TIANGEN®

Magnetic Universal Genomic DNA Kit

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Magnetic Universal Genomic DNA Kit

Cat. no. GDP705

Kit Contents

Contents	GDP705-01 (50 preps)	GDP705-02 (200 preps)
Buffer GHA	30 ml	120 ml
Buffer GHL	20 ml	80 ml
Buffer GDZ	45 ml	2 × 90 ml
Buffer PWD	20 ml	2 × 40 ml
Proteinase K	1 ml	4 × 1 ml
MagAttract Suspension GH	750 µl	3 × 1 ml
Buffer TB	15 ml	60 ml
Handbook	1	1

Optional Products

Magnetic Frame (self provide, TIANGEN, Cat.no: OSE-MF-01); Lysozyme A (50 mg/ml) (with buffer) (self provide, TIANGEN, Cat.no: GRT401-11)

Storage

This kit can be stored at room temperature (15-30°C) under dry condition for 15 months. If a precipitate has formed in Buffer, please place the buffer at 37°C for 10 min to dissolve the precipitate.

Introduction

This kit adopts magnetic beads with unique separation function and a unique buffer system to separate and purify high-quality genomic DNA from samples such as blood, saliva, oral swabs, animal tissues, etc. 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 operation process is safe and convenient, and large genomic DNA fragement can be extracted with high purity and stable quality. This kit is especially suitable for automatic extraction of high-throughput workstations.

The DNA purified by the kit is suitable for various conventional operations, including enzyme digestion, PCR, fluorescence quantitative PCR, library construction, Southern blot, chip detection, high-throughput sequencing and other experiments.

Features

- **Easy and fast:** Ultrapure genomic DNA can be obtained within 1 hour.
- **High throughput:** It can be adapted to the automated instruments of pipetting method and magnetic rod method for 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.

Notes <u>Please read these notes before using this kit.</u>

1. This product is suitable for manual extraction or automatic instrument integration.
2. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the extraction yield will also decrease.
3. If there is precipitation in Buffer GHL, it can be redissolved in 37°C water bath. Please use after shaking.
4. Self-provided reagents: Isopropanol, ethanol. For tissue samples, 1M DTT should be prepared. For bacteria samples, 1M NaOH should be prepared. If RNA residues need to be removed, RNase A(100 mg/ml) solution should be prepared. For FFPE samples, please purchase environmentally friendly deparaffinization oil DPR. To extract large volumes of blood and blood clots, please purchase Buffer CLA and Buffer GHL separately.

Reagent Dosage

	Blood	Saliva (250 µl)	Swab (300 µl)	Blood spot	Animal tissue	Mouthwash /amniotic fluid, etc	FFPE
Buffer GHA	NA	NA	500 µl	200-400 µl	300 µl	300 µl	300 µl
Proteinase K	20 µl						
Buffer GHL	300 µl						
Isopropanol	300 µl						
MagAttract Suspension GH	15 µl						
Buffer GDZ	900 µl / 500 µl						
Buffer PWD	900 µl / 300 µl						
Buffer TB	50-100 µl						

Protocol

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

A.Blood sample (anticoagulant)

1. Transfer 250 µl blood sample to 2 ml centrifuge tube.
2. Add 20 µl Proteinase K solution and 300 µl Buffer GHL, mix well by vortex. Lyse at 75°C for 15 min, and mix well upside down for 3 times (3-5 times each time) during the lysis.

   Note: When the number of samples is relatively large, the Buffer GHL and Proteinase K can be mixed in advance. Please prepare the mixture when using.
3. Add 300 µl isopropanol to the tube and vortex to mix for 10 sec.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, and let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

B. Blood samples (buffy coat and plasma-free samples)

1. Take 100-200 µl blood buffy coat sample **(the sample needs to be equilibrated to room temperature)** and add 20 µl Proteinase K solution and 350 µl Buffer GHL, and mix them upside down.
2. Lyse the sample at 75°C for 15-30 min at 1,500 rpm on a thermostatically oscillating metal bath until no lumps in the sample.

   Note: The buffy coat sample and the plasma-free blood sample are relatively viscous and have a large proportion of white blood cells. In order to fully lyse the sample, they can be mixed by vortex oscillation and appropriately extend the lysis time.
3. Add 350 µl isopropanol and vortex to mix for 10 sec.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic frame and let stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

C. Large volume blood sample (take 2 ml blood sample as an example)

1. Place the blood sample at room temperature until it is completely thawed, add 2 ml of Buffer CLA to the sample tube containing 2 ml of blood, mix it upside down for 5 times, and centrifuge at 3,600 rpm for 5 min. Discard the supernatant, add 3 ml of Buffer CLA and centrifuge at 3,600 rpm for 5 min. Leave 100-200 µl of Buffer CLA in the cell precipitate and vortex until it is thoroughly mixed.
2. Add 20 µl Proteinase K and 350 µl Buffer GHL. Lyse the sample at 75°C for 15-30 min at 1,500 rpm on a thermostatically oscillating metal bath until no lumps exist in the sample.
3. Add 350 µl isopropanol and vortex to mix for 10 sec.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

