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Micro Cells Total RNA Extraction Kit Instructions

Composition

Micro Cells Total RNA Extraction Kit Cat. No.	5 Preps 5004005	50 Preps 5004050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Carrier RNA	20 µl	180 µl
β-mercaptoethanol	50 µl	500 µl
Buffer RLT	2 ml	18 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WBR (concentrate)	1.5 ml	10 ml
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

1. Carrier RNA should be store 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 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 extracting total RNA from ≤10^5 cells. The kit's special addition of Carrier RNA assists in binding the RNA to the Spin Column, improving the efficiency of trace RNA recovery without affecting the RT-PCR reaction. After the RNA binds to the Spin Column, the PCR inhibitors remaining on the Spin Column are washed away by two wash Buffer, and the total RNA is eluted with RNase-free water and is ready for immediate use in RT-PCR reactions.

Equipment and reagents to be supplied by users

1. Absolute ethanol and 70% ethanol.
2. RNase-Free 1.5 ml centrifuge tubes.
3. Pipettes and tips (RNase-free pipette tips with filters are recommended to avoid RNase contamination).
4. Disposable gloves and protective equipment and tissues.
5. Microcentrifuge (s) (with rotors for 1.5 ml and 2 ml tubes).
6. RNase-free use labs.

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add 10 µl β-mercaptoethanol and 10 µl Carrier RNA to per 1 ml Buffer RLT and mix well. The addition of β-mercaptoethanol to Buffer RLT for one month did not affect the experimental results.
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. Because saliva and skin contain RNases, latex gloves and masks are required during the whole process of RNA extraction.

Protocol

1. **Collect the cells with a 1.5 ml centrifuge tube, flick the bottom of the tube to spread the cells apart. Add 300 µl Buffer RLT with β-mercaptoethanol and Carrier RNA and pipette the cells 5~10 times directly with a pipette tip to lyse them all.**

* If RNA is being extracted from cells in a single-well culture (e.g., a 96-well cell culture plate), follow these steps: Discard the culture medium from the cell culture plate, add 300 µl Buffer RLT with β-mercaptoethanol and Carrier RNA, pipette with a tip to the adherent cells to lyse the cells. Transfer all the cell lysate to a 1.5 ml centrifuge tube and proceed directly to step 2.

* If cells are in suspension, collect the cells as follows: Centrifuge at 300×g for 5 min and discard the culture.

2. **Add 300 µl 70% ethanol, do not discard the tip, pipette three times directly with the tip to mix well, and transfer the mixture to 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.**
3. **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.

4. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 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.

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 subsequent RT-PCR effect may be affected due to the ethanol mixed in the extracted nucleic acid.

6. **Discard the 2 ml tube, place the Spin Column in a clean RNase-free 1.5 ml tube, add 50 µl RNase-free Water to the Spin Column center, 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 1 min to avoid the lid coming off and damaging the centrifuge.

7. **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.**

* If DNA is need to be removed completely, digest the residual DNA with RNase-free DNase I.