TGuide Smart Envir-DNA Kit

(Prefilled 96-Deepwell plate)

For purification DNA from various environmental samples such as soil, stool, fermentation products, water, pond sludge, marine sediment, and intestinal contents, etc.

TECHNICAL MANUAL

Cat. no. GDP613-E

Note: To use the TGuide Smart Envir-DNA Kit, you must have the TGuide Smart Envir-DNA program (No. DP613) installed on the TGuide S16/S32/S32 Pro/S96 Dex 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

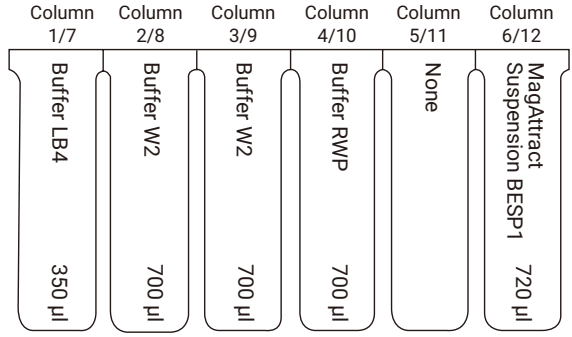
TGuide Smart Envir-DNA Kit

Cat. no. GDP613-E

Kit Contents

Contents	GDP613-E (96 preps)
Buffer SNA	80 ml
HA Removal Buffer CN	30 ml
RNase A (10 mg/ml)	1 ml
Buffer TB	15 ml
Buffer TE	15 ml
Envir-DNA Reagents	6 plates
Envir-DNA Grinding Beads Tube	2x48 tubes/pack
TGuide Smart Tip Comb	12 pcs

Envir-DNA reagents composition



Storage

The kit can be stored for 12 months under dry conditions at room temperature (15-30℃); if the solution produces precipitation, it can be preheated in a 37℃ water bath for 10 min to dissolve the precipitate.

Introduction

This kit uses a unique lysis solution combined with a specially optimized and highly efficient HA removal buffer to extract genomic DNA of Gram-negative bacteria, Gram-positive bacteria, fungi and other microorganisms from a variety of environmental samples, such as soil, feces, fermentation products, water, pond sludge, marine sediments, and intestinal contents. The grinding beads and homogenizer can effectively crush the microorganisms in the environmental samples to ensure the high efficiency and integrity of the extracted genomic DNA.

Genomic DNA purified by this kit has few impurities and good integrity, and can be directly used in downstream experiments of molecular biology such as PCR, library prep and NGS.

Features

Quick and easy: Quickly remove humic acid in 1 min.

High purity: Combined with magnetic bead purification, the extracted DNA has high purity and can be directly used in downstream experiments.

Wide application: Can be applied to microbial genomic DNA extraction for many types of samples.

Good compatibility: It can be applied to a wide range of TGuide S16/S32/S32 Pro automated nucleic acid extractor and can be extended for use with the TGuide S96 Dex Automated Nucleic Acid Extractor.

Notes <u>Please be sure to read this precaution before using the kit.</u>

1. The newly collected samples will get higher yield, and different samples should consult the corresponding optimal preservation conditions before sampling.
2. Excess DNA may inhibit downstream PCR reactions, and it is recommended to dilute the DNA template for use if inhibition appeared.
3. HA Removal Buffer CN needs to be pre-cooled at 2-8°C before use in order to achieve its optimal humic acid removal effect.
4. Buffer TB does not contain EDTA. In order to minimize the degradation of the extracted nucleic acids for long time storage, customer can replace the Buffer TB with the EDTA-containing Buffer TE if necessary, but pay attention to the fact that the EDTA will affect the downstream experiments, such as PCR, library construction, and so on.
5. Due to the complexity of environmental samples, which usually have more impurities, the concentration of extracted nucleic acids is low, and degradation of nucleic acids may occur in long time storage, etc. It is recommended to carry out downstream experiments as soon as possible after extraction.

6. This kit is equipped with two elution buffers. Choose corresponding one for the blank control.

Protocol

1. Prefilled 96-Deepwell plate

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/Buffer TE of appropriate volume (60~100 μl) into the Column 5/11 of the plate.

2. Suggested dosage for different samples

1) For common soil or fecal samples: Take 250 mg of soil samples (loam, clay, subsoil, garden soil, farmland soil, etc.) and 100 mg of fecal samples (e.g., cattle and sheep feces, poultry feces, poultry intestinal contents, fish and shrimp intestinal contents, etc.) in Envir-DNA Grinding Beads Tubes; for fecal samples preserved in alcoholic preservation solution, take an appropriate amount of the sample (not more than 100 mg of the dry mass) by high-speed centrifugation to remove the preservation solution. For fecal samples containing alcohol preservative, take an appropriate amount of sample (dry matter mass not more than 100 mg) and centrifuge it at high speed to remove the preservative, then transfer it to the Envir-DNA Grinding Beads Tube.