D. Blood clot sample (take 2 ml blood sample as an example)

1. Treatment of blood clots or blood containing blood clots:
   - For blood clots preserved with colloid, first remove the colloid with the flat end of a 1 ml pipette tip before the blood is completely thawed, and remove the jelly as much as possible.
   - Directly add 1 ml of Buffer CLA to the sample tube containing blood clots (about 2 ml). Blow the blood clots back and forth using plastic Pasteur dropper or long pipette tips until they are relatively uniform and containing no large obvious blood clots. Then add 2 ml Buffer CLA, mix upside down for 5 times and centrifuge at 3,600 rpm for 10 min.
   - Take out the sample tube, pour out the supernatant gently and leave nuclear precipitation.
   - Add 3 ml of Buffer CLA, mix it upside down or shake it on a shaker to disperse the lumps as much as possible. Centrifuge at 3,600 rpm for 5 min, discard the supernatant, leave about 100-200 µl of solution volume, vortex and oscillate until the lumps are dispersed and mixed evenly.
2. Add 20 µl Proteinase K and 1 ml Buffer GHL, place at 75°C for 1 h or incubate overnight, and blow up the remaining lumps as much as possible. Centrifuge at 3,600 rpm for 5 min to remove residual impurities.
3. Transfer the supernatant to a 2 ml centrifuge tube, add 800 µl isopropanol, spank to mix or shake to mix evenly.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

E. Blood spot sample

1. Sample treatment: Add 3-10 slides of 3×3 mm dried blood spot samples into a 1.5 ml centrifuge tube, and add 200- 400 µl of Buffer GHA and 20 µl of Proteinase K solution.

Blood spot slide numbers	Dosage of Buffer GHA
3	200 µl
5	300 µl
10	400 µl

2. After vortex for 10 sec, transfer the tube into a thermostatic oscillator preheated to 75°C and lyse at 1,500 rpm for 45 min.

   Note: When the number of samples is relatively large, the Buffer GHA and Proteinase K can be mixed in advance according to the proportion, and it's better to prepare when using.

3. Add 300 µl of Buffer GHL and 300 µl of isopropanol, and mix well by vortex.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.

5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

F. Tissue sample

1. Sample treatment: Take 10-50 mg of animal tissue, cut it into small pieces as much as possible, add 300 µl of Buffer GHA and 20 µl of Proteinase K, and grind for about 20 sec with an electric homogenizer until the tissue is completely ground.
   1) For samples with sufficient homogenate, the 65°C digestion step can be skipped.
   2) For samples with visible tissue mass, it is recommended to completely digest at 65°C for 30 min.
   3) For rat tail samples, carry out digestion overnight at 56°C.
   4) For hair and feather stem samples containing hair follicles, add 20 µl 1M DTT (self-provided) and digest for 60 min to overnight.

      Note: If there are tissue debris after sample digestion, it is recommended to centrifuge at 12,000 rpm for 1 min to remove residual impurities. If RNA removal is required, add 4 µl of RNase A and incubate at room temperature for 10 min.

2. Transfer 300 µl of the above treated sample solution to a new 1.5 ml centrifuge tube.
3. Add 300 µl of Buffer GHL and 300 µl of isopropanol, and mix well by vortex.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

G. Saliva sample

1. Take 300 µl saliva sample into 2 ml centrifuge tube.
2. Add 20 µl Proteinase K solution and 300 µl Buffer GHL, mix well by vortex, lyse at 75°C for 15 min, and mix well upside down for 3 times (3-5 times each time) during the incubation.

   Note: When the number of samples is relatively large, Buffer GHL and Proteinase K can be mixed in advance, and it is better to prepare when using.
3. Add 300 µl isopropanol and vortex to mix well for 10 sec.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

H. Swab sample

1. Sample treatment:
   1) Dry swab sample: After sample collection, add 500 µl of Buffer GHA and 20 µl of Proteinase K, vortex for 10 sec and mix evenly.

2) Swab sample containing preservation solution: If the volume of preservation solution is too large, directly take out 300 µl to 1.5 ml centrifuge tube for experiment. If the volume of preservation solution is less than 300 µl, top up to 300 µl with Buffer GHA. Add 20 µl Proteinase K, vortex for 10 sec and mix well.

2. Place at 75°C for 15 min, and mix it upside down for 3 times, 3-5 times each time. Take out 300 µl for subsequent experiments.
3. Add 300 µl of Buffer GHL and 300 µl of isopropanol, and mix well by vortex.

   Note: When the number of samples is relatively large, Buffer GHL and isopropanol can be premixed.

4 Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.

