Magnetic bead should be fully and evenly suspended before use.
5. Before the magnetic bead dry, the pipette should be used to suck up the washing liquid.
6. Excessive drying of magnetic bead should be avoided, otherwise it will seriously reduce the efficiency of nucleic acid elution.
7. It is recommended to use a good quality centrifugal tube and pipette nozzle to avoid losses caused by adhesion to magnetic beads.
8. When magnetic separation is performed in the 96-well plate, the magnetic bead adsorption time can be appropriately extended to 4~5min.