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FFPE Tissue DNA Extraction Kit Instructions

Composition

FFPE Tissue DNA Kit Cat. No.	5 Preps 4400005	50 Preps 4400050
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	1.2 ml	12 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WB (concentrate)	1.5 ml	9.5 ml
Buffer TE	1.2 ml	12 ml
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Storage

1. Proteinase K can be stored at room temperature for at least 1 year. For longer storage, it is recommended to keep at 2~8℃.
2. Other reagents and items can be stored within 2 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.(the product stored at 2~8℃ should be restored to room temperature before use).

Introduction

This kit is suitable for extracting total DNA (including genomic DNA, mitochondrial DNA, and possible viral DNA) from 3 to 8 slices 10 µm tissue sections (area less than 250 mm^2). After the tissue is lysed with proteinase K, the DNA will be bound to the Spin Column, and the degraded proteins and PCR inhibitors will be removed by filtration. After the Spin Column is washed with Buffer WA and Buffer WB, DNA will be eluted with Buffer TE and can be used for various molecular biology experiments immediately.

Equipment and Reagents to Be Supplied by User

1. Xylene, absolute ethanol.
2. 1.5 ml centrifuge tubes, pipette, and tips.
3. Disposable gloves and protective equipment and tissues.
4. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. Water bath and vortexer.
6. Old FFPE tissues may require Carrier RNA (Simgen Cat. No. 4003101).

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 25°C.
2. Set the water bath temperature to 56°C and 90°C, and incubate Buffer AT and Buffer TE to 56°C.
3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".

Protocol

1. **Remove excess paraffin blocks from the FFPE tissues with a scalpel and cut the tissue into 5~10 μm slices.**

* If the tissue surface is exposed to air, discard the surface 2~3 layers of slices.

2. **Collect 3 to 8 tissue slices into a 1.5 ml centrifuge tube, add 1 ml xylene, close the lid, vortex vigorously for 10 sec to dissolve the paraffin.**
3. **Centrifuge at 13,000 rpm for 2 min. Discard the supernatant with a tip, keeping 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 with a tip, keeping the pellet at the bottom of the tube. Open the lid and leave it at room temperature for 10 min or until the ethanol evaporates.**
6. **Add 180 μl Buffer AT and 20 μl Proteinase K, vortex to mix well.**
7. **Incubate at 56°C for 1 h (or until the tissue is completely lysed). During the incubation time, vortex several times to help the tissue digestion.**

* If there is still a small amount of insoluble matter after the incubation, centrifuge the tube at 12,000 rpm for 1 min, transfer the supernatant to another clean 1.5 ml centrifuge tube, and then follow step 8.

8. **Incubate at 90 °C for 1 h.**

* This step is to partially renature some nucleic acids that have been denatured by formaldehyde.
* If there is only one water bath, please take out the centrifuge tube and place it at room temperature. When the water bath reaches 90°C, put the centrifuge tube into the water bath.

9. **Add 200 μl Buffer SL and 200 μl absolute ethanol, invert 4~6 times to mix well. Spin down the mixture to the bottom of the tube.**

* If DNA is extracted from old FFPE tissues, please add 3 μl Carrier RNA (Simgen Cat. No. 4003101) at this step. The DNA in old FFPE tissues is severely degraded. It must be effectively adsorbed to the Spin Column with the assistance of Carrier RNA.

10. **Transfer the mixture into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), centrifuge at 12,000 rpm for 30 sec.**

* Be careful not to get the solution on the edge of the Spin Column mouth, otherwise the Spin Column will not be cleaned in subsequent washing steps.

11. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 μl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.
* The filtrate does not need to be discarded completely. If you want to avoid contamination of the centrifuge by the filtrate adhering to the centrifuge tube, you can slap the 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 WB to the Spin Column, centrifuge at 12000 rpm for 30 sec.**

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

13. **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 cannot reach 14000 rpm, centrifuge at full speed for 2 min.
* Do not pass over this step, otherwise the purified nucleic acid may be mixed with ethanol, which may affect subsequent PCR results.

14. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 60~100 μl Buffer TE incubated at 56°C to the Spin Column, and incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec.**

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

15. **Discard the Spin Column, and the eluted DNA can be used immediately for various molecular biology experiments or stored at -20°C for later use.**