User-Developed Protocol:

Isolation of total RNA from paraffin-embedded sections using the EZ1® RNA Tissue Mini Kit

This protocol has been adapted by customers and is for purification of total RNA from paraffin-embedded tissue sections using the EZ1 RNA Tissue Mini Kit in combination with the BioRobot® EZ1 workstation. Pretreatment of samples is performed according to the procedure in the *RNeasy® FFPE Handbook*. **QIAGEN has not verified the performance of this user-developed protocol.**

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *EZ1 RNA Tissue Handbook* before beginning this procedure. 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.

Equipment and reagents to be supplied by user

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.

- BioRobot EZ1 workstation, cat. no. 9000705, and disposables (see the *EZ1 RNA Tissue Handbook*)
- EZ1 RNA Card, cat. no. 9015590
- EZ1 RNA Tissue Mini Kit, cat. no. 959034
- Sterile, RNase-free pipet tips
- 1.5 ml or 2 ml microcentrifuges tubes
- Microcentrifuge (with rotor for 1.5 ml or 2 ml tubes)
- Vortexer
- 96–100 % ethanol*
- Xylene
- Disposable gloves
- Water bath or heating block capable of reaching 80°C
- QIAGEN Proteinase K, cat. no. 19131
- Buffer PKD, Proteinase K digest buffer, cat. no. 1034963; contact QIAGEN Technical Services

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

Important points before starting

- Yields will depend on both size/thickness and the type of the tissue processed. RNA integrity will depend on the fixation and embedding method as well as the age of the sample. Yields are expected to be lower than those for fresh or frozen tissues.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming at 37°C and then place at room temperature (15–25°C).
- Prepare DNase I stock solution before using the RNase-free DNase I for the first time. Dissolve the solid DNase I (750 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
- Heat a water bath or heating block to 55°C for incubation in step 12.

Procedure

1. **Using a scalpel, trim excess paraffin off the sample block.**
2. **Cut sections 5–10 µm thick.**

   If the sample surface has been exposed to air, discard the first 2 to 3 sections.
3. **Immediately place 1–4 sections in a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and close the lid.**
4. **Add 1 ml xylene to the sample. Vortex vigorously for 10 s.**
5. **Centrifuge at full speed for 2 min at 20–25°C.**
6. **Remove the supernatant by pipetting. Do not remove any of the pellet.**
7. **Add 1 ml of 96–100% ethanol to the pellet, and mix by vortexing.**

   The ethanol extracts residual xylene from the sample.
8. **Centrifuge at full speed for 2 min at 20–25°C.**
9. **Remove the supernatant by pipetting. Do not remove any of the pellet.**

   Carefully remove any residual ethanol using a fine pipet tip.
10. **Open the tube and incubate at room temperature to 37°C. Incubate for 10 min or until all residual ethanol has evaporated.**

    Note: It is important to completely evaporate all residual ethanol. Any residual ethanol may reduce RNA yield.
11. **Resuspend the pellet in 100 µl Buffer PKD, add 10 µl proteinase K, and mix by vortexing.**

12. **Incubate at 55°C for 15 min, then at 80°C for 15 min.**

    The incubation at 80°C in Buffer PKD partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented RNA. If using only one heating block, leave the sample at room temperature after the 55°C incubation until the heating block has reached 80°C.

13. **Add 200 µl Buffer RLT to adjust binding conditions and transfer the lysates into the 2 ml sample tubes provided with the EZ1 RNA Tissue Mini Kit.**
14. **Continue with step 6 of the EZ1 protocol for purification of total RNA from tissues.**