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Soil DNA Extraction Kit Instructions

Composition

Soil DNA Extraction Kit Cat. No.	5 Preps 4102005	50 Preps 4102050
2 ml Sample Tubes	5	50
Spin Columns	5	50
Proteinase K	120 µl	1.2 ml
Buffer PD	6 ml	55 ml
Buffer ST	1.2 ml	12 ml
Buffer TE	2 ml	20 ml
Buffer P	3 ml	28 ml
Buffer WB (concentrate)	1.5 ml	10 ml
Instructions	1	1

Storage

1. Proteinase K should be stored at -20°C.
2. If other reagents and articles are stored at room temperature (0~30°C), they can keep their performance unchanged for two years, and if they are stored at 2~8°C, they can extend the validity period of the product to more than two years (the product stored at 2~8°C should be restored to room temperature before use).

Technical Support

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Introduction

This product is suitable for extracting total DNA from 500 mg of fresh or frozen soil. The total DNA of various microorganisms in the lysed soil can be bound to the Spin Column, the PCR inhibitors such as degraded proteins and humic acids are filtered out, and the genomic DNA is washed by Buffer WB and eluted with Buffer TE, which can be used for various molecular biology experiments.

Equipment And Reagents to Be Supplied by Users

1. Deionized water, absolute ethanol and isopropanol.
2. 1.5 ml and 2 ml centrifuge tubes.
3. Pipettes and tips (to avoid contamination between samples, use pipette tips with filters).
4. Disposable gloves and protective equipment and tissues.
5. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Sample homogenizer, water bath, and vortexer.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25°C.
2. Set the water bath temperature to 70°C, incubate Buffer PD, Buffer ST and Buffer TE to70°C.
3. Add absolute ethanol to Buffer WB according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".

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Protocol

1. **Weigh less than 500 mg of soil and add to a 2 ml sample tube. Add 1 ml Buffer PD, close the lid, and place in a sample homogenizer for 30 sec at full speed, or vortex vigorously for 3 min if no sample homogenizer is available.**
   * If lyophilized soil powder is used, an additional 100 µl deionized water should be added to facilitate the suspension of soil particles.
2. **Add 20 µl Proteinase K, close the lid and mix well, incubate at 70°C for 15 min. During the incubation, vortex vigorously every 5 min for 30 sec.**
3. **Add 200 µl Buffer ST, vortex vigorously for 30 sec to mix well, centrifuge at 13000 rpm for 10 min.**
4. **Transfer the supernatant (about 1 ml or so) into a clean 2 ml collection tube (not provided).**
5. **Add 800 µl isopropanol, gently invert 4~6 times and mix well. Centrifuge at 13,000 rpm for 10 min.**
6. **Discard the supernatant and centrifuge at 3000 rpm for 5~10 sec to collect the residual supernatant to the bottom of the centrifuge tube. Transfer the residual supernatant with a pipette and retain the pellet at the bottom of the tube.**
7. **Add 100 µl Buffer TE incubated to 70℃, vortex until all the pellet dissolved.**
8. **Add 500 µl Buffer P and pipette directly with the tip for 6~8 times to mix well. Transfer the mixture to a Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**
9. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**
   * The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml collection tube upside down on a paper towel once.
   * Ensure that absolute ethanol has been added to Buffer WB.
10. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, and centrifuge at 14,000 rpm for 1 min.**
    * If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
    * Do not omit this step, otherwise the subsequent PCR effect may be affected due to the ethanol mixed in the purified nucleic acid.
11. **Discard the 2 ml collection tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 70°C in the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**
    * If the centrifuge does not have a leak-proof cap, change the centrifugation condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the detachment of the cap of the 1.5 ml centrifuge tube.
12. **Discard the Spin Column, the eluted DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**
    * When used for PCR amplification, the amount of DNA used as template should not exceed 1/10 of the final reaction volume (e.g., if the final reaction volume is 50 µl, the amount of DNA should not exceed 5 µl).