

QIAGEN Supplementary Protocol

Purification of genomic DNA from FFPE tissue using the QIAamp[®] DNA FFPE Tissue Kit and Deparaffinization Solution

This protocol describes how to purify genomic DNA from formalin-fixed paraffin-embedded tissue. The purification procedure requires the QIAamp DNA FFPE Tissue Kit (cat. no. 56404) and Deparaffinization Solution (cat. no. 19093).

IMPORTANT: Please read the *QIAamp DNA FFPE Tissue Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure. Handbooks can be found at www.qiagen.com/handbooks.

Product Use Limitations

QIAamp DNA FFPE Tissue Kits are intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- QIAamp DNA FFPE Tissue Kit (cat. no. 56404)
- Deparaffinization Solution (cat. no. 19093)
- 1.5 ml or 2 ml microcentrifuge tubes; 1.5 ml tubes are available from Eppendorf [Safe-Lock (cat. no. 02236204)] or Sarstedt [Safety Cap (cat. no. 72.690)]
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Thermomixer, heated orbital incubator, or water bath capable of reaching 90°C
- Microcentrifuge with rotor for 2 ml tubes
- Vortexer
- Optional: 100 mg/ml RNase A (cat. no. 19101) ▶



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Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Equilibrate all buffers to room temperature; equilibrate Deparaffinization Solution to 20–25°C.
- In the procedure below, ■ indicates the volumes to use if processing 1–2 sections per sample, while ▲ indicates the volumes to use if processing >2 sections per sample.

Things to do before starting

- Preheat a thermomixer or heated orbital incubator to 56°C for use in steps 4 and 8. If a thermomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
- If Buffer AL or Buffer ATL contain precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 13 of the *QIAamp DNA FFPE Tissue Handbook*.

Procedure

- 1. Using a scalpel, trim excess paraffin off the sample block. Cut into sections 5–10 μm thick.**
Note: If the sample surface has been exposed to air, discard the first 2–3 sections.
- 2. Immediately place the sections in a 1.5 ml or 2 ml microcentrifuge tube (not supplied).**
- 3. Add ■ 160 μl or ▲ 320 μl Deparaffinization Solution and vortex vigorously for 10 s. Centrifuge briefly to collect the sample in the bottom of the tube.**
Note: Deparaffinization Solution is not supplied with the QIAamp FFPE Kit and should be ordered separately.
- 4. Incubate at 56°C for 3 min, and then allow to cool at room temperature (15–25°C).**
- 5. Add 180 μl Buffer ATL, and mix by vortexing.**
- 6. Centrifuge for 1 min at 11,000 x g (10,000 rpm).**
- 7. Add 20 μl proteinase K to the lower, clear phase. Mix gently by pipetting up and down.**



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8. Incubate at 56°C for 1 h (or until the sample has completely lysed).

9. Incubate at 90°C for 1 h.

The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA.

Note: If using only one heating block, leave the sample at room temperature (15–25°C) after the 56°C incubation in step 8, until the heating block has reached 90°C for step 9.

10. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

11. Transfer the lower, clear phase into a new 2 ml microcentrifuge tube.

Optional: If RNA-free genomic DNA is required, add 2 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature before continuing with step 14 of the *QIAamp DNA FFPE Tissue Handbook*.

12. Continue with step 14 of the *QIAamp DNA FFPE Tissue Handbook*.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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