TGuide Smart Soil / Stool DNA Kit

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TGuide Smart Soil / Stool DNA Kit

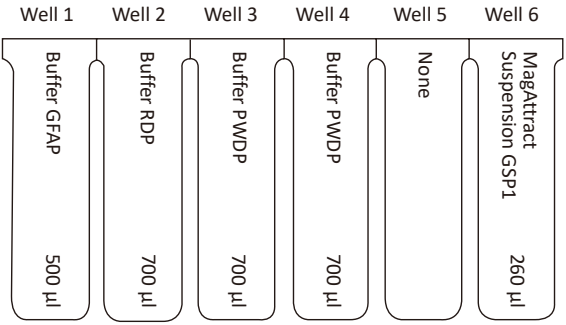
Cat. no. 4993549

Kit Contents

Contents		4993549 (48 preps)
4993542	Buffer SA	30 ml
	Buffer SC	5 ml
	Buffer SH	10 ml
	Soil /Stool DNA Reagents	48
	1 mm Grinding Beads	15 g
	RNase A (100 mg/ml)	200 µl
	Buffer TB	15 ml
4993546	TGuide Smart Tip Comb	12 pcs

Note: 4993546 is shipped and packaged separately

Soil/stool DNA reagent composition



Storage condition

All components of the kit can be stored in dry conditions at room temperature (15-30°C) for 12 months. If the solution precipitates, it can be preheated in a water bath at 37°C for 10 min before use to dissolve the precipitation, without affecting the effect.

Product

This kit adopts a unique derot buffer system to remove humic acid from soil samples as much as possible. It is also designed with grinding beads which effectively break soil samples in a variety of complex components to ensure the integrity of genomic DNA extracted from soil. Besides, it is also suitable for extracting genomic DNA from stool samples.

DNA extracted with this kit has less impurity and good integrity, which can be directly used for PCR, digestion and other downstream experiments of molecular biology.

Features

Wide applicability: It is suitable for extraction of samples from flower bed soil, flower pot soil, farmland soil, forest soil, silt, red soil, black soil, dust and other soil environmental samples extraction, as well as from stool.

Convenient operation: Ultra-pure genomic DNA can be obtained by running TGuide S16 for 42 minutes.

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

Notes <u>Be sure to read this note before using this kit.</u>

1. New samples will ensure a higher yield. For different samples, check the corresponding optimal storage conditions before sampling.
2. At the stage where the supernatant needs to be absorbed, the absorption of sediment must be avoided, otherwise the purity of the product will be affected.
3. Excessive DNA may inhibit following PCR reactions. In this case, it is recommended to dilute the DNA template before use.
4. Check buffer SC for precipitation before use. If any, please heat it at 37°C until it is completely dissolved before use.

Operational steps

1. Pre-packaged single reagent

Take out a pre-packaged single sample reagent strip and mix it upside down several times to re-suspend the magnetic beads; Gently shake the reagent and magnetic beads to concentrate at the bottom of the orifice plate. Before use, remove sealing film carefully to avoid the orifice plate from vibrating and liquid spatter.

2. Sample pre-processing

1) Soil sample processing:

Add 0.25-0.5g sample into 2 ml centrifuge tube, as well as 500 µl buffer SA, 100 µl buffer SC and 0.25g grinding beads for 15 min vortex mixing until the sample is mixed evenly; or use the TGrinder H24 tissue homogenizer (TIANGEN, OSE-TH-01, self-prepared) for mixing (oscillation at 6 M/S speed for 30 sec, with 30 sec interval and 2 cycles). Centrifuge it at 12,000 rpm (~13,400×g) for 1 min, transfer the supernatant (about 500 µl) to another 2 ml centrifuge tube.

Note: For some samples with low yield or requirements to extract fungal genome, it is suggested that after the samples are mixed by vortex mixing or tissue homogenizer, heat the mixture at 70°C for 15 min pyrolysis to improve the pyrolysis efficiency.

