

Quick-Start Protocol

miRNeasy[®] 96 Advanced QIAcube[®] HT Kit

- This protocol is for the purification of miRNA from 50–200 µl serum or plasma samples using the miRNeasy 96 Advanced QIAcube HT Kit with the QIAcube HT Prep Manager software. Download the corresponding protocol at www.qiagen.com/HB-2618. The protocol is not compatible with QCHT 4.17 software.

Further information

- *miRNeasy 96 Advanced QIAcube HT Handbook*: www.qiagen.com/HB-2618
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- Buffers RPL and RWT contain a guanidine salt and are therefore not compatible with reagents containing bleach.
- Add ethanol (96% – 100%) to Buffer RWT and Buffer RPE as indicated on the bottle label. Dissolve any precipitates in Buffer RPL by warming.
- Equilibrate buffers to room temperature (15–25°C).
- All centrifugation steps in the protocol are performed in a centrifuge (Sigma 4–16K).
- All steps should be performed at room temperature (15–25°C). Work quickly.
- Use a Collection Microtube Rack (CMTR) for sample preparation.
- Prepare a serum, plasma, or thaw frozen samples. Up to 200 µl human, mouse, or rat plasma can be used. For starting volumes that are less than 200 µl, please follow the

instructions in *miRNeasy 96 Advanced QIAcube HT Handbook* (see www.qiagen.com/HB-2618).

Preparing the samples

1. Transfer 200 µl serum or plasma per well into a CMTR rack. If using less than 200 µl starting material, please fill-up the volume with buffer TBS.
2. Add 60 µl Buffer RPL and close tubes with caps. Vortex thoroughly, and leave at room temperature for 3 min. Centrifuge shortly to spin down lysate.
3. Add 20 µl Buffer RPP. Securely cap the homogenates using the collection microtube caps. Mix vigorously by vortexing for at least 10 s. Incubate for 3 min at room temperature.
4. Centrifuge at 6,000 rpm (approximately 5,600 x g) for 3 min at room temperature to remove precipitate.

Note: Supernatant should be clear and colorless.

5. Transfer supernatant (~230 µl) to a new S-Block.

Note: Transfer procedure of Step 5 can be carried out by QIAcube HT instrument. Choose **Transfer Protocol** under setup/selected protocol. For detailed information, refer to the *miRNeasy 96 Advanced QIAcube HT Handbook*.

miRNeasy 96 Advanced Protocol on the QIAcube HT

1. Start the QIAcube HT Prep Manager software. Click the **Home** icon in the main toolbar to access the Home screen.
 2. Select miRNeasy 96 Advanced from the **Create Experiment** list. Follow the instructions in the wizard and fill in all required fields.
 3. In the **Setup** step, select **Sample type** and **Pre-treatment** for documentation.
 4. Select the protocol: miRNeasy 96 Advanced protocol. For information about optional steps and advanced options, see *miRNeasy 96 Advanced QIAcube HT Handbook*.
 5. Define samples in the **Labware selection** step.
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6. Arrange samples to the output plate in the **Assignment** step.

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

7. Follow the instructions on the virtual worktable to prepare the workbench of the instrument.

8. Add the appropriate sample volume to the selected S-Block wells as indicated in the virtual **Worktable**.

9. Click **Save** from the menu to save the experimental setup.

10. Click **Start Run**.

Important: The protocol includes an optional vacuum performance check after the first application of vacuum. This vacuum performance check can be activated during experimental setup. If activated, the software will show a dialog that needs to be confirmed after the application of vacuum is completed.

11. When the protocol is finished, cover the Elution Microtube Rack (EMTR) with the lid, and remove it from the elution chamber.

Note: If Top Elute Fluid was used, elution microtubes of the EMTR will contain two liquid phases. RNA will be in the lower phase covered by Top Elute Fluid.

Important: Please ensure that you only take the eluate from below the top layer for downstream applications.

12. Create a report (if required).

13. Follow the cleaning procedure.

Document Revision History

R1 Initial release
12/2018



Scan QR code for handbook.

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