【Product Name】 MagPure Blood DNA Kit

【Product specifications】 48 Preps, 96 Preps, 480 Preps

【Intended Use】

This product provides high quality purification of total DNA from whole blood, plasma, serum, buffy coat, or other body fluids, lymphocytes and cultured cells. There is no need to use toxic phenol chloroform extraction or time-consuming alcohol precipitation. The extraction process finish in 60 minutes. Purified DNA includes genomic DNA, mitochondrial DNA, viral DNA (e.g. HBV), or DNA from other parasitic microorganisms. The obtained DNA can be directly used in PCR, viral DNA detection and other experiments.

This kit can use on manual protocol or 96 channel automated extraction system.

【Principle】

This product is based on the purification method of high binding magnetic particles. The sample is lysed and digested under the action of lysate and Protease. DNA is released into the lysate. After adding magnetic particles and binding solution, DNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing buffer to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer.

【Kit Contents】

Cat.No.	D631100	D631101	D631102	D631103
Purification Times	20	48	96	480
MagPure Particles	0.6 ml	1.2 ml	2.5 ml	11 ml
Proteinase K	12 mg	24 mg	50 mg	220 mg
Protease Dissove Buffer	1.8 ml	1.8 ml	5 ml	15 ml
Buffer AL	10 ml	15 ml	30 ml	120 ml
Buffer GW1*	13 ml	22 ml	53 ml	220 ml
Elution Buffer	10 ml	15 ml	30 ml	100 ml

【Storage conditions and Validity】

Proteinase K, MagPure Particles should be stored at 2–8°C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

【Preparation before Use】

- Add 17ml (20 Preps) or 28ml (48 Preps) or 67ml (96 Preps) or 280ml (480 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 0.6ml (20 Preps) or 1.2ml (48 Preps) or 2.5ml (96 Preps) or 11ml (480 Preps) Protease Dissolve Buffer to the bottle of Proteinase K and store at -20~8°C after dissolve.
- 75% Ethanol
- Isopropanol

【Plate Protocol】

1. **Pipet 20 µl Proteinase K into the bottom of a 96 well Plate (2.2ml).**
2. **Add 200 µl sample to the 96 well plate.** Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^6 lymphocytes or Culture Cells in 200 µl PBS.
3. **Add 200 µl Buffer AL to the sample and seal 96-well plate with seal film. Mix by shaking at 900~1200rpm for 2 min.** Incubate at 70°C for 10 min in an incubator oven.
   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.
4. **Remove the seal film and add 300 µl Isopropanol and 20 µl MagPure Particles to the sample.** Mix again by shaking at 700~900rpm for 5 min.
5. **Place the deep well plate on an Magnet Plate and allow beads to separate for 2 minutes.** With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.
6. **Wash the beads by adding 0.5ml Buffer GW1, resuspend the beads by shaking for 1 min.**
7. Place the deep well plate on an Magnet Plate and allow beads to separate for 1 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.
8. Repeat step 6-7 once.

9. Wash the beads by adding 0.5ml 75% ethanol and resuspend the beads by shaking for 1 min.

10. Place the deep well plate on an Magnet Plate and allow beads to separate for 1 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

11. Repeat step 8-9 once.

12. Select one of the following ethanol removal steps:

- A. Leave the plate on the magnetic separation device. Add 500µL ddH2O, leave on magnet for 30 seconds, and then aspirate. Do not leave ddH2O on MagPure Particles for more than 60 seconds. Continue to Step 13.

- B. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Pure Particles for an additional 10 minutes.

13. Add 100µl Elution Buffer to the sample and shaking at maximal speed for 10 minteus to elute DNA.

14. Place the tube on the magnetic separation device to magnetize the Particles. Let sit at room temperature until the MagPure Particles are completely cleared from solution.

15. Transfer the cleared supernatant containing purified DNA to a new Tube). Store DNA at -20°C.

【KingFisher Flex Protocol】

1. Add the Reagents/sample to the well of f the deep well plate according to the table below.

Name of the Plate	Pre-loaded reagents	Addition before use
Sample plate	1. Pipet 20µl Proteinase K into the bottom of Plate. 2. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids or up to 5 x 10^6 lymphocytes or Culture Cells in 200µl PBS. 3. Add 200µl Buffer AL	
Wash Plate 1	500µl Buffer GW1, Put in 96 magnetic Tip, 20µl MagPure Particles	
Wash Plate 2	500µl Buffer GW1	
Wash Plate 3	500µl 75% Ethanol	
Wash Plate 4	500µl 75% Ethanol	
Elution plate	100µl Elution Buffer	

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.

3. Start the D6311_Flex protocol with the KingFisher Flex 96 and load the plates.

4. Add 300µl Isopropanol to the Sample plate during the dispense step.

5. Place the sample plate back into the instrument and press Start.

6. After the run is completed, remove the plates and store the purified DNA at -20°C.

Troubleshooting Guide

1. Low or no recovery

- Buffer GW1 did not contain ethanol: Ethanol must be added to Buffer GW1 before used. Repeat procedure with correctly prepare Buffer.

- Low concentration of target DNA in the Sample: Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided. Low concentration of cells or viruses in the sample

- Inefficient cell lysis due to insufficient mixing with Buffer AL: Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing.

- Low-percentage ethanol used instead of 100%: Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

2. A260/A280 ratio for purified nucleic acids is low

- Inefficient cell lysis due to insufficient mixing with Buffer AL: Repeat the procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse vortexing.

- Inefficient cell lysis due to decreased protease activity: Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Besure to store the stock solution at -20–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer AL.

- No ethanol added to the lysate before loading onto the column: Repeat the purification procedure with a new sample.

3. A260/A280 ratio for purified nucleic acids is high

- High level of residual RNA: In future DNA preparations, use the optional RNase step included in the protocols.

4. DNA does not perform well (e.g. in ligation reaction)

- Salt concentration in eluate too high: Modify the wash step by incubating the column for 3 min at room temperature after adding 500ul of Buffer GW2, then centriufge or Vacuum.

- Eluate contains residual ethanol: Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >10,000 x g for 1min, then dry.

- Inappropriate elution volume used: Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.