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Buccal Swab DNA Extraction Kit Instructions

Composition

Buccal Swab DNA Extraction Kit Cat. No.	5 Preps 4300005	50 Preps 4300050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Proteinase K	120 µl	1.2 ml
Buffer AT	2.5 ml	25 ml
Buffer SL	2.5 ml	25 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WB (concentrate)	1.5 ml	9.5 ml
Buffer TE	1.2 ml	12 ml
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Storage

1. Proteinase K should be stored at -20℃.
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

R&D Department of Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This product is suitable for total DNA (including genomic DNA, mitochondrial DNA, and possibly viral DNA) extraction from buccal swabs. The released DNA is bound to the Spin Column, the degraded protein and PCR inhibitors are filtered out, and the DNA is washed with Buffer WA and Buffer WB, and then eluted with Buffer TE for various molecular biology experiments.

Equipment and reagents to be supplied by users

1. Absolute ethanol
2. 1.5 ml and 2 ml centrifuge tubes
3. Pipettes and tips
4. Disposable gloves and protective equipment and tissues
5. Microcentrifuge (s) (with rotors for 1.5 ml and 2 ml tubes)
6. Thermostatic shaker or water bath and vortexer

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the thermostatic shaker or water bath temperature to 56℃ and 70℃ and incubate Buffer AT and Buffer TE to 56℃.
3. 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. **Cut off the swab part of the buccal swab and place it in a 2 ml centrifuge tube (not provided).**

* Sampling method: Wipe 10 times on each side of the cheeks in the mouth with a swab.

* The person being sampled should not eat or drink for half an hour before sampling.

2. **Add 400 µl Buffer AT incubated to 56℃, then add 20 µl Proteinase K and vortex for about 15 sec to mix.**
3. **Incubate the 2 ml centrifuge tube at 56℃ 900 rpm for 1 h.**

* If you incubate at a water bath, vortex several times every 15 min to help the sample lyse.

4. **Add 400 µl Buffer SL and vortex for about 15 sec to mix well. Incubate the 2 ml centrifuge tube at 70℃ 900 rpm for 10 min.**

* If you incubate at a water bath, vortex several times every 3 min to help the sample lyse.

5. **Add 200 µl absolute ethanol and vortex for about 15 sec. Spin down the solution on the cap to settle to the bottom of the tube.**
6. **Transfer 700 µl 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.**

* Be careful not to get the solution on the edge of the Spin Column, so that subsequent washing steps fail to wash the Spin Column.

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 its 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 extracted DNA.

10. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 80~150 µl Buffer TE preheated at 56℃ to the Spin Column, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000 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 and the eluted DNA can be immediately used in a variety of molecular biology experiments or stored at -20℃ for later use.**