5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

I. Mouthwash/amniotic fluid and other samples

1. Sample treatment: Add 1-20 ml mouthwash or amniotic fluid sample to a 50 ml sterile tube, centrifuge at 800 rpm (~ 1,800 x g) for 5 min, and carefully discard the supernatant.
2. Add 300 µl of Buffer GHA to resuspend the precipitate, and transfer all the liquid to a 1.5 ml centrifuge tube. Add 20 µl Proteinase K solution, vortex for 10 sec and mix evenly, place at 75°C for 15 min, vortex and mix evenly several times during this period.
3. Add 300 µl of Buffer GHL and 300 µl of isopropanol, and mix well by vortex.
4. Add 15 µl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.

5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

J. FFPE sample

1. Sample treatment:

   a. Take 2-8 FFPE sections (5-10 μm thick and 1×1 cm^2 in size) and put them into a 1.5 ml sterile centrifuge tube. Add 300 μl environmental protection deparaffinization oil DPR(self-provided), 300 μl Buffer GHA and 20 μl Proteinase K into the centrifuge tube and 10 sec digest for 30-60 min at 75°C until the tissue mass disappears.

   b. Digest at 90°C for 1 h (add the sample after the temperature of the temperature control equipment has raised to 90°C.)

2. Transfer 300 μl solution from the lower layer to a new 1.5 ml centrifuge tube for subsequent experiments.
3. Add 300 μl of Buffer GHL and 300 μl of isopropanol, and mix well by vortex.
4. Add 15 μl of MagAttract Suspension GH, vortex to mix for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.
5. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

K. Bacteria sample

1. Simple treatment: Take 1-5 ml of bacterial culture solution, centrifuge at 10,000 rpm(~11,500×g) for 1 min, and discard the supernatant.
2. Add 300 μl of Buffer GHA to the thallus precipitate and shake until the thallus is completely suspended.

   Note: For Gram-Positive Bacteria that are difficult to lyse, skip step 2 and add lysozyme solution for cell wall disruption. The specific method is: Add 110 μl buffer (20 mM Tris, pH 8.0; 2 mM Na_2-EDTA; 1.2% TritonX-100) and 50 μl Lysozyme A (50 mg/ml, customer-prepared, TIANGEN, Cat.no.: GRT401-11), treat at 37°C for 30 min or more, vortex mixing several times during incubation.

   The extraction and treatment steps of bacteria in sputum samples include: 1) Add 1 M NaOH solution (self-prepared) to the sputum samples according to the volume ratio of 1: 1 for liquefaction for 30 min. If the sputum is viscous, a certain volume of 1 M NaOH solution can be added appropriately. 2) Place the centrifuge tube in a centrifuge, centrifuge at 4,700 rpm for 5 min, and discard the supernatant. 3) Add

300 µl Buffer GHA, fully vortex to suspend the precipitate, then put it into a metal bath, heat and lyse it at 95°C for 10 min, and cool it to room temperature.

3. Add 300 µl of Buffer GHL and 20 µl of Proteinase K, vortex until the thallus is completely suspended, and stand at 75°C for more than 15 min until the thallus becomes clear.
4. Add 300 µl isopropanol and 15 µl MagAttract Suspension GH, mix evenly by vortex for 1 min, let it stand for 9 min. Vortex to mix for 1 min every 3 min during the incubation.

   Note: In order to ensure complete resuspension of magnetic beads, please vortex to mix evenly before use.
5. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

After the lysis and DNA binding with MagAttract Suspension GH of the above samples, continue to the following purification and elution steps:

6. Add 900 µl Buffer GDZ **(check whether 96-100% ethanol has been added before use)** and shake to mix for 2 min.
7. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.
8. Add 500 µl Buffer GDZ and mix well for 2 min.
9. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.
10. Remove the centrifuge tube from the magnetic frame, add 900 µl of Buffer PWD **(check whether 96-100% ethanol has been added before use)**, and mix evenly for 2 min.
11. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.
12. Remove the centrifuge tube from the magnetic frame, add 300 µl of Buffer PWD, and mix evenly for 2 min.
13. Place the centrifuge tube on a magnetic frame and let it stand for 30 sec. After the magnetic beads are completely adsorbed, carefully remove the liquid.

14. Place the centrifuge tube on a magnetic frame 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, or it will be hard to elute DNA.

15. Remove the centrifuge tube from the magnetic frame, add 50-100 µl Buffer TB, shake and mix evenly. Incubate at 56°C for 10 min, and mix evenly upside down for 3 times (3-5 times each time) during the incubation.

16. Place the centrifuge tube on a magnetic frame and let it stand for 2 min. After the magnetic beads are completely adsorbed, carefully transfer the DNA solution to a new centrifuge tube and store it under appropriate conditions.

Detection of DNA Concentration and Purity

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

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

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If ddH_2O is used instead of elution buffer, the ratio will be lower, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.