Purification of total RNA or total RNA, including miRNA, from animal tissue and cells on the QIAcube® HT and the QIAxtractor® using the RNeasy® 96 QIAcube HT Kit

Store RNeasy 96 plates and buffers at room temperature (15–25°C).

Further information

- *RNeasy 96 QIAcube HT Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- This protocol is for the purification of total RNA or total RNA, including miRNA, from human or animal cells and tissue with the RNeasy 96 QIAcube HT Kit.
- For this protocol, QIAzol Lysis Reagent (cat. no. 79306), Collection Microtube Racks (cat. no. 19560), and Collection Microtube Caps (cat. no.19566) need to be ordered separately.
- If carrying out optional on-plate DNase digestion, refer to Appendix D of the *RNeasy 96 QIAcube HT Handbook*.
- Do not overload the RNeasy membrane as this can lead to impaired RNA extraction and/or performance in downstream assays. See the kit handbook for instructions on preparing Buffers RWT and RPE and for more information on handling various sample types.
- Avoid repeated freezing and thawing of samples as this may reduce RNA yield and quality.
- Prepare Buffers RWT and RPE according to the instructions in the kit handbook.
- Ensure that software version 4.17.1 or higher, along with the following instrument run files, are installed: "RNeasy 96 QIAcube HT pre-treatment V1.QSP" and "miRNeasy 96 QIAcube HT V1.QSP", "RNeasy 96 QIAcube HT total RNA Tissue V1.QSP", or "RNeasy 96 QIAcube HT total RNA Tissue with DNase V1.QSP". This is mandatory to process the RNeasy 96 QIAcube HT Kit.

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Pretreatment procedure prior to purification

1. Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube), and transfer the collection microtube rack to a box with dry ice.
2. Remove the tissue sample from RNA*later*® RNA Stabilization Reagent or from cold storage and determine the amount of tissue. For information on various starting materials, please refer to the *RNeasy 96 QIAcube HT Handbook*.
3. Transfer the sample immediately to a cooled collection microtube. Please refer to the *RNeasy 96 QIAcube HT handbook* for further information.
4. Immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube and close the collection microtube.
5. Homogenize the sample:

   a. For cells, pipet up and down 3 times.

   b. For tissue material, lyse sample on the TissueLyser for 2 x 5 min at 25 Hz.
6. Place the collection microtube rack containing the homogenate on the benchtop at room temperature for 5 min.
7. Centrifuge at 6,000 x *g* for 1 min to collect residual liquid from the caps of the tubes.
8. Add 150 µl chloroform. Securely cap the collection microtube rack, and shake it vigorously while inverting the rack for 15 s.
9. Place the collection microtube rack on the benchtop at room temperature for 2–3 min.
10. Centrifuge at 6,000 x *g* for 15 min at 4°C.
11. Place the tip discard chute on the worktable so that the chute is over the tip disposal box.
12. Switch on the instrument. The switch is located at the back of the instrument, on the lower left.
13. Launch the QIAcube HT Software and select the **QProtocols** tab.

    Note: For samples that were lysed in a collection microtube rack using the Tissuelyser and a 5 mm steel bead, phase separation and the transfer of upper aqueous phase into an S-Block can be automated. Please follow the steps below. For samples prepared differently, phase separation needs to

be carried out manually. Please place an S-block containing the aqueous phase in worktable position B1 and proceed with step 1 of the purification procedure below.

13a. Select the "RNeasy 96 QIAcube HT pre-treatment V1.QSP" run file from the **QProtocol** tab.

13b. If processing <96 samples, follow the steps below. Otherwise, skip to step 13c.

i. Unlock the opened protocol.

ii. Select the appropriate wells on position C1 on the worktable setup. Right-click using the mouse and select **Add selected wells to sample bank**.

iii. Name the bank and click on **Add Selection and Close**.

iv. Double click the right-hand panel to open the reaction configuration. Select the appropriate sample bank set and click **OK**.

13c. Load tips. Place the collection Microtube rack containing your sample in position C1. Load an empty S-Block in position B1.

13d. Start the run immediately and perform the pre-run check. After completing the pre-run check, close the instrument hood and click **OK**. Click **Cancel** when the **Save as** dialog box appears. The protocol run begins.

13e. After the run, remove the collection microtube rack containing the organic phase and dispose of according to laboratory guidelines.

13f. If continuing directly with RNA purification, leave the S-Block in position B1and proceed with step 1. If not, remove the S-Block, and seal it with tape. Samples should be frozen if RNA isolation is not planned within the next 8 h.

Purification procedure

1. Open the "miRNeasy 96 QIAcube HT.QSP" run file by selecting **New file** in the toolbar of the QIAcube HT software, selecting the protocol from the **QProtocol** tab, and clicking **Open**. A **Protocol Description** of the selected Q Protocol will be displayed and the QIAGEN Protocol icon will appear in the toolbar.

2. Check that the Q Protocol meets your requirements, and then click **Close**. Click  in the toolbar.

3. Select the appropriate number of samples arranged in columns in the 96-well plate. Ensure that the **Turn the HEPA filter on automatically** option is checked, and click **Jump to End**.

4. Confirm the protocol by clicking **Finish**. The wizard closes.

   IMPORTANT: Ensure that there are sufficient numbers of tips for the protocol run, that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes.

5. Prepare the vacuum chamber. See the *QIAcube HT User Manual* for further information.

   Note: If less than 12 columns are to be processed, seal unused columns of the RNeasy 96 plate with adhesive tape (supplied).

6. Transfer the indicated volumes of all reagents into the corresponding reagent troughs, close the lids, and place them on the indicated positions on the worktable.

7. Start the run immediately and perform the pre-run check. After completing the pre-run check, close the instrument hood and click **OK**. Click **Cancel** when the **Save as** dialog box appears. The protocol run begins.

8. Cover the elution plate (EMTR) with the lid, and remove from the elution chamber, when the protocol is complete.

   Note: Two liquid phases might be found in the Elution Microtubes. If this is the case, Top elute fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

9. Discard used plasticware. We recommend discarding leftover reagents in the reagent troughs.

10. Clean the carriage, channeling block, channeling block holder, and tip chute. Turn on the UV lamp to decontaminate the worktable.