TIANGEN®

TGuide Smart Universal DNA Kit

(Prefilled 96-Deepwell plate)

For purification genomic DNA from blood, dried blood spot, bacteria, cells, saliva, oral swabs, animal tissues, etc.

TECHNICAL MANUAL

Cat. no. GDP605-E

Note: To use the TGuide Smart Universal DNA Kit, you must have the TGuide Smart Universal DNA(program no. DP605-01/02) installed on the TGuide S16/S32 Nucleic Acid Extractor.

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This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetic

Table Contents

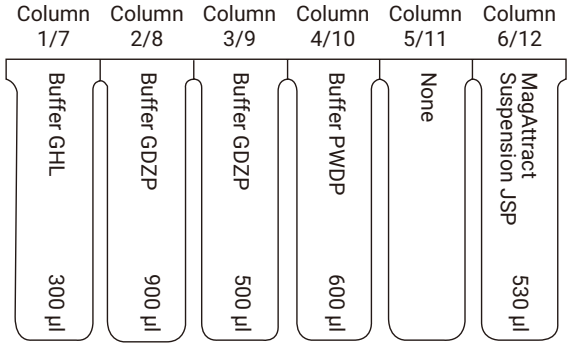
TGuide Smart Universal DNA Kit

Cat. no. GDP605-E

Kit Contents

Contents	GDP605-E (96 preps)
Buffer GHA	50 ml
Universal DNA Reagents	6 plates
Proteinase K	2×1 ml
RNase A (100 mg/ml)	400 μl
Buffer TB	15 ml
TGuide Smart Tip Comb	12 pcs

Universal DNA reagents composition



Storage condition

The kit can be stored in dry conditions at room temperature (15~30°C) for 12 months. If the solution has precipitation, it can be incubated in 37°C water bath for 10 min to dissolve the precipitation, without affecting the effect.

Materials to Be Supplied by the User

Isopropanol, 1M DTT, Lysozyme solution ,1M NaOH

1. Isopropanol should be prepared to extract any sample's genomic DNA with this kit. Please read the relevant operational steps carefully and add isopropanol into Column1/7 of the 96-Deepwell plate in the specified order and volume.
2. 1M DTT should be self-provided for extracting genomic DNA from samples of hair and feather with hair follicles;
3. Lysozyme solution (50 mg/ml) (TIANGEN, Cat. no.: GRT401) and reaction buffer for preparing lysozyme (20 mM Tris, pH 8.0; 2 mM Na_2-EDTA; 1.2% TritonX-100) should be self-provided for extracting bacterial genomic DNA.
4. 1M NaOH should be self-provided for extracting bacterial genomic DNA from sputum samples.

Product

The kit adopts magnetic beads and a unique buffer system, to isolate and purify genomic DNA with high quality from blood, dried blood spot, bacteria, cells, saliva, oral swabs, animal tissues and other samples. The uniquely embedded magnetic beads have a strong affinity for nucleic acid under certain conditions. When the conditions are changed, the magnetic beads can release the absorbed nucleic acid to rapidly separate and purify it. The whole process is safe and convenient, and the extracted genomic DNA has large fragments and high purity and is stable and reliable in quality. It can perfectly fit with TGuide S16 Nucleic Acid Extractor of TIANGEN for automated extraction.

DNA purified by this kit is suitable for downstream experiments including enzymatic digestion, PCR, qPCR, library construction, Southern Blot, chip analysis and high-throughput sequencing.

Features

- **Simple and fast:** Ultra-pure total DNA can be obtained by running TGuide S16 within 1 hr.
- **Safe and non-toxic:** No toxic reagents such as phenol/chloroform.
- **Strong commonality:** It can be used to extract genomic DNA from blood, tissues, swabs, bacteria, cells and other samples.
- **High purity:** The DNA obtained has high purity and can be directly used for chip detection, high-throughput sequencing and other experiments.

