

Magnetic Blood Spots DNA Kit

Magnetic bead method kit capable of purifying DNA in highthroughput rapid from dry blood spot

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Magnetic Blood Spots DNA Kit

Cat.no. 4992720/4992721

Kit Contents

Contents	4992720 (50 preps)	4992721 (200 preps)
Buffer GAS	25 ml	100 ml
Buffer GHC	20 ml	80 ml
Buffer PD	120 ml	2 × 240 ml
Buffer PWB	15 ml	50 ml
Proteinase K	1 ml	3 × 1 ml
MagAttract Suspension G	0.5 ml	2 × 1 ml
Buffer TBC	15 ml	30 ml
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Storage

This kit can be stored at room temperature (15-30°C) for 15 months. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C before use.

Introduction

The kit adopts magnetic beads with unique separation function and a unique buffer system to separate and purify high-quality genomic DNA from dried blood spots. The unique embedded magnetic beads have strong affinity for nucleic acid under certain conditions, and when the conditions change, the magnetic beads will release adsorbed nucleic acid, thus achieving the purpose of fast separation and purification of nucleic acid. The whole process is safe and convenient. The extracted genomic DNA fragments are large, with high purity, stable and reliable quality. The method is especially suitable for automatic extraction of high-throughput workstations.

Features

- **Easy and fast:** Ultrapure genomic DNA can be obtained within 1 h.
- **High Throughput:** It can be adapted to the automated instruments of pipetting-based method and magnetic rod method to carry out high throughput extraction experiments.
- **Safe and non-toxic:** No reagent such as phenol/chloroform is needed.
- **High purity:** The obtained DNA has high purity and can be directly used in chip detection, high-throughput sequencing and other experiments.

Important Notes Before Using

1. This product is suitable for both manual extraction or automatic instrument integration.
2. Self-prepared reagents: Isopropanol, ethanol.
3. If there are precipitates in Buffer GAS and Buffer GHC, they can be redissolved in a 37°C water bath and used after thoroughly mixing by shaking.

Protocol

Before using, please add 96%-100% ethanol into Buffer PWB according to the label on the bottle.

I. Manual operation:

1. Sample treatment: Add 3-10 pieces of blood spot samples with a diameter of 3 mm into a 1.5 ml centrifuge tube, and add 200-400 μ l of Buffer GAS and 15 μ l of Proteinase K solution.

Blood spots pieces	Buffer GAS volume (μl)
3	200
5	300
10	400

- After vortex for 10 sec, put the mixture into a thermostatic oscillator preheated to 75°C and lysis for 45 min at 900 rpm.

Note: When the number of samples is relatively large, Buffer GAS and Proteinase K can be premixed in proportion. Please use the mixture within 1 h.

- During the sample lysis, add 10 μl of MagAttract Suspension G and 600 μl of Buffer GHC (**ensure that isopropanol has been added before use**) to a new centrifuge tube, pipette and mix evenly or shake and mix evenly for 10 sec.

Note: When the number of samples is relatively large, premix MagAttract Suspension G and Buffer GHC in proportion and beat or vortex to mix for 20 sec. The amount of mixture for each sample is 610 μl.

- After the sample lysis, centrifuge the centrifuge tube in step 2 briefly and then place at room temperature for 2 min, transfer the supernatant to the centrifuge tube in step 3, and then place in a thermostatic oscillator to oscillate at 900 rpm for 10 min at room temperature.

Note: While pipetting, try not to touch the filter paper sheet, otherwise it will affect the binding of magnetic beads and nucleic acid, resulting in lower yield.

- Place the centrifuge tube on the magnetic stand and let it stand for 1 min. Carefully remove the liquid when the magnetic beads are completely attached.
- Remove the centrifuge tube from the magnetic stand, add 900 μl of Buffer PD, slap and mix evenly or shake and mix evenly for 2 min.
- Place the centrifuge tube on the magnetic stand and let it stand for 1 min. Carefully remove the liquid when the magnetic beads are completely attached.
- Remove the centrifuge tube from the magnetic stand, add 900 μl of Buffer PD, pipette and mix evenly or shake and mix evenly for 2 min.
- Place the centrifuge tube on a magnetic stand and let it stand for 1 min. Carefully remove the liquid after the magnetic beads are completely attached.

10. Remove the centrifuge tube from the magnetic stand, add 900 μl of Buffer PWB (**ensure that 96%-100% ethanol has been added before use**), slap and mix evenly or shake evenly for 2 min.
11. Place the centrifuge tube on a magnetic stand and let it stand for 1 min. Carefully remove the liquid after the magnetic beads are completely attached.
12. Place the centrifuge tube on a magnetic stand and air-dry at room temperature for 10-15 min.
Note: The ethanol residue will inhibit the subsequent enzyme reaction, so make sure the ethanol volatilizes completely when drying. However, do not dry for too long, for over-drying will lead to low yield.
13. Add 30-50 μl Buffer TBC or deionized water, slap and mix evenly or shake and mix evenly, place at 56°C, and incubate for 5-10 min.
14. Place the centrifuge tube on a magnetic stand and let it stand for 2 min. When the magnetic beads are completely attached, carefully transfer the DNA solution to a new centrifuge tube and store it under appropriate conditions.

II. Automated purification (Pipetting-based method)

Preparation and precautions

1. This product can be adapted with automatic pipetting instruments such as Hamilton Microlab STAR, Beckman Coulter Biomek® FX and Capitalbio LabKeeper for high-throughput blood genome extraction.
2. Preparation of magnetic bead diluent: Mix according to the proportion of 10 μl MagAttract Suspension G and 40 μl isopropanol, and the dosage of each sample after mixing is 50 μl .
3. Considering that there is a certain deviation between the set temperature of the instrument and the actual temperature in the 96-well plate, it is recommended that the set temperature of the instrument should be 10°C higher than the actual temperature during lysis and elution.

