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Blood/Cell/Tissue Genomic DNA Extraction Kit

Catalog No.	Specification	Storage/Shelf life
EP007-50T	50T	Room temperature/1 year
EP007-100T	200T	Room temperature/1 year

Introduction

The kit is suitable for extracting high purity total DNA from fresh or frozen animal tissues, cells, blood, bacteria and other samples. DNA fragments with a maximum molecular weight of 50 KB can be purified without the use of toxic solvents such as phenol or chloroform and ethanol precipitation. The optimized buffer system was used to efficiently and specifically bind DNA from the pyrolysis solution to the silica-based centrifugal adsorption column. PCR and other inhibitors of enzymatic reaction could be effectively removed by two-step washing steps. Finally. high purity DNA could be obtained by using low-salt buffer or water elution. The purified DNA can be directly used in enzyme digestion, PCR, Real-Time PCR, library construction, Southern Blot, molecular markers and other downstream experiments.

Kit Components

Component	EP007-50T	EP007-200T
Proteinase K	1 vial	1 vial
Solution GA1	15 ml	60 ml
Solution GS	15 ml	60 ml
Solution GA2	25 ml	100 ml
Wash Buffer	30 ml	120 ml
Solution RP	60 ml	240 ml
Solution GE	15 ml	60 ml
Adsorption column G column	50 set	200 set
User manual	1 copy	1 copy

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Before starting

Wash Buffer and Solution RP: Add an appropriate amount of absolute ethanol to the Wash Buffer and Solution RP (labeled on the reagent bottle) before use.

Enzymatic Lysis Buffer (Must be prepared when extracting genomic DNA from Gram-positive bacteria, or you can purchase this product from our company)

Attention

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extraction amount will decrease。
2. If extracting the genome of a bacterial culture with a large accumulation of secondary metabolites or cell wall thickness, it is recommended to collect samples early in the logarithmic growth phase.
3. Add absolute ethanol to Wash Buffer and Solution RP as described in the label of the reagent bottle before the first use.
4. Before use, please check whether Solution GA1. Solution GS. Solution GA2 crystallize or precipitate. If there is crystallization or precipitation, please dissolve Solution GA1, Solution GS and Solution GA2 in 37 °C water bath.
5. If downstream experiments are sensitive to RNA contamination, add 20µL of DNase-Free RNase A (20 mg/ml) before adding Solution GA2. RNase A is not available in this kit and can be ordered separately from the company if needed.

Operation steps

1. Sample processing

a. When extracting 200µL of blood sample,add the sample to the centrifuge tube (self-prepared) and proceed directly to the next experiment.

Note: To handle larger volumes of blood,such as 300 µ L-1 ml, follow these steps: add 3 volumes of red blood cell lysate to the sample (for example, add 300 µ L of blood to 900 µ L of red blood cell lysate), mix by inversion, place it at room temperature for 5 min, then invert and mix several times. Centrifuge at 10000 rpm (~11.500 × g) for 1 min (if the maximum speed of the centrifuge is not allowed, centrifuge at 3000 rpm (~3.400×g) for 5 min), and aspirate the supernatant, leave leukocyte pellet,add 200 µ L of Solution GA1, shake until thoroughly mixed; when the blood sample volume is less than 200µL, add Solution GA1 to make up to 200µL.

and then proceed to the next experiment.

b. If the blood sample to be processed is anticoagulated blood from poultry, birds, amphibians or lower organisms, the red blood cells are nucleated cells, and the blood sample volume is 5-20 μL. Solution GA1 can be added to make up to 200 μL for subsequent experiments.

c. When extracting a sample of (2×10^7 cells). collect the cells by centrifugation. and resuspend the cells with 200 μ L of Solution GA1 to proceed to the next step

d. When the sample is treated as animal tissue, 200μL of Solution GA1 is quickly added after the liquid nitrogen is fully ground, and after resuspending sufficiently (can be properly ground), the next step can be carried out

e. When the sample is treated with 1-30 ml of bacteria, add the sample to the centrifuge tube (self-prepared),centrifuge,collect the precipitate and resuspend with 200μL of Solution GA1

Note: If the downstream experiments are sensitive to RNA, add 4 μ L of RNase A (100 mg/ml) solution, shake for 15 sec and place it at room temperature for 2 min.

