TIANGEN®

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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For Research Use Only. Not for use in diagnostic procedures.

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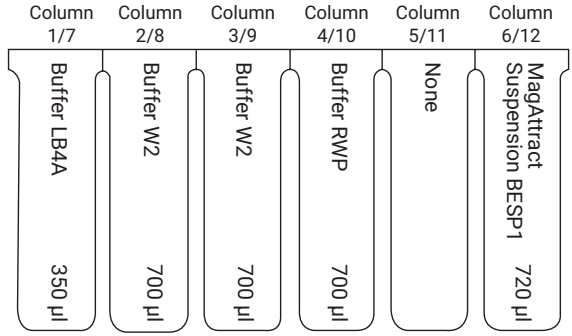
TGuide Smart Envir-DNA Kit

Cat. no. GDP613-E

Kit Contents

Contents	GDP613-E (96 preps)
Buffer SNA	80 ml
Buffer SNE	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 **Please be sure to read this precaution before using the kit.**

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 includes two elution buffers. The pre-filled buffer in the single cartridges is Buffer TB. If concentration and purity measurement using a spectrophotometer is required after extraction, use the included Elution Buffer TB (1 ml) as the blank control. Alternatively, if you replace the elution buffer with Buffer TE as mentioned in Note 4, use Buffer TE as the blank control for spectrophotometric measurement.

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) Soil samples: Use 250 mg for various soil types (e.g., loam, garden soil, farmland soil - common soils; sandy soil, deep-sea sludge, dry saline soil - lean soils). Use 150-200 mg for soils with high humic acid content (e.g., black soil, compost).
2) Fecal samples: For pelleted feces from mice, hamsters, etc., take 2-3 pellets roughly the size of rice grains (approx. 50-100 mg). For feces from livestock/poultry (pig, cow, sheep, poultry) or gut contents (without tissue), use 50-100 mg. For samples preserved in alcohol-based solution, take an appropriate amount (dry weight ≤100 mg), perform high-speed centrifugation, and remove the preservation solution.

   Note: If the soil or fecal sample has high impurity content and rich microbial load, the sample input amount can be appropriately reduced. Otherwise, it may lead to varying degrees of color residue in the eluate.
3) Liquid Samples: For samples like fermentation broth, pit yellow water, bacterial culture, milk, etc., take an appropriate volume, centrifuge at 12,000 rpm (~13,400×g) for 1-2 min, remove the supernatant, and collect approximately 10-150 mg of precipitate. If no significant precipitate is observed after centrifugation, retain 50-100 μl of liquid when aspirating the supernatant.
4) Water or Air Filter Membranes: Cut the filter membrane into pieces. For a 47 mm diameter filter, place the pieces into a 2 ml Envir-DNA Grinding Beads Tube. For a 147 mm diameter filter, distribute the pieces into 2-3 of the 2 ml Envir-DNA Grinding Beads Tubes or one 5 ml grinding tube.

   Note: The kit is standardly equipped with 48 of the 2 ml Envir-DNA Grinding Beads Tubes. Customers need to provide their own additional 2 ml or 5 ml grinding tubes if needed.
5) Other samples: Use 10-50 mg for tissue samples and 10-100 mg for plant samples. Cut into small pieces and place into the Envir-DNA Grinding Beads Tube.

3. Sample pre-treatment

1) According to section "2. Suggested dosage for different samples," weigh the sample into an Envir-DNA Grinding Beads Tube.
2) Add 700 μl of Buffer SNA or Buffer SNE and 10 μl of RNase A (10 mg/ml). Vortex to mix, ensuring solid samples are completely dispersed and resuspended in the

solution. Homogenize using the TGrinder H24 Tissue Homogenizer (customer provided, TIANGEN, Cat. No.: OSE-TH-01). Use the homogenization program: oscillate at 6 m/s for 20 sec, pause for 20 sec, repeat for 6 cycles.

