

## Paraffin-Embedded Tissue DNA Extraction Kit

**Cat:** D1710

**Size:** 50 T/100 T

**Storage:** RT, valid for 1 year. (Annex 1: RNase A (FJD1710-1); Annex 2: Proteinase K (FJD1710-2). Store at -20°C.)

### Product Composition

Name	50 T	100 T	Storage
RNase A	100 µL	100 µL×2	-20°C
Proteinase K	1 mL	1 mL×2	-20°C
Dewaxing Solution	200 mL	400 mL	RT
Solution A	10 mL	20 mL	RT
Solution B	10 mL	20 mL	RT
Washing Solution	15 mL	15 mL×2	RT
Elution Buffer	10 mL	20 mL	RT
Adsorption Columns	50 pieces	100 pieces	RT
Collection Tubes	50 pieces	100 pieces	RT
Product Manual	1 copy	1 copy	

### Introduction

This kit employs specialized dewaxing solution and optimized lysis conditions to extract DNA from formalin-fixed paraffin-embedded (FFPE) tissue sections, overcoming the inhibitory effects caused by formalin crosslinking. Through a spin column that specifically binds to DNA and a unique buffer system, the kit purifies high-quality DNA into a small elution volume. The extracted genomic DNA exhibits excellent integrity, high purity, and stable, reliable quality.

### Protocols *(for reference only)*

Before use, add anhydrous ethanol to the Washing Solution as per the volume indicated on the bottle label (45 mL of anhydrous ethanol per bottle is required). All centrifugation steps are performed at room temperature using a benchtop centrifuge.

1. Sample Preparation
  - a. Paraffin sections: Take 5-8 paraffin sections (5-10 µm thick, 1×1 cm<sup>2</sup> in size).
  - b. Paraffin blocks: Scrape approximately 30 mg of tissue sample with a scalpel (remove excess paraffin as much as possible). Note: If the sample surface has been exposed to air, discard the first 2-3 scraped pieces.
  - c. Samples in fixatives (e.g., formalin): Take 30 mg of sample, cut into small pieces with a scalpel, and transfer to a 1.5 mL centrifuge tube. Add 500 µL PBS (0.01 M, pH 7.4), vortex to mix thoroughly, centrifuge at 12,000 rpm for 1 min at room temperature, discard the supernatant, and repeat this step 3 times. Then proceed to Step 7.
2. Dewaxing (Choose One Method)
  - a. Dewaxing with Dewaxing Solution: Add 1 mL Dewaxing Solution, vortex thoroughly, incubate in a 65°C water bath for 30 min, vortex again thoroughly, centrifuge at 15,000 rpm for 15 min at 4°C, discard the supernatant. Add another 1 mL Dewaxing Solution and repeat this process 3 times. Then proceed to Step 7.
  - b. Dewaxing with Xylene: Transfer paraffin sections or paraffin block samples to a 1.5 mL sterile centrifuge tube, add 1 mL xylene, and vortex vigorously for 10-15 seconds. Then proceed to Step 3 (xylene is to be prepared by the user).

3. Centrifuge at 12,000 rpm for 2 min at room temperature, discard the supernatant. Note: Do not discard the pellet.
4. Add 1 mL anhydrous ethanol to the tube, vortex to mix for 10 seconds.
5. Centrifuge at 12,000 rpm (~13,400×g) for 2 min at room temperature, discard the supernatant. Note: Do not discard the pellet.
6. Allow the tube to stand at room temperature for 5-10 min to fully evaporate ethanol.
7. Add 200 µL Solution A, 20 µL Proteinase K, and 2 µL RNase A to the pellet, mix thoroughly. Incubate in a 56°C water bath for 1-3 h until the sample is completely lysed. During digestion, invert the centrifuge tube several times to mix. Complete digestion is indicated by a clear and viscous solution.
8. Add 200 µL Solution B, invert thoroughly to mix. If white precipitation forms, incubate at 75°C for 15-30 min to dissolve the precipitate (this will not affect subsequent experiments). If the solution remains turbid, it indicates incomplete sample digestion, which may result in low yield and poor purity of extracted DNA, or clog the adsorption column.
9. Add 200 µL anhydrous ethanol, mix thoroughly. Flocculent precipitation may form, which does not affect DNA extraction. Transfer the entire solution (including flocculent precipitation) to the adsorption column.
10. Centrifuge at 12,000 rpm for 1 min, discard the waste liquid, and place the adsorption column back into the collection tube.
11. Add 600 µL Washing Solution (**check if anhydrous ethanol has been added before use**) to the adsorption column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid, and place the adsorption column back into the collection tube.
12. Add another 600 µL Washing Solution to the adsorption column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid, and place the adsorption column back into the collection tube.
13. Centrifuge at 12,000 rpm for 2 min. Leave the adsorption column uncovered and incubate at room temperature or in a 50°C incubator for several minutes to remove residual Washing Solution. Residual ethanol in the Washing Solution may interfere with subsequent experiments (e.g., restriction enzyme digestion, PCR).
14. Transfer the adsorption column to a clean centrifuge tube. Add 50-200 µL preheated Elution Buffer (65°C water bath) to the center of the adsorption membrane (avoid touching the membrane), allow to stand at room temperature for 5 min, then centrifuge at 12,000 rpm for 2 min.
15. (Optional) Transfer the eluate back to the same adsorption column, centrifuge at 12,000 rpm for 2 min to obtain high-quality genomic DNA.

### Note

1. After opening the kit, store RNase A and Proteinase K at -20°C.
2. Avoid repeated freeze-thaw cycles of samples, as this will result in shorter extracted DNA fragments and reduced yield.
3. If precipitation forms in any solution of the kit, re-dissolve it in a 65°C water bath before use; this will not affect the experimental results.
4. The volume of Elution Buffer should be at least 50 µL—too small a volume will reduce recovery efficiency. The pH value of the elution buffer also affects elution efficiency: if using water as the elution buffer, ensure its pH is approximately 8.0 (adjust with NaOH if necessary). A pH below 7.0 will decrease elution efficiency.
5. The integrity of the DNA extracted using this product depends on the sample type, storage duration, and fixation conditions. Prolonged formalin fixation (exceeding 24 hours) or long-term sample storage (more than 1 year) may impair DNA integrity, making it impossible to amplify long fragments.