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FFPE Tissue Total RNA Extraction Kit Instructions

Composition

FFPE Tissue Total RNA Extraction Kit Cat. No.	5 Preps 5009005	50 Preps 5009050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Proteinase K	120 µl	1.2 ml
Buffer AT	1.5 ml	15 ml
Buffer SL	1.5 ml	15 ml
Buffer WBR (concentrate)	1.5 ml	12.5 ml
RNase-free Water	1.5 ml	2 ml×3
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Storage

1. Proteinase K should be stored at -20℃.
2. If other reagents and articles are stored at room temperature (0~30℃), they can keep their performance unchanged for two years, and if the product is stored at 2~8℃, the validity period of the product can be extended to more than two years.

Technical Support

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Introduction

This product is suitable for total RNA extraction from 3-8 tissue sections (less than 250 mm^2) of 10 µm. After the solubilized animal tissue is digested by proteinase K, the free RNA will be bound to the Spin Column, the degraded protein and PCR inhibitors will be filtered and removed, and the RNA will be washed by Buffer WBR and eluted with RNase-free water, which can be used for various molecular biology experiments.

Equipment And Reagents to Be Supplied By Users

1. Xylene, absolute ethanol.
2. RNase-Free 1.5 ml centrifuge tubes.
3. Pipettes and tips.
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.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the water bath temperature to 56℃ and 80℃ and incubate Buffer AT and RNase-free Water to 56℃.
3. Add absolute ethanol to Buffer WBR according to the instructions on the label of the bottle and tick the box on the label to mark "Ethanol added".

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Protocol

1. **Remove excess paraffin blocks on paraffin-embedded tissue samples with a scalpel and cut the tissue pieces into 5-10 μm thin slices.**

* If the tissue surface is exposed to air, discard 2-3 layers of flakes from the surface.
* RNA is very easily degraded, and the best sample should be freshly prepared (no more than 24 hours) paraffin-embedded tissue, most of the RNA in paraffin-embedded tissue that has been made for more than half a year has been degraded into 100-200 nt fragments, which seriously affects the sensitivity of detection.

2. **Immediately collect 3-8 tissue sections into an RNase-free 1.5 ml centrifuge tube, add 1 ml xylene, close the lid, and vortex vigorously for 10 sec to dissolve the paraffin.**
3. **Centrifuge at 13,000 rpm for 2 min. Discard the supernatant and retain the pellet at the bottom of the tube.**
4. **Add 1 ml absolute ethanol, vortex for a few seconds to suspend the pellet, and centrifuge at 13,000 rpm for 2 min.**

* Ethanol will wash away residual xylene.

5. **Discard the supernatant and retain the pellet at the bottom of the tube. Open the lid, stand at room temperature for 10 min or until the ethanol has evaporated.**
6. **Add 180 μl Buffer AT and 20 μl Proteinase K, vortex to mix well.**
7. **Incubate at 56℃ for 15 min, then incubate at 80℃ for 15 min.**

* If there is only one water bath, take the centrifuge tube out of the water bath and place it at room temperature and wait for the water bath to rise to 80℃ before placing the centrifuge tube in the water bath.

8. **Add 200 μl Buffer SL, gently invert 4-6 times to mix well. Centrifuge at 12,000 rpm for 5 min.**
9. **Transfer the supernatant to a clean RNase-free 1.5 ml tube, add 660 μl absolute ethanol, gently invert 4-6 times to mix well. Spin down for a few seconds to allow the solution on the cap to settle to the bottom of the tube.**
10. **Transfer 600 μl mixture from step 9 into 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.**
11. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add the remain mixture 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.

12. **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.

13. **Repeat step 12 once.**
14. **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 PCR effect may be affected due to the ethanol mixed in the extracted nucleic acid.

15. **Discard the 2 ml Collection Tube, place the Spin Column in an RNase-free 1.5 ml centrifuge tube, add 50-100 μl RNase-free Water incubated at 56℃ to the Spin Column, 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 cap, change the centrifugation condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the detachment of the cap of the 1.5 ml centrifuge tube.

16. **Discard the Spin Column and the eluted RNA can be used immediately for a variety of molecular biology experiments or stored at -70℃ for later use.**

* RNA extracted from paraffin-embedded tissue is usually mixed with a certain amount of genomic DNA that has been degraded into small fragments, so if you need to remove the DNA completely, digest the residual DNA with DNase I.
* RNA degradation in paraffin-embedded tissues that have been left for more than half a year is very severe, and if used for detection, the designed RT-PCR amplification fragment should not be larger than 200 bp.