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TGuide S32 Magnetic Soil/ Stool DNA Kit

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TGuide S32 Magnetic Soil/Stool DNA Kit

Cat.no. 4992991

Kit Contents

Contents	4992991 (96 preps)
Buffer SA	2×30 ml
Buffer SC	2×5 ml
Buffer SH	25 ml
Soil and Stool DNA Extraction Reagents	6 plates
1 mm Grinding Beads	30 g
S32 Tip Comb	12 sets
Handbook	1

Storage

This kit can be stored at room temperature (15-25°C) under dry condition for 12 months. For longer storage, we suggest storing the kit at 2-8°C.

Introduction

The kit adopts a unique buffer system, which can remove humic acid from soil samples as much as possible. The grinding beads supplied in this kit can effectively crush various complex components in the soil sample to ensure the integrity of extracted genomic DNA from the soil. The kit is also suitable for extracting genomic DNA from stool samples.

The DNA recovered by the kit has few impurities and good integrity, and can be directly used in downstream molecular biology experiments such as PCR, enzyme digestion, etc.

Features

Wide application: It is applicable for the extraction of environmental samples such as flowerbed soil, flowerpot soil, farmland soil, forest soil, silt, laterite, black soil, dust and other soil types, and also applicable for the extraction of stool samples.

Easy operation: The extraction can be completed in a relatively short time.

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

Notes **Please read these notes before using this kit.**

1. Higher yield can be achieved with fresh samples. Please refer to the preservation instruction for different samples before sampling.
2. Be careful not to touch the precipitate in any supernatant collection steps, or else the column would be blocked and the product purity would be affected.
3. Excessive DNA may inhibit the downstream PCR reaction. In this case, it is recommended to dilute the DNA template and use it.
4. Before use, check whether Buffer SC has precipitation. If there is precipitation, please heat it at 37°C until it is completely dissolved before use.

Protocol

1. Preparation of DNA extraction reagent

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

2. Sample treatment

1) Treatment of soil sample:

Add 0.25-0.5 g of sample into a 2 ml centrifuge tube, add 500 μl of Buffer SA, 100 μl of Buffer SC and 0.25 g of grinding beads, vortex for 15 min until the sample is uniformly mixed or uniformly mixed using TGrinder H24 Tissue Homogenizer (TIANGEN, OSE-TH-01, self-provided) (6 m/s speed oscillation for 30 sec, interval for 30 sec, 2 cycles in total). Centrifuge at 12,000 rpm (~ 13,400 × g) for 1 min and transfer supernatant (about 500 μl) to a new 2 ml centrifuge tube.

Note: For some samples with low yield or for the extraction of fungal genomes, it is suggested to mix evenly with vortex or tissue homogenizer, and then heat the sample at 70°C for 15 min to improve the lysis efficiency.

2) Treatment of stool sample:

Add 0.25-0.5 g of sample to the 2 ml centrifuge tube, transfer 200 μl to the centrifuge tube if the sample is liquid, add 500 μl of Buffer SA, 100 μl of Buffer SC and 0.25 g of grinding beads, (RNA residue may exist in stool sample. To remove RNA, it is recommended to add 10 μl RNase A (self-provided)) and mix evenly with vortex or tissue homogenizer, then heat at 70°C for 15 min to improve lysis efficiency. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min and transfer the supernatant (about 500 μl) to a new 2 ml centrifuge tube.

Notes: For gram-positive bacteria that are difficult to break the cell wall, the temperature can be increased to 95°C to promote lysis.

2.1 Add 200 μl Buffer SH, mix well, vortex for 5 sec, and place at 4°C for 10 min.

2.2 Centrifuge at 12,000 rpm for 2 min at room temperature and transfer supernatant (about 400 μl) to column 1 and column 7 of 96-deep-well plate.

3. Operation steps of TGuide S32 Nucleic Acid Extractor

3.1 Place the 96-deep-well plate on the 96-deep-well plate base of TGuide S32 Automated Nucleic Acid Extractor.

3.2 Insert the S32 Tip Comb into the magnetic rod slot.

3.3 Run “Soil automatic extraction program” of TGuide S32 Extractor.

Turn on the supporting Windows Pad, double-click the “Purification” icon to enter the TGuide S32 control program, click “Run”, select the "DP612" experimental program file and click the “Run” button in the lower right corner to start the experiment.

The specific experimental procedures are shown in the following table:

Step	Slot	Name	Waiting time (min)	Mixing time (min)	Adsoption time (s)	Mixing speed	Volume (μl)	Temper-ature (°C)	Strong adsorption mode
1	6	Beads transfer	0	0.5	60	Fast	260	--	Yes
2	1	Beads adsorption	0	8	60	Fast	900	--	Yes
3	2	Washing 1	0	5	60	Fast	700	--	Yes
4	3	Washing 2	0	3	60	Fast	700	--	Yes
5	4	Washing 3	0	3	60	Fast	700	--	Yes
6	5	Elution	5	8	120	Fast	100	65	Yes
7	6	Beads disposal	0	0.5	0	Fast	260	--	--

3.4 Upon the completion of the automatic program, pipette out DNA sample in the 5th and 11th columns of the 96-deep-well plate and store under appropriate conditions.

Determination of DNA Concentration and Purity

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shearing force during operation. The concentration and purity of the obtained DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

DNA should have a significant absorption peak at OD_{260}, with OD_{260} value of 1 equivalent to about 50 μg/ml double stranded DNA and 40 μg/ml single stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If deionized water is used instead of elution buffer, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.