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Rapid Cultured Cells Total RNA Extraction Kit Instructions

Composition

Rapid Cultured Cells Total RNA Extraction Kit Cat. No.	5 Preps 5024005	50 Preps 5024050	250 Preps 5024250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
Buffer RLY	3 ml	30 ml	150 ml
Buffer WBR (concentrate)	2 ml	20 ml	50 ml×2
RNase-free Water	1.5 ml	2 ml×2	30 ml
Instructions	1	1	1

Storage

If the kit is stored at room temperature (0~30℃), it can maintain no significant change in performance 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 does not involve the use of phenol/chloroform and is suitable for rapid extraction of total RNA from 1-2×10^6 cultured cells. Cultured cells are lysed in Buffer RLY to release RNA, which binds to the Spin Column without the addition of absolute ethanol, and solubilized proteins and PCR inhibitors are filtered out. After washing with the Buffer WBR, the RNA is eluted with RNase-free water, which can be used for various molecular biology experiments such as RT-PCR, Northern blot, Dot blot, mRNA extraction, etc.

Equipment And Reagents to Be Supplied by Users

1. Absolute 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 rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.
7. RNase-free use labs.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WBR according to the instructions on the label of the reagent bottle and tick the box on the label to mark it with "Ethanol Added".
3. Because saliva and skin contain RNases, latex gloves and masks are required during the whole process of RNA extraction.

Protocol

1. **Collect 1-2×10^6 of cultured cells in a 1.5 ml centrifuge tube and flick the tube wall to scatter the cells.**

* Cell Collection Method:

A. Suspension cultured cells: Centrifuge at 300×g for 5 min to collect approximately 1-2×10^6 cultured cells, discard the supernatant, proceed to step 2.

B. Adherent cultured cells: discard the culture supernatant, trypsinization and suspend the cells, centrifuge at 300×g for 5 min to collect about 1-2×10^6 cultured cells, discard the trypsin supernatant, proceed to step 2.

C. Cells cultured in a single well in a cell culture plate (if the number of cells in a single well ≤ 1×10^5, use the Micro Cells Total RNA Extraction Kit: Simgen Cat. No. 5001050): Discard the culture supernatant, add 500 µl Buffer RLY directly, and pipette the cells several times with a pipette tip to lyse the cells, proceed directly to step 3.

2. **Add 500 µl Buffer RLY, pipette and mix until there are no clumps of cells, and incubate at room temperature for 1 min.**
3. **Transfer the mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at its full speed for 2 min.

4. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, and centrifuge at 12,000 rpm for 30 sec.**

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

5. **Repeat step 4 once.**
6. **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 its 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.

7. **Discard the 2 ml Collection Tube, place the Spin Column in a clean RNase-free 1.5 ml centrifuge tube, add 50-100 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 for 1 min to avoid the tube lid coming off and damaging the centrifuge.

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

* The DNA may be residues in the extracted RNA, if DNA needs to be removed completely, digest the residual DNA with DNase I. (Simgen Cat. No. 8003050).