Quick-Start Protocol April 2016

exoRNeasy Serum/Plasma Midi Kit, Part I: Vesicle Isolation

The exoRNeasy Serum/Plasma Midi Kit (cat. no. 77044) is shipped at ambient temperature. Store the RNeasy® MinElute® spin columns immediately at 2–8°C. Store the miScript® Primer Assay at –30 to –15°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 purifying exosomes and other extracellular vesicles (EVs) from 0.1 to 1 ml serum or plasma. The protocol to isolate total RNA, including small RNAs, from EVs is included in Part II.
- 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*.
- The RNA purification part of the protocol (following step 7) is compatible with QIAGEN MaXtract High Density Tubes (cat. no. 129056).

1. It is recommended to only use pre-filtered plasma, excluding particles larger than 0.8 µm (e.g., using Sartorius® Minisart® NML (cat. no. 16592) or Millipore® Millex®-AA (cat. no. SLAA033SB) syringe filters). Alternatively, centrifuge samples for 10 min at 16,000 x *g* and 4°C.
2. Add 1 volume Buffer XBP to 1 volume of sample. Mix well immediately by gently inverting the tube five times. Let mixture warm to room temperature.
3. Add the sample/Buffer XBP mix onto the exoEasy spin column and spin the device for 1 min at 500 x *g*. Discard the flow-through and place the column back into the same collection tube.

   Note: In case any liquid remains on the membrane, spin again for 1 min at 5000 x *g* to make sure all liquid has passed through the membrane.
4. Add 3.5 ml Buffer XWP and spin 5 min at 5000 x *g* to remove residual volume from the column. Discard the flow-through together with the collection tube.

   Note: It is possible to reduce the steps performed at 5000 x *g* down to a minimum force of 3000 x *g* without performance loss.
5. Transfer the spin column to a fresh collection tube.
6. Add 700 µl QIAzol to the membrane. Spin for 5 min at 5000 x *g* to collect the lysate and transfer completely to a supplied 2 ml tube. Continue with Part II of the Quick-Start Protocol for the RNA isolation protocol, starting with Step 7.

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