Supplementary Protocol September 2019

Purification of Genomic DNA and Total RNA From Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue Sections in the Same Eluate; Spin Procedure

This protocol describes how to purify genomic DNA and total RNA from FFPE tissue sections in the same eluate. The purification procedure requires use of the Allprep DNA/RNA FFPE Kit.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *AllPrep DNA/RNA FFPE Handbook* before beginning this procedure. The Allprep DNA/RNA FFPE Kit is intended for molecular biology applications. The product is not intended for the diagnosis, prevention, or treatment of disease.

Further information

- *AllPrep DNA/RNA FFPE Handbook*: **www.qiagen.com/HB-0373**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Equipment and reagents

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

- Allprep DNA/RNA FFPE Kit (cat. no. 80234)
- Sterile, RNase-free pipette tips (to avoid cross-contamination, we recommend pipette tips with aerosol barriers)
- 1.5 ml or 2.0 ml Safe-Lock microcentrifuge tubes (Eppendorf®, cat. nos. 022363204 and 022363352, respectively) or 1.5 ml SafeSeal tubes (Sarstedt®, cat. no. 72.706)*
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- 96–100% ethanol†
- 96–100% isopropanol
- For deparaffinization of FFPE tissue sections:
  - Deparaffinization Solution (cat. no. 19093), or
  - 99–100% heptane and methanol and 96–100% ethanol, or
  - 99–100% xylene and 96–100% ethanol

* This is not a complete list of suppliers and does not include many important vendors.

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Starting material

The starting material for nucleic acid purification should be freshly cut sections of FFPE tissue, each with a thickness of 10–20 µm. Thicker sections may result in lower nucleic acid yields, even after prolonged incubation with proteinase K. Thinner sections can be used but are more difficult to pellet. Do not use more than four 10 µm sections of 150 mm^2 surface area or two 20 µm sections of 150 mm^2 surface area.

Avoid using too much starting material, as this affects lysis efficiency and purification and can lead to reduced yields and nucleic acid fragmentation.

Do not overload the RNeasy® MinElute® spin column, as this will significantly reduce DNA/RNA yield and quality.

Important points before starting

- If using the AllPrep DNA/RNA FFPE Kit for the first time, read "Important Notes" in the *AllPrep DNA/RNA FFPE Handbook*.
- If working with RNA for the first time, read "Appendix A: General Remarks on Handling RNA" of the *AllPrep DNA/RNA FFPE Handbook*.
- Unless otherwise stated, perform all steps quickly at room temperature (15–25°C).
- Perform all centrifugation steps using a microcentrifuge at room temperature. If using a refrigerated microcentrifuge, set the temperature to 20–25°C, otherwise significant cooling may occur below 15°C.
- Buffer RLT, Buffer FRN, and Buffer AL contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. For "Safety Information", see page 6 of the *AllPrep DNA/RNA FFPE Handbook*.

Things to do before starting

- If using Buffer FRN and Buffer RPE for the first time, reconstitute as described in "Preparation of buffers" in the *AllPrep DNA/RNA FFPE Handbook*.
- If necessary, warm and gently agitate Buffer RLT, Buffer ATL, and Buffer AL to redissolve any precipitates that may have formed.
- Equilibrate all buffers to room temperature. Mix reconstituted Buffer FRN and Buffer RPE by shaking.
- Set a thermal mixer or heated orbital incubator to 56°C for use in steps 5 and 10 of the protocol. If a thermal mixer or heated orbital incubator is not available, a heating block or a water bath can be used instead.

Procedure

1. Cut FFPE sections 10–20 µm in thickness.

   Note: Do not use more than four 10 µm sections of 150 mm^2 surface area or two 20 µm sections of 150 mm^2 surface area.

   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 Safe-Lock microcentrifuge tube (not supplied), and close the lid.
3. Remove the paraffin according to step 4a, 4b, or 4c in the *AllPrep DNA/RNA FFPE Handbook.*
4. Resuspend the pellet obtained after deparaffinization by adding 150 µl Buffer PKD and flicking the tube to loosen the pellet. Add 10 µl proteinase K and mix by vortexing.
5. Incubate at 56°C for 15 min.
6. Incubate on ice for 3 min.
7. Centrifuge for 15 min at 20,000 x *g* (14,000 rpm).
8. Carefully transfer the supernatant without disturbing the pellet to a new 2 ml Safe-Lock microcentrifuge tube. Store the supernatant at 4°C for RNA crosslink removal in step 12.

DNA crosslink removal

9. Resuspend the pellet from step 8 in 180 µl Buffer ATL, add 40 µl proteinase K, and mix by vortexing.
10. Incubate at 56°C for 1 h.
11. Incubate at 90°C for 2 h without agitation.

    Note: Agitation during this incubation step leads to lower DNA yields.

RNA crosslink removal

12. Incubate the supernatant from step 8 at 80°C for 15 min.

Simultaneous DNA and RNA purification

13. Briefly centrifuge the microcentrifuge tube from step 11 to remove drops from the inside of the lid.
14. Add 200 µl Buffer AL to the microcentrifuge tube from step 13 to adjust binding conditions and mix by vortexing. Add 200 µl ethanol (96–100%) and mix by vortexing or pipetting.

    Note: Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

15. Transfer the entire sample to an RNeasy MinElute Spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 1 min at ≥8000 x *g*. Discard the flow-through. Reuse the collection tube.

16. Briefly centrifuge the microcentrifuge tube from step 12 to remove drops from the inside of the lid.
17. Add 320 µl Buffer RLT the microcentrifuge tube from step 16 to adjust binding conditions and mix by vortexing or pipetting. Add 700 µl ethanol (96–100%) and mix well by vortexing or pipetting. Proceed immediately to step 18.
18. Transfer 700 µl of the sample, including any precipitate that may have formed, to the RNeasy MinElute spin column from step 15, placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube.
19. Repeat step 18 until the entire sample has passed through the RNeasy MinElute spin column.
20. Add 700 µl Buffer FRN to the RNeasy MinElute spin column. Close the lid gently and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube.
21. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.
22. Reuse the collection tube and repeat step 21.
23. Place the RNeasy MinElute spin column in a new 2 ml collection tube. Open the lid and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.
24. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube.
25. Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently and incubate for 1 min at room temperature. Centrifuge at full speed for 1 min to elute the DNA and RNA.

Document Revision History

Date	Changes
09/2019	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.