Quick-Start Protocol March 2016

miRNeasy Micro Kit

The miRNeasy Micro Kit (cat. no. 217084) is shipped at ambient temperature. Store the RNeasy® MinElute® spin columns immediately at 2–8°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 Micro Handbook*: **www.qiagen.com/HB-1001**
- 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 amounts of animal cells (up to 1 x 10^6 cells) and tissue (up to 5 mg tissue).
- If necessary, redissolve any precipitate in Buffer RWT by warming.
- Except for phase separation (step 5), 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, select disruption and homogenization methods according to recommendations in the *miRNeasy Micro Handbook*.

1. Add 700 µl QIAzol Lysis Reagent to the sample and disrupt and homogenize using an appropriate method.
2. Incubate the homogenate at room temperature (15–25°C) for 5 min.
3. Add 140 µl chloroform and cap tube securely. Shake vigorously for 15 s.
4. Incubate at room temperature for 2–3 min.

5. Centrifuge for 15 min at 12,000 x *g* at 4°C.
6. Transfer the upper aqueous phase to a new collection tube (not supplied). Avoid transferring any interphase. Add 1.5 volumes (usually 525 µl) of 100% ethanol, and mix thoroughly by pipetting.
7. 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.
8. Repeat step 7 using the remainder of the sample.
9. **Optional**: Perform DNase digest according to instructions in the Appendix of the handbook (not required for detecting **mature miRNA** using the miScript PCR System).
10. 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.

    This step is optional if working with cultured cells.
11. 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.
12. 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.
13. 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.
14. 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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