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Rapid Total RNA Extraction Kit Instructions

Composition

Rapid Total RNA Extraction Kit Cat. No.	5 Preps 5012005	50 Preps 5012050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Buffer RLF	4 ml	30 ml
Buffer WN	3 ml	28 ml
Buffer WBR (concentrate)	1.5 ml	12 ml
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

It can be stored at room temperature (0~30°C) for 2 years. For longer storage, it is recommended to keep at 2~8°C.(the product stored at 2~8°C should be restored to room temperature before use).

Technical Support

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Introduction

This product uses the latest RNA column purification technology, which can quickly extract high-purity total RNA from 10^6-10^7 animal cultured cells or 25~50 mg animal tissues. The special Buffer RLF can efficiently lyse biological samples, and impurities such as genomic DNA, pigments, and proteins are centrifuged to form a precipitate. The adsorbed RNA on the Spin Column is washed with Buffer WN and Buffer WBR, then dissolved in RNase-free water, which can be used for various molecular biology experiments such as RT-PCR, Northern blot, Dot blot, mRNA extraction, etc.

Regents And Equipment to Be Supplied by Users

1. Absolute ethanol .
2. RNase-free 1.5 ml centrifuge tubes, Grinding Rods (Simgen Cat. No. D-050) may be needed.
3. Pipettes and tips (RNase-free pipette tips with filters are recommended to avoid RNase contamination).
4. Disposable gloves, masks and protective equipment and paper towels.
5. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml tubes).
6. Vortexer.
7. RNase-free use labs.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25°C.
2. Add absolute ethanol to Buffer WBR according to the instructions on the label the reagent bottle 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.

Protocol

For cultured cells, follow step 1a; For animal tissues that are easily homogenized (liver, brain, etc.), follow step 1b; For tough animal tissues (skin, connective tissue, etc.), follow 1c; For bacteria, follow step 1d

1a. Collect cells from 10^6~10^7. For suspension cultured cells, centrifuge at 300×g for 5 min to discard the supernatant, flick the wall the centrifuge tube to disperse the cell pellet, add 500 µl Buffer RLF, and repeatedly pipette or vortex until the cells are completely lysed. For adherent cells or cells cultured in a single well, aspirate the culture medium and add 500 µl Buffer RLF and pipette repeatedly until the cells are completely lysed.

1b. Weigh 15~50 mg of human or animal tissue, quickly cut it into small particles, immediately transfer it to a clean 1.5 ml centrifuge tube, add 300 µl Buffer RLF, grind with a grinding rod (Simgen Cat. No. D-050) until there are no obvious particles, then add 200 µl Buffer RLF, vortex for 15 sec to mix well.

* Do not use more than 25 mg in tissues with high nucleic acid content such as pancreas and liver, otherwise genomic DNA may remain in the extracted total RNA.

1c. Add 25~50 mg of tissue to the mortar , grind the tissue into powder form with liquid nitrogen, immediately add 500 µl Buffer RLF, wait for the solution to be re-liquefied and then grind several times, transfer the homogenate to a 1.5 ml centrifuge tube, if the homogenate is less than 500 µl, add Buffer RLF to 500 µl, and then vortex and shake to mix. The tissue can also be ground with a homogenizer or beads with the addition of Buffer RLF.

* When grinding the tissue, liquid nitrogen should be added in time to avoid the tissue melting, so as to avoid the degradation of RNA due to the reactivation of endogenous RNase.

1d. Collect 10^8-10^9 bacteria cultured with a 1.5 ml centrifuge tube, resuspend the pellet with 50 µl DEPC-treated water, and then add 50 µl lysozyme solution (3 mg/ml, prepared with DEPC-treated water), vortex to mix, and incubate at 37°C for 10~30 min. Add 500 µl Buffer RLF and repeatedly pipette or vortex until all bacteria are lysed.

* 1.0×10^9 bacteria are equivalent to the number of bacteria in a bacterial culture with an OD600=1 in 1 ml.

2. **Centrifuge at full speed for 2 min. Add 250 µl absolute ethanol to a clean RNase-free 1.5 ml centrifuge tube and set aside.**
3. **Transfer all the supernatant from step 2 into an RNase-free 1.5 ml centrifuge tube prefilled with absolute ethanol, close the lid, and shake the centrifuge tube vigorously to mix well.**

* If there is floating matter (usually lipids) on top of the centrifuged supernatant, use a pipette to transfer the supernatant to an RNase-free 1.5 ml centrifuge tube prefilled with absolute ethanol instead of bringing in floating matter and pellets.

4. **Add all 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.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube. Add 500 µl Buffer WN to the Spin Column, 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. Add 750 µl Buffer WBR 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, slap the 2 ml Collection Tube upside down on a paper towel once.

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

7. **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 top speed could not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise, it may cause the residual ethanol in the eluate.

8. **Discard the 2 ml Collection Tube, place the Spin Column into a new 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, centrifuge at 12000 rpm for 30 sec.**

* Even if the genomic DNA are not detected by electrophoresis, it does not mean that there is no genomic DNA in the obtained RNA. It is recommended to use cDNA First Strand Synthesis Kit with DNase I digest (Simgen Cat. No. 7306100) for RT-PCR.

9. **Discard the Spin Column, the eluted RNA can be used immediately or stored below -70°C for later use.**