Notes

1. The sample should avoid repeated freezing and thawing, otherwise the DNA fragments extracted will be small and the amount of extraction will be reduced.
2. If there is precipitation in the Buffer GHA, it can be re-dissolved in 37°C water bath and used after mixing.
3. The pre-processing methods of different samples can be different. Please read the manual carefully before the experiment and prepare the reagent by users.
4. If you need a TGrinder H24R tissue homogenizer for electric homogenization of tissue, users need to purchase and ask TIANGEN for the grinding method.
5. After sample pre-processing, take an appropriate amount of the sample and add it to Column1/7 of the 96-Deepwell plate.
6. There are two automated extraction programs in the operational steps of this kit, Pause program DP605-01 and Non-stop program DP605-02. Wherein, DP605-01 is recommended to use in nucleic acid extraction from blood samples, and the instrument will automatically pause after the end of step 6. At this moment, users need to add 350 μl isopropanol in Column1/7 of the 96-Deepwell plate. Besides, if the sample is cells and users have a high demand for the purity of cell genomic DNA, it is also recommended to use the program DP605-01. Non-stop program DP605-02 is recommended to use in the nucleic acid extraction from other samples. Before the 96-Deepwell plate operation, users need to add 350 μl isopropanol in the Column1/7, and then run the DP605-02 program.
7. The elution buffer is not prefilled, users need to add the elution buffer to the Column 5/11 of the 96-Deepwell plate before performing on the instrument. The recommended range of elution buffer is 60~100 μl. The smaller the elution volume, the higher the nucleic acid concentration, but the lower the total yield may be. On the contrary, the larger the elution volume, the lower the nucleic acid concentration, and the higher the total yield may be. You can adjust it in the range of 60~100 μl as needed.

Operational steps

I. Preparation of universal DNA reagent composition

1. Take out a prefilled 96-Deepwell plate and invert it to re-suspend the magnetic beads; Gently shake to concentrate the reagent and magnetic beads to the bottom of the plate. Before use, remove the sealing film carefully to avoid liquid spatter or spills.
2. Add the Buffer TB of appropriate volume (60~100 μl) into the Column 5/11 of the 96-Deepwell plate.
3. Please read the operation manual carefully, and pay attention to the pause step of DP605-01 in the extraction process. If users use the non-stop program DP605-02, please add 350 μl isopropanol into the Column1/7 before operation.

II. Sample pre-processing

A. Blood sample (anticoagulant)

Blood sample type	Sample volume (μl)
Whole blood	200 μl
Buffy coat	100 μl

1. Please balance the blood sample or buffy coat sample to room temperature.

   Note1: For buffy coat samples, mix it with a vortex mixer for 2 min before adding to the Column1/7 of the 96-Deepwell plate.

   Note2: If the blood samples have cell clusters, they should also be mixed with a vortex mixer for 1~2 min before adding to the Column1/7 of the 96-Deepwell plate.

2. Add the sample (200 μl whole blood or 100 μl buffy coat) and 20 μl Proteinase K to the Column1/7 of the 96-Deepwell platee. Proceed to Part III, step 1.

B. Dried blood spot sample

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

Number of dried blood spot pieces	Buffer GHA (μl)
3 pcs	200 μl
5 pcs	300 μl
10 pcs	400 μl

2. After 10 sec of vortex mixing, put it into a thermostatic oscillator preheated to 75°C, and then dissolve by thermostatic oscillation for 45 min at 1500 rpm.

(TIANGEN TGrade Bath Shaker, OSE-DB-03, self-provided)

3. Add no more than 300 µl of the above digested solution into the Column1/7 of the 96-Deepwell plate.

   Proceed to Part III, step 1.

C. Tissue sample

1. Take 10~50 mg of animal tissue, use a liquid nitrogen or high-throughput tissue grinding homogenizer (TGrinder H24R tissue homogenizer, self-prepared, TIANGEN: OSE-TH-02) to adequately grind the tissue, or cut tissue into small pieces as much as possible, then add 300 µl Buffer GHA and 20 µl Proteinase K.

