SIMGEN
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

Blood Total RNA Extraction Kit

Composition

Blood Total RNA Extraction Kit Cat. No.	5 Preps 5201005	50 Preps 5201050
Spin Columns	5	50
2 ml Collection Tubes	5	50
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
Instructions	1	1

Storage

1. Store Buffer L9 at 2~8°C.
2. Other articles and items can be stored at room temperature without showing any reduction in performance and would be stable within 2 years. If the product is stored at 2~8°C, 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 extracting total RNA (including viral RNA in blood) from 500 µl fresh or below -70°C frozen blood or bone marrow. This kit uses a strong lysis buffer to lyse and precipitate hemoglobin and genomic DNA. After adding ethanol to the supernatant containing RNA, the RNA was added to the Spin Column. The RNA was bound to the Spin Column, and the remaining proteins and PCR inhibitors were filtered and removed. The RNA was washed by Buffer WA and Buffer WBR and eluted with RNase-free Water, which could be used in various molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tubes (RNase-free 1.5ml centrifuge tube MUST be used), 2 ml centrifuge tubes.
3. Pipettes and tips (To avoid RNase contamination, please choose RNase-free pipette tip with filter).
4. Latex gloves, protective items such as disposable face masks, and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.
7. A lab that does not use RNase.

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 25°C.
2. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the bottle label and tick the box on the label to mark "Ethanol Added".
3. Since RNase are present in saliva and skin, wear a mask and latex gloves during the entire RNA extraction process.
4. Try to use fresh whole blood or bone marrow within 3 hours in vitro for RNA extraction; otherwise, the final recovery efficiency of RNA will be affected due to the degradation of RNA. If fresh whole blood cannot be extracted in time, the whole blood can be frozen at -70°C or frozen at -20°C after being lysed with Buffer L9. (See Step 1 for details.)

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Protocol

1. **Add 1 ml Buffer L9 to a 2 ml centrifuge tube (not provided), then add 500 µl whole blood or bone marrow, shake the tube vigorously for 3-5 times, and vortex for 30 sec to mix well.**

* If fresh whole blood or bone marrow cannot be extracted in time for RNA extraction, the whole blood or bone marrow can be frozen at -70°C. The samples stored within one year will not affect the RNA extraction. It should be noted that the samples should not be frozen and thawed repeatedly before RNA extraction.

* If a -70°C refrigerator is not available, the lysed whole blood can be frozen at -20°C after this step. Freezing for two weeks does not affect the efficiency of RNA extraction.

* Buffer L9 is corrosive, please wear protective equipment for operation.

2. **Centrifuge at 13,000 rpm for 10 min. Add 500 µl absolute ethanol to a clean 1.5 ml centrifuge tube.**
3. **Transfer 700 µl supernatant to the 1.5 ml centrifuge tube (absolute ethanol added). Pipette twice to mix well.**

* The hematin contained in the supernatant can be removed during the washing step without affecting the purification effect of the final RNA.

4. **Transfer 600 µl 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 12000 rpm for 30 sec.**
5. **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 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded. To avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can slap the collection tube once on a paper towel.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA, close the lid and centrifuge at 12000 rpm for 30 sec.**

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

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 700 µl Buffer WBR, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to the Buffer WBR.

8. **Discard the filtrate, place the Spin Column back to 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 effect of the subsequent experiment may be affected because the purified nucleic acid is mixed with ethanol.

9. **Discard 2 ml Collection Tube, place the Spin Column in an RNase-free 1.5 ml centrifuge tube (not provided), add 50 µl RNase-free Water to the Spin Column, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cover, change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the tube lid falling off.

10. **Discard the Spin Column, and the eluted RNA can be immediately used in various molecular biology experiments or stored below -70°C for later use.**

* Even if the 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 decontaminate the DNA, treat the obtained RNA with DNase I (Simgen Cat. No. 8003050), which does not contain RNases.

* If used for viral RNA detection, an appropriate increase in the amount of template can improve the sensitivity of the detection.