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Saliva DNA Extraction Kit Instructions

Composition

Saliva DNA Extraction Kit Cat. No.	5 Preps 3521005	50 Preps 3521050
Saliva Collector	5	50
Spin Columns	5	50
2 ml Collection Tubes	5	50
Buffer WA (concentrate)	1.9 ml	19 ml
Buffer WB (concentrate)	1.5 ml	15 ml
Buffer TE	1 ml	12 ml
Instructions	1	1

Storage

If the kit is stored at room temperature (0~30℃), it can keep the performance unchanged for two years, and if the product is stored at 2~8℃, the validity period of the product can be extended to more than two 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 rapid (10-15 min) extraction of 3-15 μg of high-purity, large-fragment genomic DNA from human saliva. The lysate in the saliva collector is specially designed to quickly dissolve the oral epithelial cells in the saliva and keep the released DNA in a stable state. The saliva mixed with the lysate can be transported and stored at room temperature for more than 15 days without obvious DNA degradation, and high-quality genomic DNA can be extracted within 2 years if the mixture is stored at 2-8℃. After adsorption of DNA from the saliva lysate onto the Spin Column, the wash buffers remove impurities such as PCR inhibitors, and the DNA is finally eluted with Buffer TE and can be used immediately for PCR or related molecular biology experiments.

Equipment and reagents to be supplied by Users

1. Absolute ethanol
2. 1.5 ml tubes, pipettes, and tips
3. Disposable gloves and protective equipment and tissues
4. Microcentrifuge (s) (with rotors for 1.5 ml and 2 ml tubes)
5. Vortexer
6. Water bath may be required

Prepare before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol added".

Saliva sampling process

1. Saliva samples are asked not to eat or drink within half an hour before sampling, otherwise the DNA yield will be reduced.
2. Spit 1 ml saliva into the saliva collector (see label on the centrifuge tube). Immediately after collecting the saliva, open the 5 ml centrifuge tube (pink cap) and transfer all the saliva preservation solution into the saliva collector. Then remove the discard by rotating the receiving head counterclockwise on the top of the saliva collector, close the 5 ml centrifuge tube with the blue cap and screw it tight, mix vigorously several times at once, and freeze the sample to 2-8℃, or immediately proceed to the subsequent DNA extraction step.

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Protocol

Note:
If the mixture is used immediately for DNA extraction, vortex the mixture for 30 seconds before DNA extraction to ensure adequate release of saliva DNA. If the saliva and lysate are mixed by mail, as long as the mailing time is more than one day (24 hours), the vigorous shaking step can be omitted, and the operation can be carried out directly according to step 1.

1. **Add 800 µl mixture of saliva to lysate to a 1.5 ml centrifuge tube and centrifuge at the full speed (≥ 12,000 rpm) for 1 min.**

* This step is to precipitate and remove insoluble substances such as food debris.
* To increase the yield of DNA, collect 800 µl same sampler's saliva and lysate mixture each with two 1.5 ml centrifuge tubes and filter the same Spin Column in the next step. Typically, 1~7 µg of DNA can be obtained from 800 µl saliva mixture.
* The remaining lysate and saliva mixture can be stored at 2-8°C or lower for a long time.

2. **Transfer the supernatant from step 1 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.**

* If there is precipitation at the bottom of the tube, do not add it to the Spin Column.
* If extracting DNA from 1600 µl saliva mixture, filter the supernatant through the Spin Column in two fractions.

3. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 700 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* If Buffer WA is added and left at room temperature for 5-10 min, the residual salt in the last extracted DNA can be reduced.
* 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 centrifuge 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.

4. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 800 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.

5. **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 extracted nucleic acid may be mixed with ethanol and the subsequent experimental results may be affected.

6. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 80~100 µl Buffer TE to the Spin Column center, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If you want to improve the recovery efficiency of DNA, you can incubate Buffer TE to 65°C in advance, and extend the incubating time to 10~15 min after adding to the Spin Column, which can improve the DNA recovery efficiency by about 10-20%.
* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube cap coming off and damaging the centrifuge.

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