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FFPE DNA Extraction Kit

Catalog No.	Specification	Storage/Shelf life
EP016-50T	50T	Room temperature/ 2 years

Introduction

This product is suitable for separating and purifying total DNA (including genomic DNA, mitochondrial DNA and possible viral DNA) from 3-8 (area less than 250 mm^2) 10 μm tissue sections. After the dissolved animal tissue is digested and aggregated by the specific DNA separation and extraction solution, the DNA is adsorbed to the purification column through the specific DNA adsorption column, and the degraded protein and PCR inhibitors are removed by the specific washing solution, and the final DNA product Eluted with Buffer TE, it can be used in various molecular biology experiments such as PCR, sequencing, and enzyme digestion.

Kit Components

Component	EP016-50T	Storage
Proteinase K	1.2 ml	-20 ℃
Buffer GA1	15 ml	RT
Buffer GA2	12 ml	RT
Buffer RP	28 ml	RT
Buffer WB	32 ml	RT
Buffer TE	12 ml	RT
Adsorption column G	50 set	RT
User Manual	1 copy	RT

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Reagents and products that users need to prepare by themselves

1. Xylene, ethanol
2. 1.5 ml centrifuge tubes, pipettes and tips
3. Disposable gloves and protective equipment and paper towels
4. Benchtop minicentrifuge (rotor for centrifuging 1.5 ml and 2 ml tubes)
5. Water bath and vortex shaker
6. Old paraffin tissue samples, may require Carrier RNA

Preparation before use

1. If the centrifuge has refrigeration function, please set the temperature to 25°C.
2. Set the temperature of the water bath to 56°C and 90°C, and incubate Buffer GA1 and Buffer TE to 56°C.
3. Add absolute ethanol to Buffer RP and Buffer WB according to the instructions on the label of the reagent bottle, and tick the box on the label to mark "Alcohol added".

Attention:

4. Store proteinase K stock solution at -20°C.
5. If other reagents and products are stored at room temperature (0~30°C), they can maintain no significant change in performance within two years; if the product is stored at 2~8°C, the validity period of the product can be extended to more than two years (2~30°C). Products stored at 8°C should be returned to room temperature before use)

Operation steps

1. Cut off the excess paraffin block on the paraffin tissue specimen with a scalpel, and cut the tissue block into 5-10 μm thin slices.

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

2. Immediately collect 3~8 tissue slices and put them into a 1.5 ml centrifuge tube, add 1 ml xylene, cover the tube cap, and vortex vigorously for 10 seconds to dissolve the paraffin.
3. Centrifuge at 13000 rpm for 2 minutes. Discard the supernatant and keep the bottom

pellet.
4. Add 1 ml of absolute ethanol, vortex for a few seconds to suspend the pellet, and centrifuge at 13,000 rpm for 2 minutes.
* Ethanol will wash away residual xylene.
5. Discard the supernatant and keep the bottom pellet. Open the lid and place at room temperature for 10 minutes or until the ethanol evaporates completely.
6. Add 180 µl Buffer GA1 and 20 µl Proteinase K stock solution, and vortex to mix.
7. Water bath at 56°C for 1 hour (or until the tissue is completely dissolved), and vortex several times during the period to help the tissue dissolve.
* If there is still a small amount of insoluble matter after the water bath, centrifuge the 1.5 ml centrifuge tube at 12000 rpm for 1 minute, transfer the supernatant to another clean 1.5 ml centrifuge tube, and then follow step 8.
8. 90°C water bath for 1 hour.
* This step is for partial renaturation of some nucleic acids denatured by formaldehyde.
* If there is only one water bath, please take out the centrifuge tube and place it at room temperature, and put the centrifuge tube into the water bath after the water bath has risen to 90°C.
9. Add 200 µl Buffer GA2 and 200 µl absolute ethanol, invert gently 4-6 times to mix well. Centrifuge at low speed for a few seconds to allow the solution on the cap to settle to the bottom of the tube.
* If DNA is extracted from old paraffin tissue blocks, please add 3 µl Carrier RNA (additional purchase) at this step, the DNA degradation in old paraffin tissue samples is very serious, the content is very low, must be assisted by Carrier RNA In order to be effectively adsorbed on the purification column.
10. Pipette the mixture into a nucleic acid purification column (the nucleic acid purification column is placed in a 2 ml centrifuge tube), cover the tube, and centrifuge at 12000 rpm for 30 seconds.
* Be careful not to get the solution on the edge of the nozzle of the purification column, so as not to clean the purification column in subsequent washing steps.
11. Discard the filtrate in the 2 ml centrifuge tube, put the nucleic acid purification column back into the 2 ml centrifuge tube, add 500 µl Buffer RP to the nucleic acid purification column, cover the tube cap, and centrifuge at 12000 rpm for 30 seconds.
* Make sure absolute ethanol has been added to Buffer RP.

