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Instructions for Use of Genomic DNA Extraction Kit for Blood Tissue Cells

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-NA100

Introduction

This kit is used for nucleic acid extraction, enrichment, purification and other steps, and the processed products can be used for clinical *in vitro* detection.

This kit can specifically adsorb DNA with superparamagnetic microspheres and remove impurities such as proteins other than DNA by washing. The eluent can dissociate DNA adsorbed on the magnetic beads, so that high-quality nucleic acids can be isolated and purified.

Specification

100 tests/box

Main components

Serial Number	Components	Specification
1	Bead suspension ①, 50 mg/mL	1 × 2 mL
2	Lysis buffer ②	1 × 25 mL
3	Wash buffer I ③	1 × 60 mL
4	Eluent ④	1 × 30 mL
5	Proteinase K ⑤	1 × 20 mg
6	Solution A ⑥	1 × 1 mL

Storage and shelf life

The shelf life is 24 months at 2-8℃.

Applicable instrument

This kit is applicable to BIO-DL32, TIANGEN TGuide S32, TIAN LONG NP968-C and other automated nucleic acid extraction instruments, and is also applicable to manual extraction.

Sample Requirements

Fresh or frozen anticoagulated human whole blood samples, tissues and cells.

Usage

Before first use:

(1) Slowly add the specified amount (see the bottle label) of isopropanol (analytically pure, provided by the customer) into the wash buffer I ③, mark "√" in "□", mix well and store at 2-8℃.

(2) Add the specified amount (see the tube label) of solution A ⑥ into proteinase K dry powder ⑤, and mark "√" in "□"; after mixing, store the solution at 2-8℃ or store at -20℃ after dispensing.

I. Manual Operating Procedure

1. Supplies to be Provided by the Customer:

- Isopropanol (analytically pure)
- 80% ethanol
- 1.5 mL centrifuge tubes: 2 tubes/sample
- Single channel pipettes: 20 μL, 200 μL, 1000 μL
- Vortex shaker
- Vertical mixer
- Dry Bath Incubator, Cat.: 2016C or water bath (55℃)
- Magnetic separator

2. Operation procedures

(1) Lysis

Add 200 μL of anticoagulant blood sample, tissue or cells in a new 1.5-mL centrifuge tube, which can be adjusted properly as required by the experiment (make up to volume with PBS or eluent ④ when less than 200 μL), then add 10 μL of proteinase K ⑤ (check whether solution A ⑥ has been added) and 230 μL of lysis buffer ②, respectively, vortex for 10 s at maximum speed on a vortex shaker, and react at 55℃ for 5 min.

(2) Bind

Add 320 μL of isopropanol and 20 μL of magnetic bead suspension ①, vortex for 10 minutes at maximum speed on a vortex shaker (or place on the vertical mixer and then react for 10 min after vortexing for 10 s) , Fast centrifugation for 3s. Place the tubes on a magnetic separator for 2 min, remove the supernatant using a pipette and remove the centrifuge tubes from the magnetic separator.

Note: The time used for magnetic separation in this step should be no less than 2 minutes.

(3) Wash

1) Add 600 μL of wash buffer I ③ (check if isopropanol has been added), and vortex for at least 1 minute at maximum speed on a vortex shaker to fully resuspend the beads, Fast centrifugation for 3s, place the centrifuge tubes on a magnetic separator until the solution is clear, remove the supernatant and remove the centrifuge tubes from the magnetic separator.

2) Repeat this step once.

3) Add 600 μL of 80% ethanol, vortex for at least 1 minute at maximum speed on a vortex shaker to fully resuspend the beads, fast centrifugation for 3s, place the centrifuge tubes on a magnetic separator until the solution is clear, remove the supernatant and remove the centrifuge tubes from the magnetic separator.

Repeat this step once.

Note: The wash buffer should be removed as much as possible in the last washing step.

(4) Drying

Keep the centrifuge tubes on the magnetic separator and allow to stand at room temperature for 10 min; i.e., the surface of magnetic bead has no obvious luster, and then remove the centrifuge tubes.

Note: If there is liquid residue in the reaction tubes during the drying process, use a small-scale pipette to pipette and discard the liquid.

(5) Elution

Add 100 - 200 μL of eluent ④, vortex and shake, or slowly pipette the beads, so that the beads are fully resuspended. Then heat at 55℃ for 5 min, fast centrifugation for 3s, place the centrifuge tubes on the magnetic separator until the solution is clear, and transfer the supernatant to a new 1.5-mL centrifuge tube, which is the purified genomic DNA and can be stored at -20℃.