2. **Add 20μL of Proteinase K to the above solution and mix well.**
3. **Add 200μL of Solution GS,add the treated sample,mix by shaking and gently centrifuge to remove the residual liquid attached to the tube wall. Warm bath at 56 °C for 10 min. If it is difficult to digest samples such as tissue,consider digesting time to 1-3 h or even overnight digestion**

Note: After the digestion is completed, the solution in the tube should be clear and transparent. If it is not clear and transparent, it is recommended to extend the treatment time to transparent. Or carefully absorb the upper part of the transparent solution after centrifugation, but there will be more DNA loss

4. **(Optional) If the digestion in the previous step is not complete, it may cause clogging of the adsorption column in subsequent operations. In this step, centrifuge at 4 ℃ 12,000 rpm (≈13,400 × g) for 1 min, carefully pipet the supernatant into a clean EP tube, and proceed next step**
5. **Centrifuge briefly to remove water droplets from the inner wall of the cap. Add 350 μL of GA2 and mix well by vortexing. Add 350μl absolute ethanol.Fully shake evenly and centrifuge briefly (just shake off the water droplets on the tube wall).**

Note:

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1). Mix immediately after adding Solution GA2

2). After Solution GA2 is added,white precipitates may be produced, which will not affect subsequent experiments. Some organizations may form a sol-like product after adding Solution GA2. At this time, it is recommended to perform violent vibration or vortex treatment.

6. Add the mixture obtained in the previous step to the column of the adsorption column provided by the kit (if it can not be added once,it can be added in multiple times)centrifuge at 12.000 rpm (≈13.400 × g) for 1 min. and pour off the collection tube. Waste liquid. put the adsorption column back into the collection tube

7. Add 500μL of Solution RP to the adsorption column (check whether ethanol has been added before use). centrifuge at 12.000 rpm for 1 min,pour off the waste liquid from the collection tube,and put the adsorption column back into the collection tube.

8. Add 500μL of Wash Buffer to the adsorption column (check whether ethanol has been added before use). centrifuge at 12.000 rpm for 1 min. pour off the waste liquid from the collection tube. and put the adsorption column back into the collection tube.

Note: To further improve DNA purity,repeat step 7

9. Centrifuge at 12.000 rpm for 2 min and drain the waste from the collection tube. Allow the column to stand 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,and the residual ethanol will affect the subsequent enzymatic reaction (enzyme digestion. PCR. etc.)

10. Place the adsorption column in a new centrifuge tube (self-prepared),add 50-200 μ L of Solution GE or sterilized water to the middle of the adsorption column, place it at room temperature for 2-5 minutes,centrifuge at 12.000 rpm for 1 min,and collect DNA, Solution. store DNA at -20°C

Note:

1). If the downstream experiments are 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 when the pH is lower than 7.0. Time is not efficient

2). Solution GE is preheated in a 65-70 ° C water bath. Incubation at room temperature for 5 min before centrifugation can increase yield; re-elution with an additional 50-200 μ L of Solution GE or sterile water can increase 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,centrifuge at 12.000 rpm for 1 min; if the elution volume is less than 200μL, increase the final concentration of DNA,but it may Reduce total production. If the amount of DNA is less than 1μg, it is recommended to elute with 50μL of Solution GE or sterilized water

4)Because DNA stored in water is affected by acid hydrolysis,if long-term storage is required, it is recommended to use Solution GE to elute and store at -20°C

DNA concentration and purity etection

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shear force during operation. The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and ultraviolet spectrophotometry. DNA should have a significant absorption peak at OD260 with an OD260 value of 1 equivalent to approximately 50 μg/ml double-stranded DNA and 40 μg/ml single-stranded DNA. The ratio of OD260/OD280 should be 1.7–1.9. If the elution buffer is not used when eluting, and the deionized water is used,the ratio will be lower because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low

Attention

1. Samples should be protected from repeated freezing and thawing,otherwise the extracted DNA fragments will be smaller and the amount of extraction will be reduced.
2. If there is a precipitate in Solution GA1,Solution GS or Solution GA2, re-dissolve in a 56 °C water bath, shake and use.
3. All centrifugation steps are performed using a benchtop centrifuge and centrifuged at room temperature.

Frequently Questions & Answers

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A. Column blocked

Suggestion: Please lyse the sample well,without obvious flocculation,proceed to the next step; wash with Solution RP multiple times (note that multiple washes will result in low genome recovery); disposable needle filter or 200-well screen filter

B. Low genome extraction rate

Suggestion:Increase digestion time, increase sample size. etc

C. Precipitate in solution is not dissolved

Suggestion: Solution will precipitate when the temperature is low. Please check if there is any precipitate before use. If there is precipitation,please incubate at 37 °C for a while,after the solution is clarified.

D. Wash Solution was not added to ethanol as required

Suggestion: Wash Solution does not add the required amount of ethanol according to the instructions to add the required amount of absolute ethanol,tighten the bottle cap after use to prevent ethanol volatilization

E. Selection of volume and time for dissolution

Suggestion: Dissolved volume will affect the final yield,the larger the dissolved volume,the higher the yield,but the concentration will be reduced. Please use the recommended volume of dissolution in the kit to ensure the best yield and concentration. After add Elution Buffer, 2~5 min at room temperature is more favorable for dissolving.