   Note: If the soil or fecal sample is high in impurities and rich in microorganisms, reduce the sample input appropriately. Otherwise, it may result in varying degrees of color residue in the eluate.

2) Samples with high water content: e.g. fermentation broth, cellar yellow water, washed honey or nectar, milk, etc., take an appropriate volume of sample, centrifuge at 12,000 rpm (~13,400×g) for 1-2 min, remove the supernatant, and collect the precipitate amount of about 10-150 mg, or when there is no significant precipitation after centrifugation, 50-100μl of liquid can be retained in the aspirate when discarding the supernatant and then add the 600μl Buffer SNA, resuspend and mix well, then transfer to Envir-DNA Grinding Beads Tube and proceed to the next step.

3) Aqueous or Air Filter Membrane: Cut up the filter membrane, 47 mm diameter filter membrane is cut up and put into 2 ml Envir-DNA Grinding Beads Tube, 147 mm diameter filter membrane is cut up and can be dispensed into 2-3 tubes of 2 ml Envir-DNA Grinding Beads Tubes or 1 tube of 5ml Grinding Beads Tube.

   Note: Only 48×2 ml Envir-DNA Grinding Beads Tubes are included in the kit, if more 2 ml Envir-DNA Grinding Beads Tubes or 5 ml Grinding Tubes are needed, the customer needs to bring their own.

4) Highly absorbent samples: e.g., powdered dacquoise, etc. Weigh 100 mg of sample in Envir-DNA Grinding Beads Tubes.

5) Other special samples:

a) Honey: take 10-15 g of honey, add 3-4 times the volume of pure water (provided by the customer), mix well, centrifuge at 8,000 rpm (~6,000×g) for 10 min, remove the supernatant, add 1ml of pure water (provided by the customer) to resuspend the precipitate, centrifuge at 8,000 rpm (~6,000×g) for 10 min, remove the supernatant, and then add 600 µl of Buffer SNA, resuspend the precipitate and transfer to Envir-DNA Grinding Beads Tube for the next step.

b) Fruiting body of fungal, tissue samples, intestinal contents containing intestinal tissues: take 10-100 mg samples and cut them into Envir-DNA Grinding Beads Tube.

3. Sample pre-treatment

1) Weigh the sample into the Envir-DNA Grinding Beads Tube according to the procedure "2. Suggestions for different sample amounts".

2) Add 600-700 µl of Buffer SNA and 10 µl of RNase A (10 mg/ml), vortex and mix to thoroughly break up and resuspend solid samples in solution, homogenize with TGrinder H24 and homogenize with a program of 20 sec of oscillation at 6 m/s with 20 sec intervals for a total of 6 cycles.

Note:

a) For samples that have been recommended to add Buffer SNA in "2. Recommendations for different samples", it is not necessary to add Buffer SNA in this step, only 10 µl of RNase A should be added.

b) For highly absorbent samples, the amount of Buffer SNA can be increased so that the volume of supernatant obtained by centrifugation after homogenization is 400-500 µl.

c) For larger sample quantities, a proportional mixture of Buffer SNA and RNase A can be prepared freshly.

d) Drier stools, denser or centrifuged samples need to be vortex and broken up as much as possible after the buffer is added, otherwise the subsequent homogenization will be affected;

e) If a tissue homogenizer is not available, a vortexer with high speed for 10 min or an oscillator with 50 Hz for 10 min can be used instead of homogenization.

f) If the integrity of the genomic bands is required to be high, the oscillation speed of the homogenization procedure can be reduced to 4 m/s or the number of cycles can be reduced to 2 cycles (the length of the vortexer and the oscillator can be shortened to 5 min or the oscillator speed can be reduced to 25 Hz), in which case the integrity of the genomic bands will be good, but the extraction rate will be slightly reduced.

3) (Optional step) After homogenization, oscillate for 10 min at 70°C 1,200 rpm on a thermostatic metal bath.

Note: Heating is not recommended for samples with dark color and high content of humic acid and other impurities to prevent the release of too many impurities and affect the purity of nucleic acid extraction.

4) Centrifuge at 12,000 rpm (~13,400×g) for 1 min and transfer the supernatant (~400-500 µl) to a 1.5ml centrifuge tube.