   1) Samples with tissue blocks visible to naked eyes are recommended to be digested at 65°C for 30 min until completely digested;

   2) Samples with sufficient homogenization do not need the above digestion process.

   3) Rat tail samples should be digested at 56°C overnight;

   4) Hair and feather samples with hair follicles should be added with 20 µl 1 M DTT (self-provided), digesting for 60 min overnight.

   Notes: After sample digestion, if there are tissue fragments, it is recommended to centrifuge at 12,000 rpm for 1 min to remove residual impurities. To remove RNA, add 4 µl RNaseA (100 mg/mL), shake it for 15 sec, and incubate at room temperature for 5 min.

2. Add no more than 300 µl of the above digested solution into the Column1/7 of the 96-Deepwell plate. Proceed to Part III, step 1.

D. Cell sample

1. Processing methods of different cell samples:

   (1) Suspension cell: Determine the number of cells collected (the collected number should not be more than 1×10^7) and centrifugate at 300 × g for 5 min. Then collect cells into a centrifuge tube and carefully remove all supernatant of the culture medium. Wash the cells with PBS solution, and then suck out the PBS solution as much as possible. Add 100 µl PBS to the cells, and completely re-suspend the cells.

   (2) Adherent cell: Determine the number of cells and remove the culture medium. Wash cells with the PBS solution, and suck out the PBS solution. Then add the PBS solution containing 0.10~0.25% trypsin to cells for digestion. When the cells are released from the wall of the vessel, add a medium containing serum to inactivate the trypsin. Transfer the cell solution to an RNase-free centrifuge tube and centrifuge it at 300 × g for 5 min. Collect cell pellets and carefully remove all supernatant. Add 100 µl PBS to the cells, and completely

re-suspend the cells.

Note: When collecting cells, it is important to remove all cell culture medium; otherwise, it will lead to incomplete digestion.

2. Add 200 µl Buffer GHA and 20 µl Proteinase K to the collected cell pellets, and completely re-suspend the cells.

   Note: To remove RNA, add 4 µl RNaseA (100 mg/mL), shake it for 15 sec, and incubate at room temperature for 5 min.

3. Add no more than 300 µl of the above digested solution into the Column1/7 of the 96-Deepwell plate. Proceed to Part III, step 1.

E. Saliva sample

1. Balance the saliva sample to room temperature.
2. Add 300 µl Saliva and 20 µl Proteinase K to the Column1/7 of the 96-Deepwell plate. Proceed to Part III, step 1.

F. Swab sample

1. Processing methods of swab samples:

   a) Dry swab sample: Add 500 µl Buffer GHA and 20 µl Proteinase K after sample collection and vortex it for 10 sec to mix well.

   b) Swab sample containing preservation solution: If the volume of preservation solution is sufficient, take out 300 µl directly to a 1.5 ml centrifuge tube for experiment, and if the preservation solution is insufficient, use Buffer GHA to supplement it to 300 µl. Add 20 µl Proteinase K, and vortex it for 10 sec to mix well.

2. Add 300 µl processed swab sample to the Column1/7 of the 96-Deepwell plate. Proceed to Part III, step 1.

G. Mouthwash/amniotic fluid and other samples

1. Add 1~20 ml mouthwash or amniotic fluid sample in a 50 ml sterile tube and centrifuge at 800 rpm (~1,800 × g) for 5 min, and carefully pour out the supernatant.
2. Add 300 µl Buffer GHA to the pellets and re-suspend, and transfer all suspension to a 1.5 ml centrifuge tube. Add 20 µl Proteinase K , vortex it for 10 sec to mix well, and place it at 75°C for 15 min. During this period, mix it for several times.
3. Add 300 µl processed sample to the Column1/7 of the 96-Deepwell plate. Proceedto Part III, step 1.

H. Bacterial samples

1. Sample processing:

   1.1 Processing steps of bacterial culture solution: Take 1~5 ml of bacterial culture solution and centrifuge it at 10,000 rpm (~11500 × g) for 1 min and discard the supernatant.

