Soil Genome DNA Extraction KIT

NB-88-00039-50T
NB-88-00039-200T

Soil Genome DNA Extraction Kit

#Cat: NB-88-00039-50T Size: 50T
#Cat: NB-88-00039-200T Size: 200T

Catalog No.	Specification	Storage/Shelf life
NB-88-00039-50T	50T	Room temperature/1 year

Introduction

This product is suitable for the isolation of total DNA from 180~220 mg of fresh or frozen soil.The human or animal genomic DNA, viral DNA, bacterial and parasite DNA in the soil can be combined with nucleic acid purification column after dissolved. Degraded proteins and PCR inhibitors are filtered out.After genomic DNA is washed with WB and RP washing solution,elution with Buffer TE can be used in various molecular biology experiments.

Kit Components

Component	NB-88-00039-50T
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
Proteinase K	1vial(stored at -20 ° C)
Adsorption column G column	50 sets
User Manual	1 copy

I. Preparation before use

- If the centrifuge has a refrigeration function, please set the temperature to 25° C. All centrifugal operations in this experiment are performed at 25°C.
- Set the temperature of the water bath to 70℃ and 95℃, and incubate Buffer SGE and Buffer TE at 70℃.
- According to the instructions on the label of the reagent bottle, add absolute
- ethanol to Buffer WA and Buffer WB, and tick the box of the label to mark "alcohol added".

Operation steps

1. Weigh 180~220 mg of solid soil with a self-provided 2 ml centrifuge tube; if thesoil lotion is liquid, directly absorb 200 µl of the soil lotion.
   Note: If the content of the genome extracted from the soil is low, you can concentrate a larger amount of soil washing solution and take 200 µ l for subsequent experiments to improve the DNA yield.
2. Add 1.4 ml Buffer SGE and close the tube cap. Vortex until the soil is fully dispersed and no large particles are present. Water bath at 95°C for 5 minutes.
   Note: If you only need to detect the DNA of Gram-negative bacteria in the soil, you only need a 70°C water bath for 5 minutes.
3. Centrifuge at 12000 rpm for 1 minute at room temperature.
4. Pipette about 1.2ml of the supernatant from the previous step into a new EP tube, add 1/5 volume of Solution InR (vibrate and mix well before use), shake well and mix well, and let it stand for 1 min.
5. Centrifuge at 5.12000rpm for 3min, and transfer the supernatant to a new EP tube.
6. Repeat step 5 once.
7. Pipette 200 µl of the centrifuge 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. Place the centrifuge tube in a 70°C water bath for 10 minutes.
10. Add 200 µl of absolute ethanol and gently invert 4-6 times to mix evenly. Centrifuge at low speed for a few seconds to allow the solution on the tube capto settle to the bottom of the tube.
11. Add the mixture obtained in the previous step to the G column of theadsorption
12. column provided in this kit (if you cannot add it at one time, you can add it in multiple times), centrifuge at 12,000 rpm (≈13,400×g) for 1 min, and discard the collection tube Put the adsorption column back into the collection tube.
1. Add 500 µL of Solution RP to the adsorption column (check whether absolute ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
2. Add 700 µ L Wash Buffer to the adsorption column (check whether absolute ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

   Note: If you need to further improve DNA purity, repeat step 13.

3. Centrifuge at 14.12,000 rpm for 2 minutes, and discard the waste liquid in the collection tube. Leave the column at room temperature for a few minutes to dry thoroughly.

   Note: The purpose of this step is to remove the residual ethanol in the adsorption column. The residual ethanol will affect the subsequent enzymatic

   reactions (enzyme digestion, PCR, etc.).

4. Place the adsorption column in a new centrifuge tube (provided by yourself), add 50-200 µL of Solution GE or sterile water to the middle of the adsorption column, leave it at room temperature for 2-5 minutes, and centrifuge at 12,000rpm for 1 minute. Collect the DNA solution and store the DNA at -20°C.

   Note:

   1) If the downstream experiment 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 water is used as the eluent, the pH value should be 7.0-8.5(the pH value of the water can be adjusted to this range with NaOH), and the pH value is lower than 7.0 When the elution efficiency is not high.

   2) Solution GE is preheated in a 65-70 ℃ water bath. Incubate at room temperature for 5 min before centrifugation to increase the yield; eluting with another 50-200 µL Solution GE or sterile water can increase the yield.

   3) If you want to increase the final concentration of DNA, you can re-add the obtained solution to the adsorption column, place it at room temperature for 2-5 min, and centrifuge at 12,000 rpm for 1 min; if the elution volume is less than 200 µL, you can increase the final concentration of DNA, but May reduce total output. If the amount of DNA is less than 1 µg, it is recommended to elutewith 50 µL Solution GE or sterile water.

   4) Because DNA stored in water will be affected by acid hydrolysis, if long-term storage is required, it is recommended to elute with Solution GE and store at -20 ℃.

   Precautions：

* Freshly collected soil samples should be stored at -20°C or lower in time. Even if the soil (human soil) is 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 maybe degraded very seriously, and no DNA strips visible by electrophoresis may even be observed.