ELK Biotechnology

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Bacterial DNA Extraction kit

Catalog No.	Specification	Storage/Shelflife
EP021-50T	50T	Room temperature/1year
EP021-200T	200T	Room temperature/1year

Introduction

This product is suitable for the separation and purification of total DNA from 1-5 ml bacterial culture. After the bacteria are broken by lysozyme, they are dissolved by Buffer BL-1, and then precipitated by Buffer BL-2 to remove protein and cell debris. The genomic DNA in the centrifugal supernatant can be bound to the purification column. After washing with Buffer RP and Buffer WB to remove the proteins and PCR inhibitors remaining on the membrane, the genomic DNA is eluted with Buffer TE and can be used in various molecular biology experiments immediately.

Kit Components

Components	EP021-50T	EP021-200T	Storage
Buffer BL-1	15 ml	60 ml	RT
Buffer BL-2	15 ml	60 ml	RT
Buffer RP	30 ml	120 ml	RT
Lysozyme	600mg	2400mg	-20℃
Buffer WB	40ml	160ml	RT
Buffer TE	15 ml	60 ml	RT
Nucleic acid purification column	50 set	200 set	RT
User Manuals	1 Copy	1 Copy	RT

Reagents & items that users need to prepare:

1. Deionized pure water and absolute ethanol.
2. Pipette and tip (in order to avoid contamination between samples, it is recommended to use a pipette tip containing a filter)
3. Disposable gloves and protective equipment and paper towels
4. Desktop small centrifuge (can be equipped with a rotor for centrifuging 1.5ml centrifuge tubes and 2ml centrifuge tubes)

5. Water bath and vortex shaker

Preparation before use

1. If the centrifuge has a refrigeration function, please set the temperature to 25°C.
2. Set the temperature of the water bath to 37°C and incubate Buffer TE to 37°C.
3. Prepare an appropriate amount of 100 mg/ml lysozyme solution according to the number of bacterial specimens extracted at one time (calculated by adding 100 µl of lysozyme solution to each specimen): For example, to extract bacterial genomic DNA from 6 specimens, weigh 65 mg of lysozyme Enzyme dry powder, add 650 µl deionized pure water to make 650 µl lysozyme solution.
4. Note: Repeated freezing and thawing of lysozyme solution has a great influence on its activity. If more lysozyme solution is prepared at one time, it should be divided into small aliquots and stored at -20℃. After thawing, if there is any remaining lysozyme solution after use , Should be discarded, and can not be frozen again.
5. Add absolute ethanol to Buffer RP and Buffer WB according to the instructions on the label of the reagent bottle, and tick the box of the label to mark "absolute ethanol added".

Operation steps

1. Collect 1-5 ml bacterial culture in a 1.5 ml centrifuge tube, add 200 µl **Buffer TE**, and vortex to fully suspend the bacteria.
Note:Some divalent cations can inhibit the activity of lysozyme. If the bacterial culture medium contains divalent cations (such as MRS medium, etc.), a washing step should be added after the bacteria are collected by centrifugation: add 1ml of distilled water, vortex and vortex to suspend the bacteria after 12000 Centrifuge at rpm for 30 seconds, discard the distilled water, add 200 µl Buffer TE, and vortex to fully suspend the bacteria.

Bacteria collection method:

Suspension culture of bacteria: Centrifuge at 12000 rpm for 30 seconds to collect bacteria in 1~5 ml of bacterial culture, and discard the culture medium.

Single colony in a petri dish: add 200 µl **Buffer TE** to a 1.5ml centrifuge tube, scrape the colonies with an inoculating loop, and elute the bacteria in Buffer TE.

2.Add 100 µl of **lysozyme solution**, vortex for about 15 seconds to mix, and bath at 37°C for 30-60minutes.

Note: Most bacteria have fully broken their walls after 30 minutes in a water bath, but some bacteria with thicker cell walls (such as Staphylococcus aureus) need to be treated for 1-2 hours to completely break the walls. Please adjust the water bath time appropriately according to different types of bacteria.

