TGuide S96 Magnetic Universal DNA Kit

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Cat. no. 4992995

Kit Contents

Contents	4992995 (96 preps)
Buffer GHA	50 ml
Buffer GHL	1 plate (96×300 µl/well)
Buffer GDZP	1 plate (96×900 µl/well)
Buffer GDZP	1 plate (96×500 µl/well)
MagAttract Suspension GHP2	1 plate (96×715 µl/well)
Buffer PWDP	1 plate (96×300 µl/well)
Buffer TB	1 plate (96×100 µl/well)
Proteinase K	2 × 1 ml
KF 96-Tip Comb	1 set
Handbook	1

Storage

This kit can be stored at room temperature (15-30°C) under dry condition for 12 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 process is safe and convenient. The extracted genomic DNA fragment is large, with high purity, and stable and reliable quality.

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

Features

- **Wide application:** Applicable to extract genomic DNA from blood, blood spots, animal tissues, saliva, swabs, mouthwash, amniotic fluid, FFPE, bacteria, etc.
- **Easy and fast:** Ultrapure genomic DNA can be obtained within 1 h by this kit matching with automated nucleic acid extractors.
- **High throughput:** Perfectly fit with TIANGEN's TGuide S96 to perform high throughput extraction.
- **High Purity:** The obtained DNA has high purity and can be directly used in downstream experiments such as chip detection and high-throughput sequencing.

Notes Please read these notes before using this kit.

1. This product is suitable for TGuide S96 Automated Nucleic Acid Extractor.
2. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the yield will be decreased.
3. Self-provided reagent: Isopropanol. For tissue samples extraction, please prepare 1 M DTT. For bacterial samples, 1 M NaOH should be prepared.

   If RNA residues need to be removed, please prepare RNase A (100 mg/ml). To extract FFPE samples, please purchase environmentally friendly deparaffinazation oil DPR separately.

Protocol

I. Sample treatment

A. Blood sample (anticoagulant)

1. Allow all blood sample to balance to room temperature before the experiment.
2. Add 200 µl of blood and 20 µl of Proteinase K into the deep-well plate of Buffer GHL .

B. Blood spot sample

1. Add 3-10 slides of blood spot samples with a diameter of 3 mm into a 1.5 ml centrifuge tube, and add 200-400 µl of Buffer GHA and 20 µl Proteinase K.

Blood spots silides	Volume of Buffer GHA
3	200 µl
5	300 µl
10	400 µl

2. After vortex for 10 sec, transfer the mixture into a thermostatic oscillator preheated to 75°C, and lysis for 45 min under 1,500 rpm thermostatic oscillation. (TIANGEN TGrade Dry Bath Incubator, OSE-DB-03, self-provided)
3. Add no more than 300 µl of the above lysate into the deep-well plate of Buffer GHL.

C. Tissue sample

1. Take 10-50 mg of animal tissue, cut it into small pieces, add 300 µl of Buffer GHA and 20 µl of Proteinase K, and grind it with an electric homogenizer for about 20 sec 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 digest at 65°C for 30 min for complete digestion.
   3) For rat tail samples, it is recommended to digest overnight at 56°C.
   4) For hair and feather stem samples containing hair follicles, add 20 µl of 1 M DTT (self-provided) and digest for 60 min to overnight.

Note: If there are tissue fragments 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 (self-provided).

2. Add no more than 300 µl of the above solution to the deep-well plate of Buffer GHL.

D. Saliva sample

1. Allow the saliva sample to balance to room temperature.
2. Add 300 µl saliva and 20 µl Proteinase K into the deep-well plate of Buffer GHL.

E. Swab sample

1. Swab sample processing:
   a) Dry swab sample:

      After the sample is collected, add 500 µl of Buffer GHA and 20 µl of Proteinase K and vortex for 10 sec to mix thoroughly.

   b) Swab sample containing preservation solution:

      If the volume of preservation solution is sufficient, directly pipet 300 µl of the solution to a 1.5 ml centrifuge tube for experiments, and if the volume of preservation solution is small, supplement it to 300 µl with Buffer GHA. Add 20 µl Proteinase K, vortex for 10 sec to mix well.
2. Add 300 µl of the above processed swab sample to the deep well plate of Buffer GHL.

F. Mouthwash/amniotic fluid, etc.

1. 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 the precipitate for resuspension, and transfer all the suspensions to a 1.5 ml centrifuge tube. Add 20 µl Proteinase K solution, vortex for 10 sec to mix thoroughly, incubate at 75°C for 15 min, vortex to mix several times during this period.
3. Add 300 µl of the processed sample to the deep-well plate of Buffer GHL.