5) Add 200 µl of pre-cooled HA Removal Buffer CN at 2-8°C, vortex for 3-5 sec, and centrifuge at 12,000 rpm (~13,400×g) for 1 min.

Note:

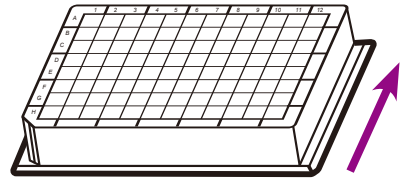
a) When the content of microorganisms and inhibitors in the sample is low, the amount of HA Removal Buffer CN can be reduced to 100 µl, and HA Removal Buffer CN cannot be added to the clarified liquid samples to improve the extraction rate.

b) When the content of impurities and inhibitors is high, the amount of HA Removal Buffer CN can be increased to 300 µl, which can improve the purity of nucleic acids.

4. TGuide S16/S32/S32 Pro/S96 Dex Automated Extraction Procedure

1) Transfer 500 µl of the processed supernatant from step "3. Sample pre-treatment" to column 1/7.

2) Place the reagent plate in the TGuide S16/S32/S32 Pro/S96 Dex Automated Nucleic Acid Extractor, insert the tip comb into the slot to ensure they are well connected and firmed.



3) Select the corresponding program and press the Start button to begin running the extraction program.

Enter the program of the TGuide S16/S32/S32 Pro/S96 Dex, select the "DP613" experimental program file and click the Run button to start the experiment.

4) At the end of the automated extraction process, take the DNA out from 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. It is recommended to store the eluted product at -30~-15℃ or -90~-65℃.

Appendix

Table 1: TGuide S16 Automatic Nucleic Acid Extraction and Purification Instrument Environmental Microbial Genomic DNA Extraction Procedure

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	0.5	5	0	700	--	5	10	0	2	2.5
2	1	Bind	8	8	0	850	--	5	10	0	2	2.5
3	2	Wash 1	5	8	0	700	--	5	10	0	2	2.5
4	3	Wash 2	3	8	0	700	--	5	10	0	2	2.5
5	4	Wash 3	3	8	0	700	--	5	10	0	2	2.5
6	6	Wash 4	3	8	8	700	--	5	10	0	2	2.5
7	5	Elution	8	7	0	100	75	5	10	0	2	2.5
8	6	Discard	0.5	8	0	700	--	1	0	0	0	--

Table 2: DNA extraction procedure for environmental microbial genome of TGuide S32/S32 Pro fully automated nucleic acid extraction and purification instrument

The automated nucleic acid extraction procedure is shown in the table below:

Lysis heating: OFF Lysis temperature: - - Lysis heating termination step: 1
Elution heating: ON Elution Temperature: 75 Elution heating start step: 7

Step	Slot	Name	Waiting time (min)	Mixing time (min)	Magnetic suction time (sec)	Mixing speed	Volume (μl)	Temperature (°C)	Adsorption mode
1	6	Transfer beads	0	0.5	120	Fast	700	--	Cycle
2	1	Bind	0	8	120	Fast	850	--	Cycle
3	2	Wash 1	0	5	120	Fast	700	--	Cycle
4	3	Wash 2	0	3	120	Fast	700	--	Cycle
5	4	Wash 3	0	3	120	Fast	700	--	Cycle
6	6	Wash 4	0	3	120	Fast	700	--	Cycle
7	5	Elution	8	8	120	Fast	100	75	Cycle
8	6	Discard	0	0.5	0	Fast	700	--	--

Table 3: TGuide S96 Dex Fully Automated Nucleic Acid Extraction and Purification Instrument Environmental Microbial Genomic DNA Extraction Procedure

Step	Well site	Name	Waiting time before mixing (mm:ss)	Mixing speed	Mixing time (mm:ss)	Mixing mode	Liquid volume (μl)	Magnetic suction time (mm:ss)	Number of magnetic aspira-tions	Heating temper-ature (°C)	Pause after comple-tion
1	6	Transfer beads	0	Fast	0:30	Normal mode	700	1:00	2	—	—
2	1	Bind	0	Fast	8:00	Normal mode	850	1:00	2	—	—
3	2	Wash 1	0	Fast	5:00	Normal mode	700	1:00	2	—	—
4	3	Wash 2	0	Fast	3:00	Normal mode	700	1:00	2	—	—
5	4	Wash 3	0	Fast	3:00	Normal mode	700	1:00	2	—	—
6	6	Wash 4	0	Fast	3:00	Normal mode	700	1:00	2	—	—
7	5	Elution	8:00	Fast	8:00	Normal mode	100	1:00	2	75	—
8	6	Discard	0	Fast	0:30	Normal mode	700	0	0	—	—