2) Stool sample processing:

Add 0.25-0.5g sample into 2 ml centrifuge tube. If the sample is liquid, then transfer 200 µl sample to the centrifuge tube, and 500 µl buffer SA, 100 µl buffer SC and 0.25g grinding beads. (Another 4 µl RNase A (100 mg/ml) is recommended for removing possible residual RNA) for vortex mixing until the sample is mixed evenly; or use the TGrinder H24 tissue homogenizer (TIANGEN, OSE-TH-01, self-prepared) for mixing. Heat the mixture at 70°C for 15 min pyrolysis to improve the pyrolysis efficiency. Centrifuge it at 12,000 rpm (~13,400×g) for 1 min, transfer the supernatant (about 500 µl) to another 2 ml centrifuge tube.

Note: For gram-positive bacteria which are difficult to break the cell wall, the temperature can be raised to 95°C to promote the pyrolysis.

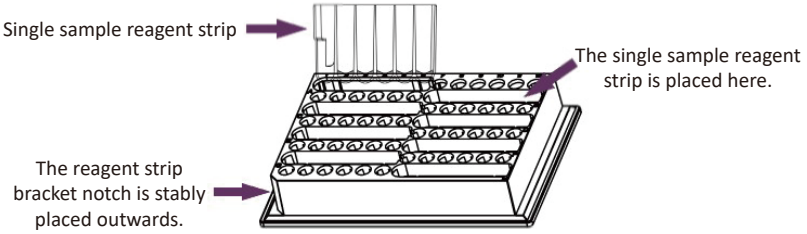
2.1 Add 200 µl buffer SH for mixing and then 5 min vortex mixing, and place it at 4 degrees for 10 min.

2.2 Centrifuge it at 12,000 rpm for 2 min, transfer the supernatant (about 400 µl) to the 1st well of a single reagent.

3. Operation steps of TGuide S16 Nucleic Acid Extractor

3.1 Add the above sample to the 1st well of the single sample reagent strip, and place the single sample reagent strip on the reagent tank bracket of TGuide S16 Nucleic Acid Extractor.

3.2 Place the reagent tank bracket on the base of the 96-hole plate of the TGuide S16 Nucleic Acid Extractor. Insert the Tip Comb into the slot of the Tip Comb to ensure that they are well connected and firmed.



3.3 If you use the TGuide S16 Nucleic Acid Extractor, select the corresponding program DP612 file on the on-board screen, click the icon in the lower right corner of the screen, or click the "RUN" button at the bottom of the screen to start the experiment.

The extraction process of S16 provided for DP612 is shown in the following table:

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Segments	Every time(s)	Magneti-zation time(s)	Cycle	Magnet speed (mm/s)
1	6	Collect beads	0.5	8	0	260	--	5	3	0	2	2.5
2	1	Bind	8	8	0	900	--	5	4	0	2	2.5
3	2	Wash 1	5	8	0	700	--	5	3	0	2	2.5
4	3	Wash 2	3	8	0	700	--	5	3	0	2	2.5
5	4	Wash 3	3	8	5	700	--	5	3	0	2	2.5
6	5	Elution	8	8	0	100	65	3	5	2	2	2.5
7	6	Discard	0.5	5	0	260	--	1	0	0	0	--

3.4 At the end of the automated extraction process, attract the DNA out of the 5th well of the single sample reagent strip and store it under appropriate conditions.

Detection of DNA concentration and purity

The size of the obtained genomic DNA fragment is affected by the sample storage time and shear force during operation. The concentration and purity of the obtained DNA fragments can be detected by agarose gel electrophoresis and UV spectrophotometer.

Ideally, the DNA should be absorbed at most at OD_{260}, where an OD_{260} value of 1 corresponds to approximately 50 μg/ ml double stranded DNA and 40 μg/ ml single stranded DNA.

The OD_{260}/OD_{280} ratio should be 1.7-1.9. The value will be lower if the eluting buffer is not used and deionized water is used. This is because pH and existing ion can affect the light absorption value, but it doesn't indicate low purity.