TIANGEN®

TIANamp FFPE DNA Kit

For purification of genomic DNA from formalin-fixed, paraffin-embedded tissues

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This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

TIANamp FFPE DNA Kit

(Spin Column)

Cat. no. GDP331

Kit Contents

Contents	GDP331-02 50 preps
Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TE	15 ml
Proteinase K	1 ml
RNase-Free Spin Columns CR2	50
Collection Tubes 2 ml	50
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Storage

TIANamp FFPE DNA Kit could be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality.

Introduction

The TIANamp FFPE DNA Kit is optimized for purification of DNA from FFPE tissue sections. It uses xylene to remove paraffin, and provides unique lysis conditions for DNA release from tissue slice, well removes formalin crosslinking of the released DNA. Combined selective-binding silica-based membrane and flexible elution system, this kit could elute high-quality DNA to small volume.

DNA purified by TIANamp FFPE DNA Kit is stable and of high purity, could be applied in PCR and Real-time PCR analysis, SNP genotype analysis and STR analysis, and pharmacogenomics analysis.

Important Notes (please read this before use)

1. Fix tissue samples in 4-10% formalin as quickly as possible after surgical removal. Use a fixation time of 8-24 hours (longer fixation time lead to more severe DNA fragmentation, resulting in poor performance in downstream assays).
2. Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit PCR reaction).
3. This kit applies in medical and scientific research.
4. The integrity of DNA obtained in this kit depends on the samples type, storage and fixation conditions. Longer fixation time or longer storage time (>1 year) will lead to DNA fragmentation, in this case, long fragments will not be amplified.
5. If a precipitate has formed in Buffer GA or Buffer GB or Buffer GD, warm it at 37°C until the precipitate has fully dissolved.
6. Each reagent in the kit should be performed according to this instruction.

Protocol

Ensure that Buffer GD and Buffer PW have been added with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly. All centrifugation steps should be carried out at room temperature (15-30°C).

1. Samples treatment:

   a. Paraffin section: take 5-8 pieces of paraffin sections (thickness of 5-10 μm, surface area of 1x1 cm^2)

   b. Paraffin block: use a scalpel to cut around 30 mg tissue sample (trim excess paraffin off the sample block).

   Note: if the sample surface has been exposed to air, discard the first 2-3 sections.

   c. Samples embedded in formalin: use a scalpel to cut 30 mg sample into pieces, and place in a 1.5 ml centrifuge tube. Add 500 μL PBS (10 mM, pH 7.4), vortex to mix, then centrifuge at 12,000 rpm (~13,400x g) for 1 min at room temperature. Repeat three times, and then start from step 7.

2. Place the paraffin section or paraffin block in a 1.5 ml centrifuge tube, and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 s.
3. Centrifuge at 12,000 rpm (~13,400x g) for 2 min at room temperature. Remove the supernatant by pipetting.

   Note: do not remove any of the pellet.

4. Add 1 ml ethanol (96-100%) to the pellet, and vortex for 10 sec.
5. Centrifuge at 12,000 rpm (~13,400x g) for 2 min at room temperature. Remove the supernatant by pipetting.

   Note: do not remove any of the pellet.

6. Open the tube and incubate at room temperature for 5-10 min or until all the residual ethanol has evaporated.
7. Re-suspend the pellet in 200 μL Buffer GA, add 20 μL Proteinase K, mix thoroughly, incubate at 56°C for 1 h (or until the sample has been completely lysed).
8. Incubate at 90°C for 1 h.
9. (Optional) To remove RNA, add 2 μL RNase A (100 mg/ml), incubate at room temperature for 2 min.

10. Add 220 µL Buffer GB to the sample, and mix thoroughly by vortex. Then add 250 µL ethanol (96-100%), and mix again thoroughly by vortex. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
11. Carefully transfer the entire lysate to the Spin Column CR2, close the lid, and centrifuge at 8,000 rpm (~6,000x g) for 2 min. Discard the flow-through and place the Spin Column CR2 back to the collection tube.

    Note: if the lysate volume exceeds 700 µL, centrifuge successive aliquots in the same column.
12. Add 500 µL Buffer GD to the Spin Column CR2, close the lid and centrifuge at 8,000 rpm (6,000x g) for 60 sec. Discard the flow-through and place the Spin Column CR2 back to the collection tube.
13. Add 600 µL Buffer PW to the Spin Column CR2, close the lid and centrifuge at 8,000 rpm (6,000x g) for 60 sec. Discard the flow-through and place the Spin Column CR2 back to the collection tube.
14. Repeat step 13.
15. Centrifuge at 12,000 rpm (~13,400x g) for 2 min, discard the flow-through. Open the lid and place the spin column at room temperature for 2-5 min to dry the membrane completely.

    Note: ethanol carryover into the eluate may interfere with some downstream applications. However, do not dry for long time, since that would be difficult to elute DNA.
16. Place the Spin Column to a clean 1.5 ml centrifuge tube, and apply 30-100 µL Buffer TE which is preheated at 65°C or ddH_2O to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min, then centrifuge at 12,000 rpm (~13,400x g) for 2 min. Store the DNA-containing tube at -20°C.

    Note: The elution buffer should be larger than 30 µL, since small volume will affect recovery ratio. To raise genomic DNA yield, the buffer obtained by centrifuge could be added to the CR2 membrane again, stand for 2 min, and then centrifuge at 12,000 rpm (13,400x g) for 2 min. The pH of elution buffer has great effect on elution efficiency. If eluted with H_2O, the pH of H_2O should be controlled between 7.0-8.5, pH<7.0 will reduce the elution efficiency. Obtained DNA should be stored at -20°C to prevent degradation.