* The filtrate does not need to be completely discarded. If you want to avoid the contamination of the centrifuge by the filtrate adhering to the nozzle of the centrifuge tube, you can slap the 2 ml centrifuge tube upside down on a paper towel once.

12. Discard the filtrate in the 2 ml centrifuge tube, put the nucleic acid purification column back into the 2 ml centrifuge tube, add 600 µl Buffer WB to the nucleic acid purification column, cover the tube cap, and centrifuge at 12000 rpm for 30 seconds.

* Make sure absolute ethanol has been added to Buffer WB.

13. Discard the filtrate in the 2 ml centrifuge tube, put the nucleic acid purification column back into the 2 ml centrifuge tube, and centrifuge at 14000 rpm for 1 minute.

* If the centrifuge does not reach a speed of 14000 rpm, centrifuge at maximum speed for 2 minutes.

* Do not omit this step, otherwise the subsequent PCR effect may be affected due to ethanol in the purified nucleic acid.

14. Discard the 2 ml centrifuge tube, put the nucleic acid purification column into a clean 1.5 ml centrifuge tube, add 60~100 µl 56°C incubated Buffer TE to the purification column, cover the tube cap, and let stand at room temperature for 1 minute , centrifuge at 12000rpm for 30 seconds.

* If the centrifuge does not have a leak-proof cover, please change the centrifugation condition to 8000 rpm for 1 minute, so as not to damage the centrifuge due to the cap falling off of the 1.5 ml centrifuge tube.

15. Discard the purification column, and the eluted DNA can be used immediately for various molecular biology experiments; or store the DNA at -20°C for future use.

DNA concentration and purity detection

The size of the obtained genomic DNA fragments is related to factors such as the storage time of the sample and the shearing force during operation. The recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer for concentration and purity. DNA should have a significant absorption peak at OD260, with an OD260 value of 1 corresponding to approximately 50 µg/ml double-stranded DNA and 40 µg/ml single-stranded DNA. The OD260/OD280 ratio should be 1.7－1.9, if eluted with deionized water instead of elution buffer, the ratio will be lower because pH and the presence of ions will affect the absorbance value, but does not indicate low purity .

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Frequently Asked Questions and Answers

A. Genome extraction yield is low:

Suggestions: prolong the digestion time, increase the sample volume, etc.

B. Precipitate in the reagent is not dissolved

Suggestion: Some reagents will precipitate when the temperature is low. Please check whether there is precipitation before use. If there is precipitation, please incubate at 37 ℃ for a while, and use it after the solution is clarified.

C. Ethanol was not added to the Wash Buffer as required

Suggestion: Add the required amount of absolute ethanol according to the instructions, and tighten the bottle cap after use to prevent the ethanol from volatilizing.

D. Selection of dissolution volume and time

Suggestion: The dissolved volume will affect the final yield, the larger the dissolved volume, the higher the yield, but the concentration will be lower. Please use the dissolution volume recommended by the kit for dissolution to ensure the best yield and concentration.
Suggestion: After adding Buffer TE, let it stand at room temperature for 2-5 minutes, which is more conducive to dissolution.