Quick-Start Protocol April 2016

exoRNeasy Serum/Plasma Maxi and Midi Kits, Part II: RNA Isolation

The exoRNeasy Serum/Plasma Maxi and Midi Kits (cat. nos. 77064 and 77044) are shipped at ambient temperature. Store the RNeasy® MinElute® spin columns immediately at 2–8°C. Store the miScript® Primer Assay at –15°C to –30°C. QIAzol® Lysis Reagent can be stored at room temperature (15–25°C) or at 2–8°C. Store the remaining components dry at room temperature. All kit components are stable for at least 9 months under these conditions if not otherwise stated on label.

Further information

- *exoRNeasy Serum/Plasma Handbook*: **www.qiagen.com/HB-1779**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for isolating total RNA, including small RNAs, from exosomes and other extracellular vesicles (EVs). For the vesicle isolation protocol, see Part I of the Quick-Start Protocol.
- If necessary, redissolve any precipitate in Buffer RWT by warming.
- Except for phase separation (step 11), all steps should be performed at room temperature (15–25°C). Work quickly.
- Add ethanol (96–100%) to Buffer RWT and Buffer RPE concentrates before use (see bottle label for volume).
- The miRNeasy Serum/Plasma Spike-In Control (cat. no. 219610) must be purchased separately. For recommendations on how to prepare a working solution, see the *exoRNeasy Serum/Plasma Handbook*.

7. Briefly vortex the tube containing the lysate and incubate at room temperature (15–25°C) for 5 min.
8. Optional: Add 3.5 µl miRNeasy Serum/Plasma Spike-In Control (at 1.6 x 10^8 copies/µl).
9. Add 90 µl chloroform and cap tube securely. Shake vigorously for 15 s.
10. Incubate at room temperature for 2–3 min.
11. Centrifuge for 15 min at 12,000 x *g* at 4°C.
12. 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.
13. 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.
14. Repeat step 13 using the remainder of the sample.
15. Add 700 µl Buffer RWT to the RNeasy MinElute spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
16. Pipet 500 µl Buffer RPE onto the RNeasy MinElute spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
17. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid, and centrifuge for 2 min at ≥8000 x *g*. Discard the flow-through and the collection tube.
18. 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.
19. 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.

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