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Saliva Total RNA Extraction Kit Instructions

Composition

Saliva Total RNA Extraction Kit Cat. No.	5 Preps 4008005	50 Preps 4008050
Saliva Collector	5	50
Filter Columns	5	50
Spin Columns	5	50
Buffer WA (concentrate)	1.9 ml	19 ml
Buffer WBR (concentrate)	1.5 ml	15 ml
RNase-Free Water	1 ml	2 ml×2
Instructions	1	1

Storage

If the product is stored at room temperature (0~30℃), it can keep the performance of the product for two years without obvious change, 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

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Introduction

This product is suitable for rapid (15-20 min) isolation and purification of human saliva to obtain 1-2 μg of high-purity total RNA (including viral RNA that may be present in saliva). The lysate in the saliva collector with a special user-friendly design can quickly lyse the oral epithelial cells in the saliva and keep the released nucleic acids in a stable state. Saliva mixed with lysate can be stored at -20℃ for more than 30 days without obvious RNA degradation, and high-quality total RNA can be extracted within 1 year if the mixture is stored at -80℃. After the genomic DNA and food residues in the saliva lysate are removed by the Filter Column, ethanol is added to the filtrate to promote the adsorption of RNA to the Spin Column, and the two wash buffers efficiently remove impurities such as PCR inhibitors, and the RNA is finally eluted with RNase-free water, which can be immediately used in PCR or related molecular biology experiments.

Equipment And Reagents to Be Supplied by Users

1. Absolute ethanol.
2. RNase-free 1.5 ml centrifuge tubes and RNase-free tips with filters.
3. Disposable gloves, masks and protective equipment and tissues.
4. Microcentrifuge (s) (rotor with 1.5 ml and 2 ml tubes)
5. Vortexer
6. Laboratories that do not use RNases.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol added".
3. Since saliva and skin contain RNases, please wear a mask and latex gloves throughout the RNA extraction process.

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Protocol

Saliva sampling process

1. **Saliva sampling process**

   Spit 1 ml saliva into the saliva collector (see label on the centrifuge tube). After collecting the saliva, open the 5 ml centrifuge tube (pink lid) immediately 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 lid and screw it tight, mix vigorously several times at once, let stand at room temperature for 5 min, and freeze the sample to -20℃ or -80℃, or immediately proceed to the subsequent RNA extraction step.

* Saliva samplers should not eat or drink for half an hour before sampling, as this will reduce the yield of RNA.
* If the sample is cryopreserved, make sure that the sample is completely thawed and mixed before proceeding to step 2.

2. **Transfer 800 µl mixture of saliva and preservation solution into the Filter Column and centrifuge at the full speed (≥ 13,000 rpm) for 5 min.**

* This step is to remove insoluble substances such as genomic DNA and food debris. If liquid remains in the Filter Column after centrifugation, the centrifugation time can be extended until all the liquid has passed through the Filter Column.
* To increase RNA yield, an additional 800 µl filtrate of the same sample can be obtained in the same way, and ethanol can be added to the next step and filtered through the same Spin Column. Typically, about 1-2 µg of total RNA can be obtained from a mixture of 800 µl saliva and preservation solution.
* Additional Filter Columns can be ordered separately from Simgen Cat. No. 7501050。
* The remaining mixture of saliva and preservation solution can be stored at -20℃ for temporary storage or -80℃ for long-term storage.

3. **Discard the Filter Column, add 500 µl absolute ethanol to the filtrate, do not discard the tip, directly use the tip to pipette 6~8 times and mix well, transfer 700 µl mixture into a Spin Column, close the lid, and centrifuge at 12000 rpm for 30 sec.**
4. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, transfer all the remaining mixture from step 3 into 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.

5. **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.**

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

6. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, add 800 µl Buffer WBR 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 WBR.

7. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, and centrifuge at the full speed (≥13000 rpm) for 1 min.**

* Do not omit this step, otherwise the extracted nucleic acid may be mixed with residual ethanol.

8. **Discard the 2 ml tube, place the Spin Column in an RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free Water to the Spin Column center, close the lid, incubate at room temperature for 2 min, and centrifuge at 12,000 rpm for 30 sec.**
9. **Discard the Spin Column, the eluted RNA can be immediately used in a variety of molecular biology experiments or stored below -70℃ for later use.**