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# Stool DNA Extraction Kit Instructions

## Composition

| Stool DNA Extraction Kit<br>Cat. No. | 5 Preps<br>4101005 | 50 Preps<br>4101050 | 250 Preps<br>4101250 |
|---|---|---|---|
| Spin Columns | 5 | 50 | 250 |
| 2 ml Collection Tubes | 5 | 50 | 250 |
| Proteinase K | 120 µl | 1.2 ml | 1.2 ml×5 |
| Buffer S | 4 ml | 35 ml | 165 ml |
| Buffer ST | 4 ml | 35 ml | 165 ml |
| Buffer SL | 1.2 ml | 12 ml | 60 ml |
| Buffer WA (concentrate) | 1.9 ml | 12 ml | 60 ml |
| Buffer WB (concentrate) | 1.5 ml | 10 ml | 50 ml |
| Buffer TE | 1.2 ml | 12 ml | 60 ml |
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## Storage

1. Proteinase K can be transported at room temperature, please store at -20°C after receiving the product.
2. If other reagents and components are stored at room temperature (0~30°C), the performance can be maintained within two years without significant change; if the product is stored at 2~8°C, the validity period of the product can be extended 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 total DNA extraction from 150~200 mg of fresh or frozen human or animal feces. The human or animal genomic DNA, viral DNA, bacterial and parasitic DNA in the sample lysate can be bound to the Spin Column, the degraded proteins and PCR inhibitors are filtered to remove, and the genomic DNA is washed by Buffer WA and Buffer WB and eluted with Buffer TE, which can be used in various molecular biology experiments.

## Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml microcentrifuge tubes and 2 ml microcentrifuge tubes (some 2 ml microcentrifuge tubes lid will burst opened during 95°C incubation, please choose a suitable 2 ml microcentrifuge tube.).
3. Pipette and tip (to avoid contamination between samples, use a pipette tip with filter element).
4. Protective equipment such as disposable latex gloves and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml tubes).
6. Water bath and vortexer.

## Preparation Before Use

1. If the centrifuge has refrigeration function, please set the temperature to 25°C.
2. Set the water bath temperature to 70°C and 95°C, incubate Buffer ST and Buffer TE at 70°C.
3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".

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# Protocol

**** Freshly collected stool samples should be stored at -20°C or lower temperature. Even if the feces (human feces) are left at room temperature for 2-3 hours, degradation of the extracted DNA will be observed; if it is left for a longer time, the extracted DNA may be so degraded that the DNA bands will be invisible by electrophoresis.***

1. **Weigh 150~200 mg stool sample with 2 ml microcentrifuge tube; If the sample is liquid, aspirate 200 µl sample directly.**

* If the stool sample is very dry (e.g., constipated stool sample), do not exceed 120 mg, as this may result in subsequent steps clogging the Spin Column or excessive levels of PCR inhibitors in the extracted DNA.

2. **Add 600 µl Buffer S, close the lid, and vortex until the stool are sufficiently dispersed and no large particles are present.**

* If the stool particles are hard and difficult to disperse, you can use a tip or grinding rod to mash the stool particles.

3. **Add 600 µl Buffer ST, close the lid, shake vigorously to mix well, incubate at 95°C for 5 min.**

* If only intestinal cell DNA or DNA of gram-negative bacteria in stool is required, only a 5-minute bath at 70°C is required.

4. **Vortex for 15 sec and centrifuge at full speed (≥12,000 rpm) for 1 minute.**
5. **Add 20 µl Proteinase K to the bottom of a 1.5 ml microcentrifuge tube.**
6. **Pipette 200 µl supernatant from step 4 into this 1.5 ml microcentrifuge tube.**

* If there is floating grease on top of the centrifugal supernatant in step 4, aspirate the supernatant with the tip through the float without bringing the float.

7. **Add 200 µl Buffer SL, vortex for about 15 sec to mix well, and incubate the 1.5 ml microcentrifuge tube at 70°C water bath for 10 min.**
8. **Add 200 µl absolute ethanol, gently invert 4~6 times to mix evenly, spin down the mixture on the lid to the bottom of the tube.**
9. **Transfer the mixture from step 8 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12000 rpm for 30 sec.**

* Be careful not to stain the solution on the edge of the mouth of the Spin Column.

10. **Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid the contamination of the centrifuge by the filtrate adhering to the nozzle of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

* Make sure absolute ethanol has been added into Buffer WA.

11. **Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, centrifuge at 12000 rpm for 30 sec.**

* Ensure ethanol has been added into Buffer WB.

12. **Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14000 rpm for 1 min.**

* If the top speed could not reach 14000 rpm, centrifuge at top speed for 2 min.

* Do not omit this step, otherwise, it may cause problems in downstream applications due to the residual ethanol in the eluate.

13. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml microcentrifuge tube, add 150~200 µl 70°C incubated Buffer TE to the center of column membrane, close the lid, incubate for 1 min at room temperature, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cap, please change the centrifugation condition to 8000 rpm for 1 min to prevent the 1.5 ml microcentrifuge tube cover from falling off and damaging the centrifuge.

14. **Discard the Spin Column, the eluted DNA can be used for molecular biology experiment immediately or store the DNA at -20°C for later use.**

* When DNA is used for PCR amplification, the volume added should not exceed 1/10 of the volume of the PCR amplification system (e.g., if the amplification system volume is 50 µl, the amount of DNA added should not exceed 5 µl).