

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 μ l β -mercaptoethanol (β -ME) or 20 μ l 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 5×10^5 cells as a cell pellet or lysed directly in the vessel. Add 260 μ l of Buffer RLT. Vortex for 30 s or homogenize.

Tissues: Disrupt the tissue (≤ 5 mg *) and homogenize the lysate in 260 μ l of Buffer RLT.

2. Add 40 μ l 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 $\geq 8000 \times g$ ($\geq 10,000$ 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 μ l Buffer RPP. Close the tube cap and mix vigorously by vortexing for >20 s. Incubate at room temperature for 3 min.
5. Centrifuge at $12,000 \times g$ for 3 min at room temperature to pellet the precipitate.
Note: Supernatant should be clear and colorless. Transfer supernatant (for cells: approx. 300 μ l; for tissue: approx. 270 μ l) 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 $\geq 8000 \times g$. 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.

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8. Pipet 700 µl Buffer RWT to the RNeasy UCP MinElute spin column. Close the lid and centrifuge for 15 s at $\geq 8000 \times g$. 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 $\geq 8000 \times g$. 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 $\geq 8000 \times g$. 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
07/2021	Updated the HB number in the trademarks line.



Scan QR code for handbook.

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