April 2021

Quick-Start Protocol

miRNeasy Tissue/Cells Advanced Micro Kit

The miRNeasy Tissue/Cells Advanced Micro Kit (cat. no. 217684) is shipped at ambient temperature. Store the RNeasy® UCP MinElute® spin columns immediately at 2–8°C. Store the remaining components dry at room temperature (15–25°C). All kit components are stable for at least 9 months under these conditions if not otherwise stated on label. This protocol is for purification of total RNA, including small RNAs from animal cells and tissues.

Further information

- *miRNeasy Tissue/Cells Advanced Micro Kit Handbook*: **www.qiagen.com/HB-2902**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Buffers RWT, AL, and RLT contain guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- If necessary, redissolve any precipitate in Buffer RLT or Buffer RWT by warming.
- Equilibrate buffers to room temperature.
- All steps should be performed at room temperature. Work quickly.
- Add ethanol (96–100%) to Buffer RWT and Buffer RPE concentrates before use (see bottle label for volume).

- The RNA Spike-in Kit, for RT (cat no. 339390), may be purchased separately. For instructions on preparing a working solution, please refer to the handbook.
- If purifying RNA from cell lines rich in RNases or tissue, add either 10 µl β-mercaptoethanol (β-ME) or 20 µl 2 M dithiothreitol (DTT) to 1 ml Buffer RLT before use. Buffer RLT containing DTT or β-ME can be stored at room temperature for up to 1 month.

Procedure

1. **Cells**: Harvest a maximum of 5 x 10^5 cells as a cell pellet or lysed directly in the vessel. Add 260 µl of Buffer RLT. Vortex for 30 s or homogenize.

   Tissues: Disrupt the tissue (≤5 mg*) and homogenize the lysate in 260 µl of Buffer RLT.
2. Add 40µl Buffer AL and mix thoroughly. Incubate at room temperature for 3 min. Transfer the homogenized lysate to a gDNA Eliminator spin column placed in a 2 ml collection tube (supplied).
3. Centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Discard the column and save the flow-through.

   Optional: Steps 4 and 5 do not need to be carried out when working with cell samples.
4. Transfer the flow-through to a new 2 ml reaction vessel (not provided). Add 20 µl Buffer RPP. Close the tube cap and mix vigorously by vortexing for >20 s. Incubate at room temperature for 3 min.
5. Centrifuge at 12,000 x *g* for 3 min at room temperature to pellet the precipitate.

   Note: Supernatant should be clear and colorless. Transfer supernatant (for cells: approx. 300 µl; for tissue: approx. 270 µl) to a new 2 ml reaction tube.
6. Add 1.3 volume isopropanol and mix well by pipetting. Transfer the entire sample to an RNeasy® UCP MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
7. Repeat step 6 using the remainder of the sample.

* The amount indicated is applicable for fresh or frozen tissue only. For stabilized tissue, use only half of the amount indicated.

8. 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.
9. 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.
10. 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.
11. 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 collection tube.
12. 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 for 1 min. Close the lid and centrifuge for 1 min at full speed to elute the RNA.

Document Revision History

Date	Changes
04/2021	Initial release

Scan QR code for handbook.

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