G. FFPE sample

1. Take 2-8 FFPE sections (thickness: 5-10 μm; size: 1×1 cm^2 in) and put them into a 1.5 ml steril centrifuge tube. Add 300 μl of the deparaffinazation oil DPR (self-supplied), 300 μl Buffer GHA and 20 μl Proteinase K into an oscillating metal bath at 1,400 rpm for 30-60 min until the tissue mass disappears. (TIANGEN TGrade Dry Bath Incubator, OSE-DB-03, self-provided)
2. Place the sample at 90°C and digest for 1 h (add the sample after the temperature of the incubator is raised to 90°C).
3. Transfer 300 μl solution from the lower layer to the deep-well plate of Buffer GHL.

   Note: The deparaffinazation oil DPR and Buffer GHA will form two layers. Please transfer the processed sample in the lower layer solution for DNA extraction.

H. Bacterial sample

1. Sample processing: Take 1-5 ml of bacterial culture, centrifuge at 10,000 rpm(~11,500×g) for 1 min, and discard the supernatant.
2. Add 300 μl of Buffer GHA to the bacteria pellet and vortex until the pellet is completely suspended.

   Note: For gram-positive bacteria that are difficult to break the wall, replace step 2 with lysozyme treatment. The specific step is: Add 180 μl of the solution containing lysozyme (20 mM Tris, pH 8.0; 2 mM Na_2-EDTA; 1.2% Triton X-100; The final concentration of lysozyme is 20 mg/ml) and incubate at 37°C for more than 30 min.
3. Treatment of bacteria in sputum samples:
   1) According to the volume ratio of 1:1, add 1 M NaOH solution (self-provided) to sputum sample to liquefy for 30 min. If the sputum is viscous, increase the volume of 1 M NaOH solution appropriately.
   2) Centrifuge the tubes at 4,700 rpm for 5 min, and discard the supernatant.
   3) Add 300 μl of Buffer GHA to fully suspend the pellet, and then place in a metal bath (TIANGEN TGrade Dry Bath Incubator, OSE-DB-01, self-provided) to lyse the cells at 95°C for 10 min. After the lysis, cool to room temperature.
4. Add 300 μl of the above treated bacterial sample and 20 μl of Proteinase K to the deep-well plate of Buffer GHL.

II. Operation steps of TGuide S96

A. Preparation

Take out the vacuum package pre-packaged 96-deep well plate from the kit, mix it upside down for several times to resuspend the magnetic beads, remove the vacuum package, gently swing the 96-deep well plate to concentrate the reagent and magnetic beads to the bottom of the 96-deep well plate (or centrifuge in a plate centrifuge at 500 rpm for 1 min), carefully tear off the aluminum foil sealing film before use to avoid vibration of the 96-deep well plate and prevent liquid spillage.

B. Reagents and plate Distribution

position	E	F	G	H
Reagent	Blank	GDZP 500 µl	GHP2 715 µl Tip Comb	Blank
position	**A**	**B**	**C**	**D**
Reagent	GHL: 300 µl Sample★: 300 µl	GDZP 900 µl	PWDP 300 µl	TB 100 µl

Note: ★ Add 300 µl of processed sample into Buffer GHL Before the machine is started, and the deep-well plate of Buffer GHL with added sample should be placed at position A.

C. TGuide S96 running program

1. Add the processed sample to the 96-well plate of Buffer GHL. Place the magnetic rod comb in the deep-well plate of MagAttract Suspension GHP2. Put the plate on the machine according to the plate position distribution in step B.
2. Run the TGuide S96 purification program for genomic DNA.

The program is shown in the following table:

Step	Plate position setting	Mixing volume (μl)	Mixing speed	Mixing time (min)	Precipitation time (sec)	Adsorbing times	Adsorbing speed (mm/s)	Heating position	Heating temperature (°C)	Pause time (min)	Auto stop	Capture action
Capture Tip Comb	G	—	—	—	—	—	—	—	—	—	—	Capture
Collect Beads	G	715	Medium	0.5	30	1	1	—	—	—	—	—
Lysis	A	600	Medium	15	—	—	—	A	75	—	Yes	—
Binding	A	900	Middle-slow	10	30	1	1	—	—	—	—	—
Wash-I	B	900	Middle-slow	3	30	1	1	—	—	—	—	—
Wash-II	F	500	Medium	3	30	1	1	—	—	—	—	—
Wash-III	G	715	Medium	3	30	1	1	—	—	—	—	—
Wash-IV	C	300	Medium	3	30	1	1	D	—	8	—	—
Elution	D	100	Medium	8	30	2	1	D	60	—	—	—
Finish	G	—	—	—	—	—	—	—	—	—	—	Release

3. After the Lysis step is completed, pause the instrument, open the door, add 300 μl isopropanol to the position A, close the door, and continue to run the program .
4. After the extraction program of TGuide S96 Automatic Nucleic Acid Extractor is completed, aspirate the DNA in the position-D of the 96 deep-well plate and store at -20°C.