Note: For samples typically rich in microbes (e.g., common soil, feces), Buffer SNA is recommended. For samples with low microbial content (e.g., lean soil), highly water-absorbent samples (e.g., dry koji), or when high genomic DNA integrity is required, Buffer SNE is recommended. Please refer to the following table:

Environmental samples		Sample Dosage Recommend-ation	Recomme-nded Lysis Buffer	Sample library information				
Soil Type	Common Soil	250 mg	SNA	Garden Soil	Farmland Soil	Bamboo Forest Soil	Pond Bottom Sludge	Lake Sediment
				Distiller's Grains	Koji	Pit Mud		
	High Humic Acid Soil	150–200 mg	SNA	Black Soil	Sheep Manure Compost	Chicken manure fertilizer	Earthworm manure fertilizer	Deep-Sea Sediment
	Lean Soil	**250 mg**	**SNE**	**Sandy Soil**	**Gravelly Soil**	**Clay**	**Dry Saline Soil**	
				Deep Well Soil	**Tin Ore Soil**	**Sulfide**		
	Other Difficult Soils	**250 mg**	**SNE**	**Red Soil**	**Daqu Powder**	**Fungal Matrix**		
Fecal Type	Pelleted Feces	50-100 mg (2-3 pellets, rice grain size)	SNA	Mouse feces	Hamster Feces	Rat Feces		
	Hard/ Lumpy Feces	50–100 mg	SNA	Rabbit Feces	Cow Dung	Sheep Dung	Pig Dung	Dog Feces
	Loose/ Soft Feces	50–100 mg	SNA	Poultry Feces	Rat Gut Contents	Poultry Gut Contents	Fish/ Shrimp Gut Contents	Pig Chyme
Filter Membrane Type		1-2 membranes, cut into pieces	SNA	Water Filter Membrane	Air Filter Membrane			
Liquid Type		**Centrifuge, remove supernatant, collect 10-150 mg precipitate**	**SNE**	**Fermen-tation Broth**	**Pit Mud Yellow Water**	**Milk**	**Yogurt**	**Honey**
				Nectar	**Leaf Surface Rinse**	**Sample in Preservation Solution**		
Other Samples	**Tissue Type**	**10–50 mg**	**SNE**	**Insect**	**Fungal Fruiting Body**			
	Plant Type	**10–100 mg**	**SNE**	**Plant leaf**	**Plant Roots**	**Corn Stalk**	**Wheat Bran**	**Bamboo Shoot**

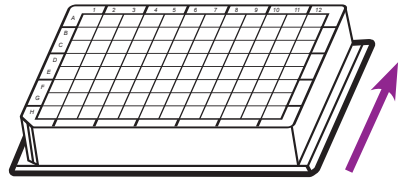
3) Centrifuge at 12,000 rpm (~13,400×g) for 1 min. Transfer the supernatant (approx. 400-500 µl) to a 1.5 ml microcentrifuge tube.

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

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	5	0	2	2.5
2	1	Bind	7	8	0	850	--	5	10	0	2	2.5
3	2	Wash 1	2	8	0	700	--	5	8	0	2	2.5
4	3	Wash 2	1	8	0	700	--	5	8	0	2	2.5
5	4	Wash 3	1	8	0	700	--	5	5	0	2	2.5
6	6	Wash 4	1	8	8	700	--	5	5	0	2	2.5
7	5	Elution	7	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	60	Fast	700	--	Cycle
2	1	Bind	0	7	120	Fast	850	--	Cycle
3	2	Wash 1	0	2	90	Fast	700	--	Cycle
4	3	Wash 2	0	1	90	Fast	700	--	Cycle
5	4	Wash 3	0	1	60	Fast	700	--	Cycle
6	6	Wash 4	0	1	60	Fast	700	--	Cycle
7	5	Elution	8	7	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	7:00	Normal mode	850	2:00	2	–	–
3	2	Wash 1	0	Fast	2:00	Normal mode	700	1:30	2	–	–
4	3	Wash 2	0	Fast	1:00	Normal mode	700	1:30	2	–	–
5	4	Wash 3	0	Fast	1:00	Normal mode	700	1:00	2	–	–
6	6	Wash 4	0	Fast	1:00	Normal mode	700	1:00	2	–	–
7	5	Elution	8:00	Fast	7:00	Normal mode	100	2:00	2	75	–
8	6	Discard	0	Fast	0:30	Normal mode	700	0	0	–	–