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Micro Animal Tissue Total RNA Extraction Kit Instructions

Composition

Micro Animal Tissue Total RNA Extraction Kit Cat. No.	5 Preps 5100005	50 Preps 5100050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Grinding rods	5	50
Proteinase K	60 µl	0.6 ml
DNase I	28 µl	270 µl
Buffer RDD	250 µl	2.5 ml
β-mercaptoethanol	50 µl	500 µl
Buffer RL	2 ml	20 ml
Ultrapure water	4 ml	40 ml
Buffer WAR	5 ml	40 ml
Buffer WBR (concentrate)	2 ml	12 ml
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

1. Proteinase K and DNase I should be stored 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

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Introduction

This kit can quickly extract total RNA from 10~20 mg of animal tissue and can process a large number of different samples at the same time. The extracted total RNA is of high purity and free of genomic DNA, proteins, and other impurities, and can be used for a various experiment such as RT-PCR, Real Time RT-PCR, microarray analysis, Northern Blot, Dot Blot, PolyA screening, in vitro translation, RNase protection analysis, and molecular cloning.

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 rotors for 1.5 ml and 2 ml tubes)
6. Water bath and vortexer
7. RNase-free use labs

Preparation before use

1. Add β-mercaptoethanol to a final concentration of 1% in Buffer RL before operation, e.g., 10 µl β-mercaptoethanol in 1 ml Buffer RL. This lysate is best prepared ready-to-use and can be left for one month at 4℃ with Buffer RL with β-mercaptoethanol. Buffer RL may form a precipitate during storage, if a precipitate occurs, redissolve any precipitate by warming to 56℃.
2. Absolute ethanol should be added to the Buffer WBR according to the instructions on the bottle label before first use and tick in the box on the label to mark "Ethanol added".
3. Because saliva and skin contain RNases, latex gloves and masks are required during the whole process of RNA extraction.
4. Unless otherwise specified, the following operations are performed at room temperature.
5. Prepare a water bath for 56℃.

Protocol

1. **Homogenization treatment:**
   Add 300 µl Buffer RL with β-mercaptoethanol per 10~20 mg of tissue, grind the tissue thoroughly with a grinding rod, then add 590 µl ultrapure water and 10 µl Proteinase K to the homogenate, and incubate at 56℃ for 10~20 min.
   * Tissue must be reground with Buffer RL before grinding, otherwise RNA degradation will result. If it is difficult to grind the tissue thoroughly, an electric or glass homogenizer can be used.
   * Do not exceed 20 mg of tissue, as this will result in reduced RNA yield and quality.
2. **Centrifuge at 12,000 rpm for 2~5 min, transfer the supernatant to a clean 1.5 ml centrifuge tube.**
3. **Add 450 µl absolute ethanol, mix well (precipitation may occur at this point), transfer 700 µl mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), centrifuge at 12,000 rpm for 1 min, discard the filtrate, and place the Spin Column back into the 2 ml Collection Tube.**
   * 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, slap the 2 ml Collection Tube upside down on a paper towel once.
4. **Transfer the remaining mixture from step 3 to the Spin Column, centrifuge at 12,000 rpm for 1 min, discard the filtrate, and place the Spin Column back into the 2 ml Collection Tube.**
5. **Add 350 µl Buffer WAR to the Spin Column, centrifuge at 12,000 rpm for 1 min, discard the filtrate, and place the Spin Column back into a 2 ml Collection Tube.**
6. **Add 5 µl DNase I into a new RNase-free centrifuge tube, add 45 µl Buffer RDD, gently pipette to mix well, and transfer all the mixture to the Spin Column center, incubate at room temperature for 15 min.**
7. **Add 350 µl Buffer WAR to the Spin Column， centrifuge at 12,000 rpm for 30 sec， discard the filtrate, and place the Spin Column back into a 2 ml Collection Tube.**
8. **Add 550 µl Buffer WBR to the Spin Column, incubate at room temperature for 2 min, centrifuge at 12,000 rpm for 30 sec, discard the filtrate, and place the Spin Column back into a 2 ml Collection Tube.**
   * Ensure that absolute ethanol has been added to Buffer WBR.
9. **Repeat step 8 once.**
10. **Centrifuge at the full speed (≥12,000 rpm) for 2 min and discard the 2 ml Collection Tube.**
    * The purpose of this step is to remove the residual rinse solution from the Spin Column, and the residual ethanol may affect the subsequent RT and other experiments.
11. **Place the Spin Column into a clean RNase-free 1.5 ml centrifuge tube, add 30~100 µl RNase-free Water to the Spin Column center, incubate at room temperature for 2 min, and centrifuge at 12,000 rpm for 1 min.**
    * The volume of RNase-free Water should not be less than 30 µl, too small a volume will affect the efficiency of RNA recovery.
12. **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.**