Extraction steps:

1. Treat the samples according to the methods in steps 1 and 2 of Manual Extraction, and then transfer the samples to a 96-well plate (self-provided).
2. Add 600 μl of Buffer GHC to each well (**please confirm whether isopropanol has been added before use**) and mix well at room temperature for 5 min.
3. Add 10 μl of MagAttract Suspension G or 50 μl of diluted MagAttract Suspension G to each well, pipette up and down to mix for 6 times, and then shake and mix evenly for 10 min.
4. Place the 96-deep-well plate on a magnetic stand and let it stand for 2 min. After the magnetic beads are completely attached, discard the supernatant.
5. Remove the 96-deep-well plate from the magnetic stand, add 100 μl of Buffer PD, shake and mix well for 2 min. Then add 600 μl of Buffer PD, pipette up and down to mix for 6 times, and then shake and mix evenly for 2 min.
6. Place the 96-deep-well plate on a magnetic stand and let it stand for 2 min. After the magnetic beads are completely attached, discard the supernatant.
7. Remove the magnetic stand of the 96-deep-well plate, add 500 μl Buffer PD, shake and mix for 2 min.
8. Place the 96-deep-well plate on a magnetic stand and let it stand for 2 min. After the magnetic beads are completely attached, discard the supernatant.
9. Remove the 96-deep-well plate from the magnetic stand, add 100 μl of Buffer PWB, shake and mix well for 1 min. Then add 600 μl of Buffer PWB (**ensure that 96%-100% ethanol has been added before use**), pipette up and down to mix for 6 times, and then shake and mix evenly for 2 min.
10. Place the 96-deep-well plate on a magnetic stand and let it stand for 2 min. After the magnetic beads are completely attached, discard the supernatant.
11. Place the 96-deep-well plate on a magnetic stand and dry it at 37°C for 5 min.
12. Remove the 96-deep-well plate from the magnetic stand, add 50-100 μl of Buffer TBC, place it at 65°C and mix well for 10 min.

- Place the 96-deep-well plate on a magnetic stand and let it stand for 2 min. After the magnetic beads are completely attached, carefully transfer the DNA solution to the collection plate and store it under appropriate conditions.

III. Automated purification (Magnetic rod method)

Preparation and precautions

This product can be adapted with Thermo KingFisher Flex and other magnetic rod method automatic instruments with heating devices to extract high-throughput dried blood spot genome. This manual uses Thermo KingFisher Flex as an example, and other instruments can be adjusted according to the characteristics of the machine.

Extraction steps:

- Process the blood spot sample according to the method in steps 1 and 2 of Manual Extraction, and add 200-300 μl of the treated sample to the 96-deep-well plate containing Buffer GHC (**ensure that isopropanol has been added before use**). Put the remaining 4 96-deep-well plates into the extractor according to the program prompts.

Plate number	Buffer type	Volume
1	Buffer GHC	600 μl
2	Buffer PD/ MagAttract Suspension G	900 μl / 10 μl / Magnetic rod comb
3	Buffer PD	300 μl
4	Buffer PWB	500 μl
5	Buffer TBC	50-100 μl

- When the program starts, the magnetic rod will grab the magnetic rod comb and transfer it to No.1 deep-well plate containing sample and Buffer GHC. Slap and mix at medium speed for 2 min.
- Transfer the magnetic comb to the No.2 deep-well plate of Buffer PD to adsorb magnetic beads for 3 times, each time for 5 sec.
- Transfer the magnetic beads adsorbed by the magnetic rod and the magnetic comb to the No.1 deep-well plate containing the Sample and Buffer GHC, slap at medium speed and mix evenly for 10 min, and then adsorb the magnetic beads for 3 times with 5 sec each time.
- Transfer the adsorbed magnetic beads to No.2 deep-well plate containing Buffer PD, release the magnetic beads, rapidly slap and mix for 3 min, then absorb magnetic beads 3 times, 5 sec each time.

6. Transfer the adsorbed magnetic beads to No. 3 deep-well plate containing Buffer PD, release the magnetic beads, quickly slap and mix for 3 min, then absorb magnetic beads 3 times, 5 sec each time
7. Transfer the adsorbed magnetic beads to deep-well plate No.4 containing Buffer PWB, release the magnetic beads, slap and mix for 3 min, and then adsorb the magnetic beads for 3 times with 5 sec each time.
8. Hang the magnetic beads adsorbed by the magnetic rod and magnetic comb in the air for 5 min on deep-well plate No.4 containing Buffer PWB.
9. Transfer the magnetic beads adsorbed by the magnetic rod to the No.5 deep-well plate containing Buffer TBC, release the magnetic beads, place them at 75°C, slap and mix them evenly for 12 min. Then absorb magnetic beads 5 times, 20 sec each time.
10. Transfer the magnetic beads adsorbed by the magnetic rod and the magnetic comb to the No.4 deep-well plate containing Buffer PWB.
11. After the procedure is completed, carefully transfer the DNA solution to the collection plate and store it under appropriate conditions.

Determination of DNA Concentration and Purity

The size of the purified genomic DNA fragment is related to factors such as sample storage time and shearing force during the operation. The concentration and purity of the DNA fragments can be detected by agarose gel electrophoresis and UV spectrophotometer.

DNA should have a significant absorption peak at OD_{260} , with OD_{260} value of 1 equivalent to about 50 $\mu\text{g/ml}$ double stranded DNA and 40 $\mu\text{g/ml}$ single stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If using deionized water instead of elution buffer, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.