QIAGEN Supplementary Protocol

Simultaneous Purification of Genomic DNA and Total RNA, including Small RNAs from FFPE Cores, using the AllPrep® DNA/RNA FFPE Kit

As an alternative to sectioning using a microtome, biopsy needles can be used to obtain core punches from FFPE specimens. The diameter of these cores is typically several orders of magnitude greater than the thickness of microtome sections. This protocol has been specially adapted from the AllPrep DNA RNA FFPE protocol for the simultaneous purification of genomic DNA and total RNA, including small RNAs, from FFPE cores using 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. 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 these products.

Equipment and reagents

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

- AllPrep DNA/RNA FFPE Kit (cat. no. 80234)
- Sterile, RNase-free pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)
- Microcentrifuge (with rotor for 2 ml tubes)
- 1.5 ml Safe-Lock microcentrifuge tubes (Brinkmann, cat.no. 022363204 or Eppendorf, cat.no. 0030 120.086) or 1.5 ml SafeSeal microcentrifuge tubes (Sarstedt, cat. no. 72.706)*
- 2 ml Safe-Lock microcentrifuge tubes (Brinkmann, cat. no. 022363352 or Eppendorf, cat. no. 0030 120.094) or 2 ml SafeSeal microcentrifuge tubes (Sarstedt, cat. no. 72.695)†
- Vortexer
- 96–100% ethanol†
- 96–100% isopropanol
- 99–100% xylene
- Thermal mixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C, 80°C, and 90°C
- Mechanical disruption device (e.g., TissueLyser II)
- **Optional**: RNase A (100 mg/ml; cat. no. 19101)

* 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.

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Starting material

Starting material for nucleic acid purification should be one FFPE core with a maximum diameter of 3 mm and a length of 3 mm after excess paraffin is trimmed off. If there is no information about the nature of your starting material, we recommend starting with no more than 10 mg FFPE core per preparation. Do not use more than 25 mg of FFPE core per preparation.

Do not overload the QIAamp® and 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 of the *AllPrep DNA/RNA FFPE Handbook*.
- Buffer RLT, Buffer FRN, Buffer AL, and Buffer AW1 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*.
- Unless otherwise indicated, perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps using a microcentrifuge set at 15–25°C. If using a refrigerated microcentrifuge, set the temperature to 20–25°C, otherwise, significant cooling below 15°C may occur.
- In the procedure below, ▲ indicates specific steps for purification of total RNA that does not include small RNAs, and ● indicates specific steps for purification of total RNA that includes small RNAs.

Things to do before starting

- If using Buffer FRN, Buffer RPE, Buffer AW1, Buffer AW2, and RNase-free DNase 1 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 (15–25°C). Mix reconstituted Buffer FRN, Buffer RPE, Buffer AW1, and Buffer AW2 by shaking.
- Set a thermal mixer or heated orbital incubator to 56°C for use in step 8.

Procedure

1. **Place FFPE core material into a 2 ml Safe-Lock microcentrifuge tube and close the lid.**

   Do not use more than the recommended maximum amount of starting material. See section "Starting material".

2. **Disrupt and homogenize the FFPE core using the TissueLyser II.**

   Place a stainless steel bead (5 mm diameter) in the microcentrifuge tube containing the sample. Place the tubes in the TissueLyser Adapter Set 2 x 24. Operate the TissueLyser for 30 s at 20 Hz.

   Complete disruption and homogenization may not always be possible. However, small amounts of debris are usually digested in the proteinase K step.

   Leave the stainless steel bead in the microcentrifuge tube and proceed to step 3.

3. **Add 650 µl xylene to the sample. Vortex vigorously for 20 s, and incubate at room-temperature (15–25°C) for 3 min.**

4. **Add 650 µl ethanol (96–100%), and mix by vortexing for 20 s.**

5. **Centrifuge at maximum speed for 5 min.**

   To prevent damage to microcentrifuge tubes, do not exceed 20,000 x *g*.

6. **Remove the stainless steel bead and the supernatant without disturbing the pellet.**

   For bead removal, a magnet can be slid along the outside of the reaction tube, so that the bead is indirectly moved to the rim of the tube. Subsequently remove the supernatant by pipetting. Carefully remove any residual liquid using a fine pipet tip.

   Note: In some cases the pellet may be loose. Remove the stainless steel bead and supernatant carefully.

7. **Keep the lid of the microcentrifuge tube open and incubate at room temperature (15–25°C), or up to 37°C for 10 minutes or until all residual ethanol has evaporated. Proceed to step 8.**

8. **Resuspend the pellet by adding 150 µl Buffer PKD and flicking the tube to loosen the pellet. Add 10 µl proteinase K and mix by vortexing.**

9. **Incubate at 56°C for 15 min.**

   Depending on the sample material, the sample may not be completely lysed. This does not affect the procedure. Proceed to step 10.

10. **Incubate on ice for 3 min.**

    Complete cooling is important for efficient precipitation in step 11.

11. **Centrifuge for 15 min at 20,000 x *g*.**

12. **Carefully transfer the supernatant without disturbing the pellet to a new ▲ 1.5 ml or ● 2 ml Safe-Lock microcentrifuge tube for RNA purification. Keep the pellet for DNA purification.**

    Note: Depending on the amount and nature of the FFPE sample, the pellet may be very small or difficult to see. If the pellet is aspirated with the supernatant, allow the pellet to drop slowly to the bottom of the tube and use the pipet tip to reattach the pellet to the tube. Alternatively, centrifuge the supernatant again.

    The DNA-containing pellet can be stored for 2 h at room temperature (15–25°C), for up to 1 day at 2–8°C, or for longer periods at –20°C.

13. **Proceed to step 10 of the *AllPrep DNA/RNA FFPE Handbook* for RNA purification or to step 25 of the *AllPrep DNA/RNA FFPE Handbook* for DNA purification.**

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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