TIANquick FFPE DNA Kit

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

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TIANquick FFPE DNA Kit

(Spin Column)

Cat. no. 4992296

Kit Contents

Contents	4992296 50 preps
Buffer GL	30 ml
Buffer GP	3 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TE	15 ml
RNase-Free Spin Columns CR2	50
Collection Tubes 2 ml	50
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Storage

TIANquick FFPE DNA Kit could be stored at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C before use.

Introduction

The TIANquick FFPE DNA Kit is optimized for purification of DNA from FFPE tissue sections. It uses special method to remove paraffin, and provides unique lysis conditions for DNA release from tissue slice, well removes formalin crosslinking of the released DNA, reducing the damage of formalin to DNA maximally. Combined selective-binding silica-based membrane and flexible elution system, this kit could elute high-quality DNA. Xylene is not involved in the whole process, making the operation safe, easy and fast.

DNA purified by TIANamp FFPE DNA Kit is stable and of high purity. It could be applied in PCR and Real-time PCR analysis, SNP genotype analysis , 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. For FFPE sample with trace DNA, TIANamp FFPE DNA Kit is recommended.
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 GL 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.
7. This kit is applied in medical and scientific research.

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 1 × 1 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 (~13,400 × g) for 1 min at room temperature. Repeat three times and discard the flow-through.
2. Place the paraffin section or paraffin block in a 1.5 ml sterile microcentrifuge tube, and add 500 μl Buffer GL and 50 μl Buffer GP to the sample. Close the lid and vortex vigorously for 10 sec.
3. Heat at 98°C for 30 min, during which invert the tube up and down for three times until sample completely dissolved.

   Note: tweezers should be used to take tube out of water bath.
4. Centrifuge at 12,000 rpm (~13,400 × g) for 5 min at room temperature.
5. Carefully transfer the clear water phase in the middle layer to a new microcentrifuge tube with 200 μl pipette. The upper layer is mixture of paraffin and protein, and the bottom layer contains few contaminants. Prolong the centrifuge time if there is incomplete layering, until the upper mixture completely isolated from the clear water phase.
6. **(Optional)** To remove RNA, add 2 μl RNase A (100 mg/ml), incubate at room temperature for 2 min.
7. Add 2 volume of ethanol (96-100%) to the solution (e.g., add 900 μl ethanol (96-100%) to 450 μl clear water phase), and mix completely, incubate for 3 min.
8. Transfer the entire mixture to the Spin Column CR2, close the lid, and centrifuge at 8,000 rpm (~6,000 × 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.

9. Add 500 µl Buffer GD to the Spin Column CR2, close the lid and centrifuge at 8,000 rpm (6,000 × g) for 60 sec. Discard the flow-through and place the Spin Column CR2 back to the collection tube.
10. Add 600 µl Buffer PW to the Spin Column CR2, close the lid and centrifuge at 8,000 rpm (6,000 × g) for 60 sec. Discard the flow-through and place the Spin Column CR2 back to the collection tube.
11. Repeat step 10.
12. Centrifuge at 12,000 rpm (~13,400 × 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, drying too long time will make DNA difficult to elute.
13. Place the Spin Column CR2 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 (~13,400 × 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 Spin Column CR2 membrane again, stand for 2 min, and then centrifuge at 12,000 rpm (13,400 × 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.