3. Add **225 µl Buffer BL-1** and vortex for 30 seconds.
Note: If DNA is extracted from freshly cultured bacteria, part of the RNA in the bacteria may

be separated and purified together, but the presence of RNA does not affect PCR-related experiments. If you want to completely remove RNA, You can add RNase A solution (10 mg/ml, not provided in this kit) according to 1:200 in this step.

4. Add **225 µl Buffer BL-2**, shake the centrifuge tube vigorously 3~5 times, and then vortex for 30 seconds to mix.

5. Centrifuge at 13000 rpm for 2 minutes.

6. Pour the supernatant in step 5 into the nucleic acid purification column (the nucleic acid purification column is placed in a 2ml centrifuge tube), cover the tube, and centrifuge at 12000 rpm for 30 seconds.

7. Discard the filtrate in the 2ml centrifuge tube, return the nucleic acid purification column to the 2ml centrifuge tube, add 500 µl **Buffer RP** to the nucleic acid purification column, cover the tube, and centrifuge at 12000 rpm for 30 seconds.

Note: Make sure that absolute ethanol has been added to Buffer RP. The filtrate does not need to be completely discarded. If you want to avoid contamination of the centrifuge with the filtrate adhering to the nozzle of the centrifuge tube, you can tap the 2ml centrifuge tube upside down on a paper towel once.

8. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, add 600 µl **Buffer WB** to the nucleic acid purification column, cover the tube, and centrifuge at 12000 rpm for 30 seconds.

Note: Make sure that absolute ethanol has been added to Buffer WB.

9. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, and centrifuge at 14000 rpm for 1 minute.

10. Discard the 2ml centrifuge tube, put the nucleic acid purification column in a clean 1.5 ml centrifuge tube, add 50-100µl of **Buffer TE** incubated at 37°C in the center of the purification column, cover the tube, and let it stand at room temperature for 1 minute. Centrifuge at 12000 rpm for 30 seconds.

11. Discard the purification column, and the eluted DNA can be used in various molecular biology experiments immediately; or the DNA can be stored at -20°C for later use.

DNA concentration and purity detection

The size of the obtained genomic DNA fragment is related to factors such as the storage time of the sample and the shear force during operation. The recovered DNA fragments can be tested for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak at OD_{260}, and an OD_{260} value of 1 is equivalent to about 50 μg/ml double-stranded DNA and 40 μg/ml single-stranded DNA. The OD_{260}/OD_{280} ratio should be 1.7-1.9. If elution buffer is not used for elution and deionized water is used, the ratio will be lower, because pH and the presence of ions will affect the light absorption value, but it does not mean that the purity is low

Attentions

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extraction volume will also decrease.
2. All centrifugation steps are performed using a benchtop centrifuge and centrifuge at room temperature.

Frequently Q&A

A. Blocking the column

Suggestion: Please proceed to the next step after the sample is fully lysed and there are no obvious flocs; if there are obvious flocs, please carefully aspirate the supernatant after centrifugation to avoid clogging the adsorption column.

B. Low genome extraction yield

Suggestion: Increase the amount of sample and the amount of BL-1/BL-2 used in the same proportion to increase the genome yield.

C. Precipitation in Buffer is not dissolved

Suggestion: Buffer will precipitate when the temperature is low. Please check whether there is precipitation before use. If there is precipitation, please incubate at 37 ℃ for a while, and use it after the solution is clear.

D. Ethanol is not added to the Wash Buffer as required

Suggestion: Add the required amount of absolute ethanol according to the instructions, and tighten the cap after use to prevent the ethanol from evaporating.

E. Selection of dissolving volume and time

Suggestion: The dissolution volume will affect the final yield. The larger the dissolution volume, the higher the yield, but the concentration will decrease. Please use the recommended dissolution volume of the kit for dissolution to ensure the best yield and concentration.

Suggestion: After adding Buffer TE, leave it at room temperature for 2~5 minutes, which is more conducive to dissolution.

F. There is RNA contamination

Suggestion: Add our company's RNaseA at 1:200 in step 3 to prevent RNA contamination.