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Bacteria DNA Extraction Kit Instructions

Composition

Bacteria DNA Extraction Kit Cat. No.	5 Preps 3302005	50 Preps 3302050	250 Preps 3302250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
Lysozyme	60 mg	600 mg	3 g
Buffer L1	2 ml	14 ml	70 ml
Buffer L2	2 ml	14 ml	70 ml
Buffer WA (concentrate)	1.9 ml	12 ml	60 ml
Buffer WB (concentrate)	1.5 ml	10 ml	50 ml
Buffer TE	2.5 ml	25 ml	125 ml
Instructions	1	1	1

Storage

1. Please store lysozyme at 2~8℃.
2. If other reagents and items are stored at room temperature (0~30℃), they can keep their performance unchanged for 2 years, and if the product is stored at 2~8℃, the validity period can be extended to more than 2 years.

Technical Support

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Introduction

This product is suitable for total DNA extraction from 1-5 ml bacterial cultures. The bacteria cells are lysed by lysozyme and Buffer L1, then protein and cell debris were precipitated by Buffer L2, the genomic DNA in the supernatant can be bound to the Spin Column. After washing with Buffer WA and Buffer WB to remove the proteins and PCR inhibitors remaining on the membrane, genomic DNA is eluted with Buffer TE and can be used immediately for various molecular biology experiments.

Equipment And Reagents to Be Supplied by Users

1. Deionized water and absolute ethanol.
2. Pipettes and tips (pipette tips with filters are recommended to avoid contamination between samples).
3. Disposable gloves and protective equipment and paper towel.
4. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml tubes).
5. Water bath and vortexer

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the water bath temperature to 37℃ and incubate Buffer TE to 37℃.
3. Prepare an appropriate amount of 100 mg/ml lysozyme solution according to the number of samples extracted at one time (calculated according to the need to add 100 µl lysozyme solution to each sample): for example, if you want to extract bacterial genomic DNA from 6 samples, weigh 65 mg lysozyme dry powder and add 650 µl deionized pure water to prepare 650 µl lysozyme solution.

Note: Repeated freezing and thawing of lysozyme solution has a great impact on its activity, if more lysozyme solution is prepared, it should be divided into small portions and stored at -20℃, and if there is any remaining lysozyme solution after thawing and use, it should be discarded and not frozen again.

4. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".

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Protocol

1. **Collect 1-5 ml bacteria culture with a 1.5 ml centrifuge tube, add 200 µl Buffer TE, and vortex to fully suspend the bacteria.**

* Some divalent cations will inhibit the activity of lysozyme, if the bacterial medium contains divalent cations (such as MRS medium, etc.), a washing step should be added after centrifugation to collect the bacteria: add 1 ml deionized pure water, centrifuge at 12000 rpm for 30 sec after suspending the bacteria, discard the supernatant, then add 200 µl Buffer TE, and vortex to fully suspend the bacteria.
* Some special bacteria will change the pH value of the medium after culture (such as lactobacillus), which will inhibit the activity of lysozyme, and it is also necessary to wash once after centrifugation to collect bacteria, the method is the same as above.
* Bacteria collection method:

Suspension cultured bacteria: Centrifuge at 12,000 rpm for 30 sec to collect bacteria in 1-5 ml bacterial cultures, discard the medium.

Single colonies in petri dishes: Add 200 µl Buffer TE to a 1.5 ml centrifuge tube, scrape the colonies with an inoculation loop and elute the bacteria in Buffer TE.

2. **Add 100 µl lysozyme solution, vortex for about 15 sec to mix well and incubate at 37°C for 30-60 min.**

* Most bacteria have fully lysed the cell wall after 30 min incubation, but some bacteria with thick cell walls (such as Staphylococcus aureus) need to be treated for 1-2 hours to completely break the cell wall. Please adjust the incubating time appropriately for different types of bacteria.

3. **Add 225 µl Buffer L1, close the lid, shake vigorously for 5~6 times, vortex for 30 sec.**

* If DNA is extracted from freshly cultured bacteria, some of the RNA in the bacteria may be extracted together, but the presence of RNA does not affect PCR-related experiments. If you want to completely remove RNA, add 4 µl RNase A (100 mg/ml, not provided) at this step.

4. **Add 225 µl Buffer L2, close the lid, shake vigorously for 5~6 times, vortex for 30 sec.**
5. **Centrifuge at 13,000 rpm for 2 min.**
6. **Transfer the supernatant from step 5 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

9. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the subsequent PCR effect may be affected due to the ethanol mixed in the purified nucleic acid.

10. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 37°C in the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

11. **Discard the Spin Column, the eluted DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**