February 2019

Quick-Start Protocol

exoRNeasy Midi/Maxi Kit

Part 2: RNA Isolation

The exoRNeasy Midi/Maxi Kits (cat. nos. *77144*, *77164*, *77023*) are shipped at ambient temperature. Store RNeasy® MinElute® spin columns immediately at 2–8°C. QIAzol® Lysis Reagent and all remaining components can be stored at room temperature (15–25°C).

Further information, including more detailed protocols

- *exoRNeasy Midi/Maxi Handbook:* **www.qiagen.com/HB-2630**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol (Part 2 of 2) is for isolating total RNA, including small RNAs, from extracellular vesicle (EV) lysates prepared according to Part 1.
- If necessary, redissolve any precipitate in Buffer RWT by warming.
- Add 30 ml ethanol (96–100%) to Buffer RWT concentrate and 44 ml ethanol (96–100%) to Buffer RPE concentrate before use.
- Except for phase separation (step 5), all steps should be performed at room temperature (15–25°C). Work quickly.

(*Continued from Part 1*)

7. Briefly vortex the tube containing the lysate collected at the end of Part 1 (i.e., the *exoRNeasy Midi/Maxi Kit, Part 1: Vesicle Isolation* quick-start protocol), and then incubate at room temperature (15–25°C) for 5 min.
   Optional: RNA spike-in control may be added at this point (see handbook for recommendations).
8. Add 90 µl chloroform, and cap tube securely. Shake vigorously for 15 s.

9. Incubate at room temperature for 2–3 min.
10. Centrifuge for 15 min at 12,000 x *g* at 4°C.
11. Transfer the upper aqueous phase to a new collection tube (not supplied). Avoid transferring any interphase. Add 2 volumes of 100% ethanol (e.g., for 400 µl aqueous phase, add 800 µl ethanol). Mix thoroughly by pipetting.
12. Pipet up to 700 µl sample, including any precipitate, into an RNeasy MinElute spin column in a 2 ml collection tube. Close the lid and centrifuge at ≥8000 x *g* for 15 s at room temperature. Discard the flow-through.
13. Repeat step 12 using the remainder of the sample.
14. Add 700 µl Buffer RWT to the RNeasy MinElute spin column. Close the lid, and then centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
15. Pipet 500 µl Buffer RPE onto the RNeasy MinElute spin column. Close the lid, and then centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
16. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid, and then centrifuge for 2 min at ≥8000 x *g*. Discard the flow-through and the collection tube.
17. 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 to dry the membrane. Discard the flow-through and the collection tube.
18. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, let the column stand for 1 min, and then centrifuge for 1 min at full speed to elute the RNA.

Scan QR code for handbook.

exosomed a bio-techne brand

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