Quick-Start Protocol March 2016

miRNeasy Serum/Plasma Kit

The miRNeasy Serum/Plasma Kit (cat. no. 217184) 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

- *miRNeasy Serum/Plasma Handbook*: **www.qiagen.com/HB-1002**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for purifying total RNA, including small RNAs, from small volumes (up to 200 µl) of serum, plasma or other body fluids.
- If necessary, redissolve any precipitate in Buffer RWT by warming.
- Except for phase separation (step 7), 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).
- Before starting with step 1 for the first time, read the recommendations for preparing serum or plasma in the *miRNeasy Serum/Plasma Handbook*.
- 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 *miRNeasy Serum/Plasma Handbook*.

1. Prepare serum or plasma, or thaw frozen samples.
2. Add 5 volumes QIAzol Lysis Reagent to the sample (e.g., for 200 µl sample, add 1 ml QIAzol Lysis Reagent). Mix by vortexing or pipetting up and down.

3. Incubate the homogenate at room temperature (15–25°C) for 5 min.
4. Add 3.5 µl miRNeasy Serum/Plasma Spike-In Control (at 1.6 x 10^8 copies/µl).
5. Add chloroform of an equal volume to the starting sample and cap tube securely (e.g., for 200 µl sample, add 200 µl chloroform). Shake vigorously for 15 s.
6. Incubate at room temperature for 2–3 min.
7. Centrifuge for 15 min at 12,000 x *g* at 4°C.
8. Transfer the upper aqueous phase to a new collection tube (not supplied). Avoid transferring any interphase. Add 1.5 volumes of 100% ethanol (e.g., for 600 µl aqueous phase, add 900 µl ethanol). Mix thoroughly by pipetting.
9. 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.
10. Repeat step 9 using the remainder of the sample.
11. 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.
12. 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.
13. Add 500 µl of 80% ethanol 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.
14. 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.
15. 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, and centrifuge for 1 min at full speed to elute the RNA.

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