II. Automated Operating Procedure

1. Supplies to be Provided by the Customer:

- Magnetic rod automated nucleic acid extractor (BIO-DL 32, TIANGEN TGuide S32, TIANLONG NP968-C, etc.)
- Two 96-well magnetic rod sleeves
- One 96-well deepwell plate
- Isopropanol (analytically pure)
- 80% ethanol
- 1.5 mL centrifuge tubes: 2 tubes/sample
- Single channel pipettes: 20 μL, 200 μL, 1000 μL

2. Operation procedures

(1) Add 10 μL of Proteinase K (check whether Solution A has been added), 200 μL of sample, 230 μL of lysis buffer and 320 μL of isopropanol sequentially in Columns 1 and 7 of the 96-well deepwell plate.

(2) Add 600 μL of wash buffer I (check whether isopropanol has been added) and 20 μL of magnetic bead suspension in Columns 2 and 8.

(3) Add 600 μL of wash buffer I (check whether isopropanol has been added) in Columns 3 and 9.

(4) Add 600 μL of 80% ethanol to Columns 4 and 10.

(5) Add 600 μL of 80% ethanol to Columns 5 and 11.

(6) Add 100-200 μL of eluent in Columns 6 and 12.

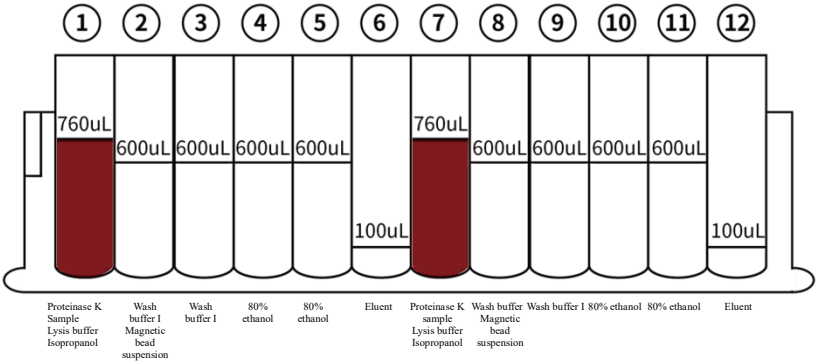

Figure 1 Schematic diagram of loading into well

Note: Columns 1 and 7 reagents are added in the following order: adding 10 μL of Proteinase K, followed by 200 μL of sample, then 230 μL of lysis buffer and finally 320 μL of isopropanol. (Incorrect order of reagent addition may lead to low nucleic acid extraction concentration and purity.)

(7) Recommendations for setting extraction parameters of automated nucleic acid extractors:

Step	Name	Slot position	Waiting time (s)	Mixing time (s)	Magnetic absorption time (s)	Magnetic absorption mode	Mixing speed	Volume (μL)	Temperature (℃)
1	Magnetic beads	2	0	60	120	Cycle	Fast	600	OFF
2	Lysis	1	0	1200	180	Cycle	Fast	760	75
3	Wash 1	2	0	120	100	Cycle	Fast	600	OFF
4	Wash 2	3	0	120	100	Cycle	Fast	600	OFF
5	Wash 3	4	0	120	30	Cycle	Fast	600	OFF
6	Wash 4	5	0	120	30	Cycle	Fast	600	OFF
7	Elution	6	350	300	100	Cycle	Fast	100	60
8	Discard the beads	2	0	60	0	Cycle	Fast	600	OFF

Note: The above program parameters can be appropriately adjusted depending on the actual situation. The critical steps are the mixing time and temperature of the lysis step, and the mixing time of the washing step, which can be appropriately extended if the extraction is not satisfactory.

(8) After completion of the automated procedure, transfer the eluates from columns 6 and 12 to new centrifuge tubes as the purified genomic DNA and can be stored at −20℃.

Precautions

1. Please read this product manual carefully before performing any operation.
2. The quality of blood samples can significantly impact on the yield from purification of product DNA, and repeated freezing and thawing of blood samples should be avoided.
3. Proteinase K dry powder can be stored in aliquots at -20℃ after dissolution, but repeated freezing and thawing cycles should be avoided.
4. Avoid freezing and centrifuging the magnetic beads.
5. The magnetic beads should be resuspended thoroughly before use.
6. Wash solution should be pipetted using a pipette before drying of the magnetic beads.
7. Excessive drying of magnetic beads should be avoided, as it can severely reduce nucleic acid elution efficiency.
8. It is recommended to use high-quality centrifuge tubes and pipette tips, so as to avoid loss due to adhering beads.
9. When performing magnetic separation operations in 96-well plates, the beads adsorption time can be extended appropriately.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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