Quick-Start Protocol March 2016

miRNeasy Mini Kit

The miRNeasy Mini Kit (cat. no. 217004) can be stored dry at room temperature (15–25°C) for at least 9 months if not otherwise stated on label. QIAzol® Lysis Reagent can be stored at room temperature or at 2–8°C.

Further information

- *miRNeasy Mini Handbook*: **www.qiagen.com/HB-1277**
- 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 animal cells and 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 Mini Kit 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. 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® Mini 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 Appendix B of the handbook (not required for detecting **mature miRNA** using the miScript PCR system).
10. Add 700 µl Buffer RWT to the RNeasy Mini 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 Mini column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
12. Add 500 µl Buffer RPE to the RNeasy Mini column. Close the lid, and centrifuge for 2 min at ≥8000 x *g*.
13. **Optional**: Place the RNeasy Mini column into a new 2 ml collection tube. Centrifuge at full speed for 1 min to further dry the membrane.
14. Transfer the RNeasy Mini column to a new 1.5 ml collection tube. Pipet 30–50 µl RNase-free water directly onto the RNeasy Mini column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x *g* to elute.
15. If expected RNA yield is >30 µg, repeat step 14 using an additional 30–50 µl RNase-free water or using the eluate from step 14 (if high RNA concentration is required). Reuse the collection tube from step 14.

Scan QR code for handbook.