Stool Genome DNA Extraction KIT

NB-88-00025-50T
NB-88-00025-200T

For research use only.

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Catalog No.	Specification	Storage/Shelf life
NB-88-00025-50T	50T	Room temperature/1 year
NB-88-00025-200T	200T	Room temperature/1 year

Introduction

This Kit is suitable for separating total DNA from 180 to 220 mg of fresh or frozen human or animal feces. The genomic DNA, viral DNA, bacteria and parasite DNA of the human or animal in the dissolved feces can be bound to the nucleic acid purification column, and the degraded protein and PCR inhibitor are removed by filtration, and the genomic DNA is washed by WB and RP. After washing, it can be used in various molecular biology experiments by eluting with Buffer TE.

Kit Components

Component	NB-88-00025-50T
Solution SGE	80 ml
Solution InR	15 ml
Solution GA2	12 ml
Wash Buffer	70 ml
Solution RP	25 ml
Elution Buffer	2.5 ml
ProteinaseK	1 vial
Adsorption column G	50 set
User Manual	1 copy

Before starting

1. If the centrifuge has a cooling function, set the temperature to 25°C. All centrifugation in this experiment is performed at 25°C.
2. The water bath temperature was set to 70°C and 95°C, and Buffer SGE and Buffer

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TE were incubated at 70 ° C.

3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the reagent bottle label, and tick the box to make the “ethanol added” mark.

Operation steps

1. Weigh 180~220 mg of solid feces with a self-contained 2 ml centrifuge tube; if the feces are in a liquid state, draw 200 µl of feces directly.

2. Add 1.4 ml Buffer SGE and cover the tube. The vortex oscillated until the feces were fully dispersed and no large particles were present. 95°C water bath for 5 minutes.

Note: If you only need to detect DNA from gut cell DNA or Gram-negative bacteria in feces, you only need a 70°C water bath for 5 minutes.

3. Centrifuge at 12000 rpm for 1 minute at room temperature.

4. Pipette the around 1.2ml supernatant from the previous step into a new EP tube, add 1/5 volume of Solution InR (fully shake and mix before use), mix well and shake, stand for 1 min.

5. Centrifuge at 12000 rpm for 3 min and aspirate the supernatant into a new EP tube.

6. Repeat step 5 for once.

7. Pipette 200 µl of the centrifugation supernatant from step 6 into a new 1.5 ml centrifuge tube.

8. Pipette 10 µl of Proteinase K stock solution and mix by pipetting.

9. Add 200 µl Buffer GA2 and vortex for about 15 seconds to mix. The tube was placed in a 70 ° C water bath for 10 minutes.

10. Add 200 µl of absolute ethanol and gently mix for 4 to 6 times to mix evenly. Centrifuge at low speed for a few seconds to allow the solution on the cap to settle to the bottom of the tube.

11. Add the mixture obtained in the previous step to the column of the adsorption column provided by the kit (if it cannot be added once, it can be added in multiple times), centrifuge at 12,000 rpm (≈13,400×g) for 1 min. discarding the waste liquid in the collection tube, and re-placing the adsorption column in the collection tube.

12. Add 500 µL of Solution RP to the column, (check whether absolute ethanol has

been added before use) centrifuge at 12,000 rpm for 1 min, drain the waste from the collection tube, and place the column back into the collection tube.

13. Add 700 µL Wash Buffer to the column (check for added absolute ethanol before use), centrifuge at 12,000 rpm for 1 min, drain the waste from the collection tube, and place the column back into the collection tube.

Note: Step 13 can be repeated to further increase DNA purity.

14. Centrifuge at 12,000 rpm for 2 min and drain the waste from the collection tube. The column was allowed to stand at room temperature for a few minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column. The residual ethanol will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.)

15. Place the adsorption column in a new centrifuge tube (self-supplied), add 50-200 µL of Solution GE or sterilized water to the middle of the adsorption column, stand it at room temperature for 2-5 minutes, and centrifuge at 12,000 rpm for 1 min. Collecting the DNA solution and storing at -20 °C.

Note: 1) If the downstream test is sensitive to pH or EDTA, it can be eluted with sterile water. The pH value of the eluent has a great influence on the elution efficiency. If the eluent is used as water, the pH should be 7.0-8.5 (the pH of the water can be adjusted to this range with NaOH), and if the pH is lower than 7.0. The elution efficiency is not high.

2) Solution GE is preheated in a 65-70 °C water bath. Incubation at room temperature for 5 min before centrifugation which can increase yield; re-elution with an additional 50-200 µL of Solution GE or sterile water can increase yield.

3) If the final concentration of DNA is to be increased, the obtained solution can be re-added to the adsorption column, placed at room temperature for 2-5 min, centrifuged at 12,000 rpm for 1 min; if the elution volume is less than 200 µL, the final concentration of DNA can be increased, but May reduce total production. If the amount of DNA is less than 1 µg, it is recommended to elute with 50 µL of Solution GE or sterilized water.

4) Because the DNA stored in water is affected by acid hydrolysis, if it needs to be stored for a long time, it is recommended to use Solution GE to elute and store at -20°C.

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Attention

Freshly collected fecal samples should be stored at - 20℃ or lower in time. Even if feces (human feces) were stored at room temperature for 2-3 hours, degrading phenomena could be observed after DNA extraction; if placed for a longer time, the degraded DNA might be very serious, and even the DNA bands visible in electrophoresis could not be observed.