Quick-Start Protocol April 2016

miRNeasy 96 Kit

The miRNeasy 96 Kit (cat. no. 217061) can be stored at room temperature (15–25°C) for up to 9 months if not otherwise stated on label.

Further information

- *miRNeasy 96 Handbook*: **www.qiagen.com/HB-1253**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- For more information, including preparation of miRNA-enriched fractions, optional DNase treatment and general handling advice, refer to the *miRNeasy Mini Handbook*, which can be found at **www.qiagen.com/HB-1277**.
- QIAzol® Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- Add ethanol (96%–100%) to Buffer RWT and Buffer RPE as indicated on the bottle label. Dissolve any precipitates in Buffer RWT by warming.
- Equilibrate buffers to room temperature (15–25°C).
- A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary. After switching on, apply vacuum until transfer is complete (1–5 min). Switch off the vacuum and ventilate the manifold.
- All centrifugation steps in the protocol are performed in a Centrifuge 4–16K.
- Step 6 and step 9 should be carried out at 4°C. All other steps should be performed at room temperature.

1. Prepare the QIAvac 96 vacuum manifold and harvest cells as described in the handbook.
2. Loosen the cell pellet by flicking the tube. Add 700 µl QIAzol Lysis Reagent to each plate well/collection microtube. Pipet up and down 3 times.

3. If the lysates are in plate wells, transfer to collection microtubes (supplied).
4. Close the collection microtubes using the supplied caps, and vortex for 1 min at maximum speed. If cell number is >3 x 10^6 cells, homogenize with the TissueLyser II as described in the *miRNeasy 96 Handbook*.
5. Incubate homogenates at room temperature (15–25°C) for 5 min.
6. Centrifuge at 5600 x *g* (approximately 6000 rpm) for 1 min at 4°C.
7. Add 140 µl chloroform. Securely cap the homogenates using new strips of collection microtube caps. Shake the rack vigorously for 15 s.
8. Incubate at room temperature for 2–3 min.
9. Centrifuge at 6000 x *g* for 15 min at 4°C.
10. Transfer the upper aqueous phases to a new S-Block. Add 1.5 volumes of 100% ethanol and mix. Proceed immediately to step 11.
11. Pipet the samples into the wells of the RNeasy® 96 plate and apply vacuum.
12. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate. Apply vacuum.
13. Lift the top plate carrying the RNeasy 96 plate off the base, and empty the waste tray. Reassemble the QIAvac 96 vacuum manifold.
14. Add 800 µl Buffer RPE to each well and apply vacuum. Repeat.
15. Place the RNeasy 96 plate on top of an S-Block. Seal with an AirPore Tape Sheet. Load into the holder and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm for 10 min at room temperature.
16. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.
17. Add 45–70 µl RNase-free water to each well and seal with a new tape sheet. Incubate for 1 min. Centrifuge at 6000 rpm for 4 min at room temperature.
18. Remove the AirPore Tape Sheet. Repeat the elution step (step 17) with a second volume of 45–70 µl RNase-free water.

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