Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

Sperm Total RNA Extraction Kit Instructions

Composition

Sperm Total Extraction RNA Kit Cat. No.	5 Preps 5203005	50 Preps 5203050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Buffer SP	180 μL	1.8 ml
Proteinase K	120 μL	1.2 ml
Buffer L9	6 ml	55 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WBR (concentrate)	1.5 ml	12 ml
RNase-free Water	1.5 ml	2 ml×2
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Storage

1. Buffer SP and Proteinase K should be stored at -20℃, Buffer L9 should be stored 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, if stored at 2~8℃, the validity period can be extended to more than 2 years.

Technical Support

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

Introduction

This product is suitable for total RNA extraction from 450 μl freshly obtained sperm or sperm stored below -70℃. This kit uses a strong lysis buffer to lyse and remove proteins and genomic DNA from sperm. After adding absolute ethanol to the supernatant containing RNA, the RNA in the mixture is bound to the Spin Column, the residual protein and PCR inhibitors are filtered and removed, and the RNA is washed by Buffer WA and Buffer WBR, and then eluted with RNase-free water, which can be used for various molecular biology experiments immediately.

Equipment And Reagents to Be Supplied By Users

1. Absolute ethanol.
2. 1.5 ml and 2 ml tubes (RNase-free 1.5 ml and 2 ml tubes must be selected).
3. Pipettes and tips (RNase-free pipette tips with filters can avoid RNase contamination).
4. Protective equipment such as latex gloves, disposable masks, and paper towels.
5. Microcentrifuge (s) (rotor with 1.5 ml and 2 ml tubes).
6. Vortexer, incubate, or dry bath.
7. Laboratories that do not use RNases.

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the incubate or dry bath to 56℃.
3. 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".
4. Since saliva and skin contain RNases, please wear a mask and latex gloves throughout the RNA extraction process.
5. Try to use fresh sperm within 3 hours exvivo for RNA extraction, otherwise the recovery efficiency of the final RNA will be affected due to RNA degradation. If RNA extraction of freshly collected sperm cannot be performed in time, the collected sperm should be frozen below -70℃.

Protocol

1. **Add 450 µl liquefied sperm, 30 µl Buffer SP and 20 µl Proteinase K to the 2 ml centrifuge tube (not provided), vortex to mix well. Incubate at 56℃ for 10 min.**
2. **Add 1 ml Buffer L9 and vortex for 30 sec to mix well.**

* Buffer L9 is corrosive, please wear protective equipment when operating.

3. **Centrifuge at 13,000 rpm for 10 min. Add 500 µl absolute ethanol to an RNase-free 1.5 ml centrifuge tube for later use.**
4. **Carefully transfer 700 µl supernatant into the 1.5 ml centrifuge tube filled with absolute ethanol, do not discard the tip, pipette twice directly with the tip to mix well.**

* Do not aspirate the pale-yellow lower phase, so as not to affect the purification of the final RNA.

5. **Transfer 600 µl mixture from step 4 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.**
6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining mixture 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 centrifuge tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

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.**

* 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 700 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.

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 minutes.

* Do not omit this step, otherwise the extracted nucleic acid may be mixed with ethanol and the subsequent experimental results may be affected.

10. **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, 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.

* Do not elute RNA with less than 50 µl volumes of RNase-free Water, as this may affect the elution efficiency of RNA due to the inability of RNase-free Water to penetrate the Spin Column.

11. **Discard the Spin Column, the eluted RNA can be immediately used in a variety of molecular biology experiments or store the RNA below -70℃ for later use.**

* Even if the bands of genomic DNA are not detected by electrophoresis, it does not mean that there is no genomic DNA in the obtained RNA. If you want to completely remove contamination from the DNA, treat the obtained RNA with RNase-free DNase I.