Quick-Start Protocol April 2021

miRNeasy Serum/Plasma Advanced Kit

The miRNeasy Serum/Plasma Advanced Kit (cat. no. 217204) is shipped at ambient temperature. Upon arrival, store the RNeasy® UCP MinElute® spin columns at 2–8°C. Store the remaining components dry at room temperature (15–25°C). This protocol is for purification of total RNA including small RNAs from serum or plasma.

Further information

- *miRNeasy Serum/Plasma Advanced Kit Handbook*: **www.qiagen.com/HB-2390**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Buffer RWT and RPL contain guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- If necessary, re-dissolve any precipitate in Buffer RPL or Buffer RWT by warming
- Equilibrate buffers to room temperature (15–25°C).
- 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, read method for preparing serum or plasma in the handbook.

Procedure

1. Prepare serum or plasma or thaw frozen samples.
2. Transfer 200 µl serum or plasma into a 2 ml tube.
3. Add 60 µl Buffer RPL. Close the tube caps and vortex for > 5 s. Incubate at room temperature for 3 min.

4. Add 20 µl Buffer RPP. Close the tube caps and mix vigorously by vortexing for >20 s. Incubate at room temperature for 3 min.
5. Centrifuge at 12000 *x g* for 3 min at room temperature to pellet the precipitate.

   Note: Supernatant should be clear and colorless.
6. Transfer the supernatant (~230 µl) to a new reaction tube. Add 1 volume isopropanol. Mix well by vortexing. Transfer the entire sample to an RNeasy UCP MinElute column. Close the lid, and centrifuge for 15 s at ≥8000 *x g*. Discard the flow-through.
7. Pipet 700 µl Buffer RWT to the RNeasy UCP MinElute spin column. Close the lid, and centrifuge for 15 s at ≥8000 *x g*. Discard the flow-through.
8. Pipet 500 µl Buffer RPE onto the RNeasy UCP MinElute spin column. Close the lid, and centrifuge for 15 s at ≥8000 *x g*. Discard the flow-through.
9. Add 500 µl of 80% ethanol to the RNeasy UCP MinElute spin column. Close the lid, and centrifuge for 2 min at ≥8000 *x g*. Discard the flow-through and the collection tube.
10. Place the RNeasy UCP 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.
11. Place the RNeasy UCP MinElute spin column in a new 1.5 ml collection tube (supplied). Add 20 µl RNase-free water directly to the center of the spin column membrane and incubate 1 min. Close the lid, and centrifuge for 1 min at full speed to elute the RNA.

Document Revision History

Date	Changes
04/2021	Removed the reference to the miRNeasy Serum/Plasma Spike-In Control.

Scan QR code for handbook.

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