   1.2 Processing steps for bacteria in sputum samples:

   1) Add 1 M NaOH solution (customer self-provided) to the sputum sample at a volume ratio of 1:1 and liquefy it for 30 min. If the sputum is thick, add twice the volume of the 1 M NaOH solution.

   2) Place the tube in the centrifuge, centrifuge it at 4,700 rpm for 5 min, and then discard the supernatant.

2. Add 300 µl Buffer GHA to the bacterial precipitation, shake until the bacteria is completely suspended.

   Note1: For bacteria in sputum sample, fully suspend and shake the precipitation and then put it in a metal bath(TIANGEN TGrade Lite Dry Bath Incubator, OSE-DB-05/06, self-provided) for heating and lysis at 95°C for 10 min, and then cool it to room temperature.

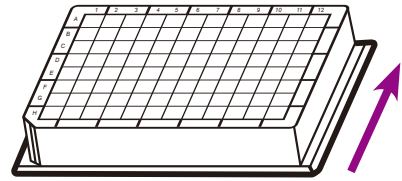
   Note2: For gram-positive bacteria that are difficult to break the wall, step 2 can be skipped and lysozyme solution can be added for breaking the wall. The specific method is: Add 110 µl Buffer (20 mM Tris, pH 8.0; 2 mM Na_2-EDTA; 1.2% Triton), and 70 µl Lysozyme solution (50 mg/ml, customer self-provided, Cat. no.: GRT401), process it at 37°C for more than 30 min, and vortex it for several times to mix well during the period. To remove RNA, users can add 4 µl RNase A (100 mg/ml) solution, shake it for 15 sec, and then place it at room temperature for 10 min.

3. Add 300 µl processed bacteria sample (add all the volume of processed grampositive bacteria sample if user is applying Note 2) and 20 µl Proteinase K to the Column1/7 of the 96-Deepwell plate. Proceed to Part III, step 1.

III. Operation steps of TGuide S16 Nucleic Acid Extractor

1. Place the plate on the reagent tank bracket.

2. Place the reagent plate on the base in the TGuide S16 Nucleic Acid Extractor. Insert the Tip Combs into the slots to ensure that they are well connected and firmed.



3. If you use the TGuide S16 Nucleic Acid Extractor, select “Pause program DP605-01” or “Non-stop program DP605-02” on the touch screen, click the icon  in the lower right corner of the screen and click the "RUN" button at the bottom of the screen to start the experiment.

   Notes: (1) The program DP605-01 involves a pause step. After step 6, the instrument will automatically pause. At this time, add 350 μl isopropanol to Column1/7 of the 96-Deepwell plate and click the icon to continue the program. This program is suitable for extracting blood samples, which can better ensure the nucleic acid yield of blood samples. If the samples are whole blood or blood cells, users are recommended to use this program for extracting nucleic acid. Besides, if the sample is cells and users have a high demand for the purity of cell genomic DNA, it is also recommended to use the program DP605-01.

   (2) The program DP605-02 is a non-stop program, and is suitable for extracting other samples except blood samples. Before the operation of the 96-Deepwell plate, users need to add 350 μl isopropanol to Column1/7, and then run the program.

4. At the end of the automated extraction process, take the DNA out of the Column 5/11 of the 96-Deepwell plate and store it under appropriate conditions. 96-Deepwell plate and tip comb are for single use only.

Appendix

1.Program

The automated extraction program of Universal DNA kit is shown in the following table.

Table 1-1

DP605-01:Automated Extraction Program of Universal DNA (Blood Samples) by TGuide S16 Nucleic Acid Extractor.

