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Quick-Start Protocol

miRNeasy 96 Tissue/Cells Advanced Kit

This protocol is for the purification of miRNA from cells and easy-to-lyse tissue samples using the miRNeasy 96 Tissue/Cells Advanced Kit (cat no. 217661).

Further information

- *miRNeasy 96 Tissue/Cells Advanced Kit Handbook*: **www.qiagen.com/HB-3134**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Buffers RLT and 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.
- Equilibrate buffers to room temperature (15–25°C).
- All centrifugation steps in the protocol are performed in a Centrifuge 4–16K.
- All steps should be performed at room temperature (15–25°C). Work quickly.
- Before starting, read the *miRNeasy 96 Tissue/Cells Advanced Kit Handbook*, **www.qiagen.com/HB-3134**
- If purifying RNA from cell lines rich in RNases or from tissue, we recommend adding either β-mercaptoethanol (β-ME) or 2 M dithiothreitol (DTT) to Buffer RLT before use (10 µL β-ME or 20 µL DTT per 1 mL Buffer RLT). Buffer RLT containing DTT or β-ME can be stored at room temperature for up to 1 month.

Preparing the samples

- **Symbols**: RNA purification from ● cells/▲ tissue samples.

1. ● Harvest cells as a cell pellet or, for cells grown in a monolayer, aspirate the cell-culture medium from the cell-culture vessel. Add 300 µL Buffer RLT to either the pellet or the cell-culture vessel, vortex, or pipet to mix and homogenize.

   ▲ Add 300 µL Buffer RLT to tissue sample (not more than 30 mg fresh/frozen or 15 mg stabilized tissue, for further information please refer to the handbook), then disrupt and homogenize. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting and transfer to an S-Block.
2. Add 80 µL Buffer AL to each sample and mix by pipetting. Incubate at room temperature for 3 min. Transfer the lysate to a gDNA Eliminator 96 plate and centrifuge at 6000 rpm for 4 min at room temperature. Discard the plate and save the flow through.
3. Add 75 µl RNase-free Water and 25 µL proteinase K, mix and incubate for 10 min at room temperature.
4. Place an RNeasy® 96 plate on top of a square-well block.
5. Add 1 volume of 100% isopropanol and mix. Proceed immediately to step 6.
6. Pipet the samples into the wells of the RNeasy 96 plate, seal with an AirPore Tape Sheet and centrifuge for 4 min at full speed. Empty the square well block.
7. Add 800 µl Buffer RWT to each well of the RNeasy 96 plate, seal with an AirPore Tape Sheet and centrifuge for 4 min at full speed. Empty the square well block.
8. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, seal with an AirPore Tape Sheet and centrifuge at full speed for 4 min. Empty the square block and repeat this step.
9. 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.

10. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing elution microtubes.
11. Add 70–100 µL RNase-free water to each well and seal with a new tape sheet. Incubate for 1 min. Centrifuge at full speed for 4 min.

Document Revision History

Date	Changes
01/2023	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.