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Animal Tissue/Cultured Cells Total RNA Extraction Kit Instructions

Composition

Animal Tissue/Cultured Cells Total RNA Extraction Kit Cat. No.	5 Preps 5001005	50 Preps 5001050
Filter Columns（with 2 ml Collection Tubes）	5	50
Spin Columns（with 2 ml Collection Tubes）	5	50
β-Mercaptoethanol	50 µl	500 µl
Buffer RLT	4 ml	32 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

If the kit is stored at room temperature (0~30°C), it can maintain no significant change in performance within 3 years; if stored at 2~8°C, the validity period can be extended to more than 3 years.

Technical Support

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

Introduction

This kit is no need of phenol and chloroform extraction and is suitable for extracted total RNA from ≤30 mg tissue or ≤1×10^7 cultured cells. The tissue/cultured cells are lysed in the lysis buffer and released RNA, the RNA is bound to the spin column after adding ethanol, and the protein and PCR inhibitor are filtered to remove it. After RNA is washed by two wash buffers and eluted with RNase-free Water, it can be used in RT-PCR, Northern blot, Dot blot, mRNA isolation and other molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol and 70% ethanol.
2. RNase-free 1.5 ml centrifuge tubes.
3. Pipettes and pipette tips (RNase-free pipette tips must be selected, DNase-free & RNase-free tips with filter element recommended).
4. Protective equipment such as disposable latex gloves and paper towels.
5. Centrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.
7. Laboratory without RNase use.

Preparation Before Use

1. If the centrifuge has refrigeration function, please set the temperature to 25°C.
2. Add 10 µl β-mercaptoethanol to every 1 ml Buffer RLT and mix well. The use of Buffer RLT with β-mercaptoethanol within one month does 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. Since saliva and skin contain RNase, disposable gloves and masks are required during the whole process of RNA extraction.

Animal Tissue Protocol

1. **Grind 200~400 mg chopped animal tissue in the mortar into powder quickly with liquid nitrogen. Then weigh 20~30 mg tissue into a 1.5 ml centrifuge tube precooled with liquid nitrogen.**

* When grinding tissues, liquid nitrogen should be added in time to avoid tissue thawing, to avoid endogenous RNase recovery and degradation of RNA.
* Do not use tissue larger than 30 mg, as this may cause clogging of the Filter Column and contamination of the purified RNA with genomic DNA.

2. **Add 600 μl Buffer RLT with β-mercaptoethanol, vortex until all tissues lyse (the lysate should be translucent), and centrifuge at 13,000 rpm for 2 min.**

* Buffer RLT is corrosive, please wear protective equipment to operate.

3. **Transfer the supernatant into a Filter Column, close the lid and centrifuge at 13,000 rpm for 1 min.**

* Do not omit this step, as this may cause the spin column to be blocked in subsequent steps.
* If the lysate cannot be filtered through the filter column, it means that the nucleic acid content in the tissue is too high. At this time, transfer 300 μl filtrate to a clean 1.5 ml centrifuge tube, add 300 μl 70% ethanol to the 1.5 ml centrifuge tube, pipet 6-8 times to mix well, and then transfer the mixture to a spin column, centrifuge at 13000 rpm for 1 min. Discard the filter column and proceed to step 6.

4. **Keep the collection tube and discard the Filter Column, add 600 μl 70% ethanol to the filtrate and mix well by pipetting 6-8 times, transfer 600 μl mixture to a Spin Column, close the lid and centrifuge at 13000 rpm for 1 min.**

* If there is a precipitate after mixing with 70% ethanol, please add the precipitate together to the Spin Column.

5. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube. Transfer the remaining mixture into the Spin Column, and centrifuge at 13,000 rpm for 1 min.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the collection tube, you can slap the 2 ml Collection Tube upside down 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 to the Spin Column, close the lid and centrifuge at 13,000 rpm for 1 min.**

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

7. **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 13,000 rpm for 1 min.**

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

8. **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 speed does not reach 14000 rpm, centrifuge at the full speed for 2 min.
* Do not omit this step, otherwise the subsequent RT-PCR effect may be affected by the mixing of ethanol in the purified nucleic acid.

9. **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 center of the column, close the lid, incubate for 1 min at room temperature, and centrifuge at 13000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation conditions to 8000 rpm for 1 min to avoid damaging the centrifuge by detaching the tube lid of the 1.5 ml centrifuge tube.

10. **Discard the Spin Column. Eluted RNA can be used for various molecular biology experiments or store below -70°C for later use.**

* Even if DNA cannot be observed by electrophoresis, purified RNA should not be considered free of genomic DNA contamination, if you need to completely remove DNA, please digest the residual DNA with DNase I (Simgen Cat. No.8003050).

Culture Cells Protocol

1. Collect ≤1×10^7 cultured cells in a 1.5 ml centrifuge tube and flick the tube to disperse the cells.

* Cell collection method:

a. Cells cultured in suspension: Centrifuge at 300×g for 5 min to collect approximately 1×10^7 cultured cells and discard the supernatant.

b. Adherent-walled cultured cells: discard the culture supernatant, digest, and suspend the cells with trypsin, centrifuge at 300×g for 5 min to collect about 1×10^7 cultured cells, discard the trypsin supernatant.

c. Cells cultured in a single well in a cell culture plate (if the number of cells in a single well ≤ 1×10^5, please use the Micro Cells Total RNA Kit: Cat. No.: 5004050): Discard the culture supernatant, directly add 600 µl Buffer RLT with β-mercaptoethanol and use the tip to pipette the cells several times to lyse, directly into step 3.

2. Add 600 µl Buffer RLT with β-mercaptoethanol and vortex until the cells are completely lysed and the lysate is transparent.

* Do not use too many cells, as this may clog the Filter Column and lead to serious DNA contamination of the final purified RNA with genomic DNA.

3. Transfer all the cell lysate to a Filter Column, close the lid, and centrifuge at 13,000 rpm for 2 min.

* Do not omit this step, as this may cause the Spin Column to be blocked in subsequent steps.

* If there is insufficient liquid filtration at this step, too many cells are being used. In step 4, an equal volume of 70% ethanol with the filtrate can be added to continue the operation, and the other steps remain unchanged.

4. Keep the collection tube and discard the Filter Column, add 600 µl 70% ethanol to the filtrate and mix well by pipetting 6-8 times, transfer 600 µl mixture to a Spin Column, close the lid, and centrifuge at 13000 rpm for 1 min.

5. Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube. Transfer the remaining mixture into the Spin Column, and centrifuge at 13,000 rpm for 1 min.

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the collection tube, you can clap the 2 ml Collection Tube upside down 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 to the Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min.

* Ensure absolute ethanol has been added into Buffer WA.

7. 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 13,000 rpm for 1 min.

* Ensure absolute ethanol has been added into Buffer WBR.

8. 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 speed does not reach 14000 rpm, centrifuge at the full speed for 2 min.

* Do not omit this step, otherwise the subsequent RT-PCR effect may be affected by the mixing of ethanol in the purified nucleic acid.

9. 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 center of the column, close the lid, incubate for 1 min at room temperature, and centrifuge at 12000 rpm for 30 sec.

* If the centrifuge does not have a leak-proof lid, change the centrifugation conditions to 8000 rpm for 1 min to avoid the tube lid falling off and damaging the centrifuge.

10. Discard the Spin Column. Eluted RNA can be used for a variety of molecular biology experiments or store at -70°C for later use.

* Even if the DNA cannot be observed by electrophoresis detection, it does not mean that the purified RNA does not contain DNA contamination, if you need to completely remove the DNA, please digest the residual DNA with DNase I (Simgen Cat. No.8003050).