February 2019

Quick-Start Protocol

RNeasy® FFPE Kit

RNase-Free DNase I and RNeasy MinElute® spin columns should be stored at 2–8°C upon arrival. All other reagents and components of the RNeasy FFPE Kit (cat. no. 73504) should be stored at room temperature (15–25°C). Proteinase K is stable for at least 1 year after delivery when stored at room temperature. If longer storage is required or if ambient temperatures often exceed 25°C, we recommend storage at 2–8°C.

Further information

- *RNeasy FFPE Handbook*: **www.qiagen.com/HB-0375**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the purification of total RNA from FFPE tissue sections. For purifying total RNA from microdissected FFPE tissue sections, refer to the *RNeasy FFPE Handbook*.
- Buffer RBC contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the "Safety Information" section in the *RNeasy FFPE Handbook*.
- Unless otherwise indicated, all steps should be performed at room temperature (15–25°C). 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.
- If using Buffer RPE and the RNase-Free DNase I for the first time, reconstitute them as described in the *RNeasy FFPE Handbook*.
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer RPE by shaking.
- Set a thermal mixer, heat block or water bath to 56°C for use in step 5 and step 9. If possible, set a second thermal mixer, heat block or water bath to 80°C for use in step 9.
- ▲ indicates volumes to use if processing 1–2 sections per sample, while ● indicates volumes to use if processing >2 sections per sample.

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections 5–20 µm thick.
3. Immediately place the sections in ▲ a 1.5 ml or 2 ml microcentrifuge tube or ● a 2 ml microcentrifuge tube (not supplied) and close the lid.
4. Add ▲ 160 µl or ● 320 µl Deparaffinization Solution, vortex vigorously for 10 s and centrifuge briefly to bring the sample to the bottom of the tube.

   Note: Deparaffinization Solution is not supplied with the RNeasy FFPE Kit and should be ordered separately (cat. no. 19093).
5. Incubate at 56°C for 3 min, and then allow to cool at room temperature.
6. Add ▲ 150 µl or ● 240 µl Buffer PKD, and then mix by vortexing.
7. Centrifuge for 1 min at 11,000 x *g* (10,000 rpm).
8. Add 10 µl proteinase K to the lower, colorless phase. Mix gently by pipetting up and down.

9. Incubate at 56°C for 15 min, and then at 80°C for 15 min. Ensure that the heating block has reached 80°C before starting the 15 min incubation.

   If the heating block that you used has no shaking function, vortex the mixture briefly every 3–5 min.

10. Transfer the lower, colorless phase into a new 2 ml microcentrifuge tube.
11. Incubate the mixture on ice for 3 min, and then centrifuge for 15 min at 20,000 x *g* (13,500 rpm).
12. Transfer the supernatant to a new microcentrifuge tube (not supplied). Be careful not to disturb the pellet.
13. Add DNase Booster Buffer equivalent to one-tenth of the total sample volume (approximately ▲ 16 µl or ● 25 µl) and 10 µl DNase I stock solution. Mix by inverting the tube. Centrifuge briefly to collect residual liquid from the sides of the tube.
14. Incubate at room temperature for 15 min.
15. Add ▲ 320 µl or ● 500 µl Buffer RBC to adjust binding conditions, and then mix the lysate thoroughly.
16. Add ▲ 720 µl or ● 1200 µl ethanol (100%) to the sample, and then mix well by pipetting. Do not centrifuge. Proceed immediately to step 17.
17. Transfer 700 µl of the sample – including any precipitate that may have formed – to an RNeasy MinElute spin column placed inside a 2 ml collection tube (supplied). Close the lid gently, and then centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube in step 18.
18. Repeat step 17 until the entire sample has passed through the RNeasy MinElute spin column. Reuse the collection tube in step 19.
19. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and then centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube in step 20.

20. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and then centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.
21. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.
22. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14–30 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

1116192 02/2019 HB-2634-001