Quick-Start Protocol March 2016

miRNeasy FFPE Kit – Part 1

Store the RNeasy® MinElute® spin columns and RNase-Free DNase I from the miRNeasy FFPE Kit (cat. no. 217504) immediately at 2–8°C. Buffers and Proteinase K can be stored at room temperature (15–25°C). Kit components are stable for at least 9 months under these conditions if not otherwise stated on label. If longer storage of Proteinase K is required or if ambient temperatures often exceed 25°C, we recommend storage at 2–8°C.

Further information

- *miRNeasy FFPE Handbook*: **www.qiagen.com/HB-0374**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for purification of total RNA, including miRNA, from FFPE tissue sections. For purification from microdissected FFPE tissue sections, refer to the *miRNeasy 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 *miRNeasy FFPE Handbook*.
- Unless indicated, all steps should be performed at room temperature (15–25°C). Work quickly.
- Perform all centrifugation steps using a microcentrifuge placed 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 *miRNeasy 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 or ● 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.

   Deparaffinization Solution is not supplied with the miRNeasy FFPE Kit and should be ordered separately (cat. no. 19093).
5. Incubate at 56°C for 3 min, then allow to cool at room temperature.
6. Add ▲ 150 µl or ● 240 µl Buffer PKD, and 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, then at 80°C for 15 min. Ensure that the heating block has reached 80°C before starting the 15 min incubation.

   If a heating block without a shaking function is used, briefly mix by vortexing every 3–5 min.
10. Transfer the lower, colorless phase into a new 2 ml microcentrifuge tube.
11. Incubate on ice for 3 min. Then centrifuge for 15 min at 20,000 x *g* (13,500 rpm).
12. Transfer the supernatant to a new microcentrifuge tube (not supplied) taking care not to disturb the pellet. Proceed to step 13, *Quick-Start Protocol: miRNeasy FFPE Kit – Part 2*.

Scan QR code for handbook.

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