Quick-Start Protocol March 2016

miRNeasy FFPE Kit – Part 2

Refer to *Quick-Start Protocol: miRNeasy FFPE Kit – Part 1* for instructions about kit storage and reagent preparation.

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

- ▲ indicates volumes to use if processing 1–2 sections per sample, while ● indicates volumes to use if processing >2 sections per sample.
- Perform steps 1–12 detailed in the *Quick-Start Protocol: miRNeasy FFPE Kit – Part 1*. Next, proceed to step 13 below.

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.
14. Incubate at room temperature for 15 min.
15. Add ▲ 320 µl or ● 500 µl Buffer RBC and mix the lysate thoroughly.
16. Add ▲ 1120 µl or ● 1750 µl ethanol (100%) to the sample, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 17.

17. Transfer 700 µl of the sample, including any precipitate, to an RNeasy® MinElute® spin column 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 in step 18.

18. Repeat step 17 until the entire sample has passed through the column.

    Reuse the collection tube in step 19.

19. Add 500 µl Buffer RPE to the 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 in step 20.

20. Add 500 µl Buffer RPE to the column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm). Discard the collection tube with the flow-through.

    After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise carryover of ethanol will occur.

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.

    Elution with smaller volumes of RNase-free water leads to higher total RNA concentrations, but lower RNA yields. The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

* Flow-through contains Buffer RBC and is therefore not compatible with bleach. See "Safety Information" section in the *miRNeasy FFPE Handbook*.

Scan QR code for handbook.