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Swab Elution Bacterial DNA Extraction Kit Instructions

Composition

Swab Elution Bacterial DNA Extraction Kit Cat. No.	5 Preps 4310005	50 Preps 4310050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Proteinase K	120 µl	1.2 ml
Buffer A10	150 µl	1.2 ml
Carrier RNA	40 µl	400 µl
Buffer AC	3 ml	18 ml
Buffer WB	1.5 ml	12 ml
Buffer TE	1.5 ml	5 ml
Instructions	1	1

Storage

1. Proteinase K and Carrier RNA can be transported at room temperature and stored at -20℃.
2. Other reagents and items can be stored for up to 2 years at room temperature (0-30℃). For longer storage, it is recommended to keep at 2-8℃. The product stored at 2~8℃ should be restored to room temperature before use.

Technical Support

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Introduction

This product is suitable for extracting total bacterial DNA from 180 µl swab elution. The bacteria in the swab elution are lysed by Buffer A10 and proteinase K to release DNA, and then the buffer system is adjusted by Buffer AC to bind DNA to the Spin Column, and the residual protein and PCR inhibitors are filtered and removed.

Equipment And Reagents to Be Supplied by Users

1. Absolute ethanol.
2. 1.5 ml centrifuge tubes and pipette tips (filtered tips prevent cross-contamination of aerosols between samples).
3. Protective equipment such as latex gloves, disposable masks, and paper towels.
4. Microcentrifuge (s) (with rotors for 1.5 ml and 2 ml tubes).
5. Vortexer.
6. A water bath or dry bath.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the water bath temperature to 56℃ and incubate Buffer A10 (precipitation will appears when the room temperature is below 15℃, and the precipitation disappears after 56℃ incubation) and Buffer TE to 56℃.
3. Add isopropanol to Buffer AC and add absolute ethanol to Buffer WB according to the instructions on the label of the reagent bottle, tick the box on the label to mark "Isopropanol Added" and "Ethanol Added".

Protocol

1. **Transfer 1 ml swab elution (or secretion elution) containing the precipitate to a 1.5 ml centrifuge tube, centrifuge at 12,000 rpm for 1 min, discard 800 µl supernatant, and retain about 200 µl pellet and supernatant.**

* If there is any precipitate in the swab elution, be sure to transfer to a 1.5 ml centrifuge tube, otherwise the sensitivity of subsequent tests will be affected.
* The elution buffer for swab or secretion can be replaced with normal saline or PBS solution.

2. **Add 20 µl Buffer A10 and 20 µl Proteinase K, incubate at 56℃ for 10 min.**

* For large sample volumes, premix Buffer A10 and proteinase K into a mixture in a 1:1 volume, directly add 40 µl mixture to each sample. The mixture should be used immediately, as use after more than 30 min of premixing may reduce lysis effectiveness.
* Ensure that there is no precipitation in Buffer A10 before use.
* If there are still insoluble substances after Proteinase K digestion, centrifuge at 14,000 rpm for 1 min, aspirate the supernatant and transfer to another clean 1.5 ml centrifuge tube, discard the pellet and then proceed to step 3.

3. **Add 5 µl Carrier RNA and 500 µl Buffer AC, close the lid, vortex for a few seconds to mix well.**

* For larger sample volumes, premix Carrier RNA and Buffer AC into a 1:100 mixture and directly add 505 µl mixture to each sample.
* Carrier RNA can be omitted when the total DNA content in the sample is exceeds 1 µg, if the amount of DNA in the sample cannot be determined, do not omit the addition of Carrier RNA, as this will seriously affect the sensitivity of the test.
* Ensure that isopropanol has been added to Buffer AC.

4. **Add the mixture from step 3 to 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.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 700 µl Buffer WB 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 Spin Column, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WB.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, 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 extracted nucleic acid may be mixed with ethanol and the subsequent experimental results may be affected.

7. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 50 µl Buffer TE incubated to 56℃ in the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12,000 rpm for 1 min.**

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

8. **Discard the Spin Column and the eluted DNA can immediately be used in a variety of molecular biology experiments or stored at -20℃ for later use.**