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Segments	Every time (sec)	Magnetization time(sec)	Cycle	Magnet speed (mm/s)
1	6	Transfer beads	0	8	—	530	—	5	5	0	2	2.5
2	1	Lysis	3	8	0	500	90	0	0	0	0	0
3	1	Lysis	2	8	0	900	90	0	0	0	0	0
4	1	Lysis	3	8	0	500	90	0	0	0	0	0
5	1	Lysis	2	8	0	900	90	0	0	0	0	0
6	1	Lysis	3	8	0	500	—	0	0	0	0	0
7	1	Pause	Add 350 μl isopropanol in the Column1/7 after cooling to room temperature.									
8	1	Bind	3	8	0	900	—	0	0	0	0	0

9	1	Bind	5	5	0	900	–	3	20	10	1	2.5
10	2	Wash1	0.5	8	0	100	–	0	0	0	0	0
11	2	Wash2	2	8	0	900	–	5	5	0	2	2.5
12	3	Wash3	0.5	8	0	100	–	0	0	0	0	0
13	3	Wash4	2	8	0	500	–	5	5	0	2	2.5
14	4	Wash5	1	8	0	600	–	5	5	0	2	2.5
15	6	Wash6	1	6	8	300	–	3	20	0	1	2.5
16	5	Elution	8	8	0	100	75	5	12	0	2	2.5
17	6	Discard	0.5	8	0	300	–	0	0	0	0	0

Table 1-2

DP605-02, Automated Extraction Program of Universal DNA (Other Samples) by TGuide S16 Nucleic Acid Extractor

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Seg-ments	Every time (sec)	Magneti-zation time(sec)	Cycle	Magnet speed (mm/s)
1	6	Transfer beads	0	8	–	530	–	5	5	0	2	2.5
2	1	Lysis	3	8	0	500	90	0	0	0	0	0
3	1	Lysis	2	8	0	900	90	0	0	0	0	0
4	1	Lysis	3	8	0	500	90	0	0	0	0	0
5	1	Lysis	2	8	0	900	90	0	0	0	0	0
6	1	Lysis	3	8	0	500	–	0	0	0	0	0
7	1	Bind	3	8	0	900	–	0	0	0	0	0
8	1	Bind	5	5	0	900	–	3	20	10	1	2.5
9	2	Wash1	0.5	8	0	100	–	0	0	0	0	0
10	2	Wash2	2	8	0	900	–	5	5	0	2	2.5
11	3	Wash3	0.5	8	0	100	–	0	0	0	0	0
12	3	Wash5	2	8	0	500	–	5	5	0	2	2.5
13	4	Wash5	1	8	0	600	–	5	5	0	2	2.5
14	6	Wash6	1	6	8	300	–	3	20	0	1	2.5
15	5	Elution	8	8	0	100	75	5	12	0	2	2.5
16	6	Discard	0.5	8	0	300	–	0	0	0	0	0

2. Related Products

Instrument and Accessories

Product name	Packing Size	Cat.No
TGuide S16 Nucleic Acid Extractor	1 set	OSE-S16
TGuide Smart Magnetic Tip Comb	200 pieces/box	OSE-TGA-S03
TGuide Single Sample Tank Bracket	5 pieces/box	OSE-TGA-S32

TGuide Smart Reagent Kits

Product name	Cartridge	Cat.No
TGuide Smart Magnetic Plant DNA Kit	48 preps	GDP607-DE
TGuide Smart Soil /Stool DNA Kit	48 preps	GDP612-DE
TGuide Smart Magnetic Tissue/Cell DNA Kit	48 preps	GDP602-DE
TGuide Smart Magnetic Plant RNA Kit	48 preps	GDP662-DE
TGuide Smart DNA Purification Kit	48 preps	GDP642-DE
TGuide Smart Blood/Cell/Tissue RNA Kit	48 preps	GDP661-DE
TGuide Smart Blood Genomic DNA Kit	48 preps	GDP601-DE
TGuide Smart Viral DNA/RNA Kit	48 preps	GDP604-DE
TGuide Smart Magnetic Tissue/Cell DNA Kit	96 preps	GDP602-E
TGuide Smart Blood/Cell/Tissue RNA Kit	96 preps	GDP661-E
TGuide Smart Universal DNA Kit	96 preps	GDP605-E