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RNeasy® 96 QIAcube® HT Handbook

For automated purification of total RNA from animal and human cells and tissue using QIAcube HT and QIAcube HT Prep Manager Software

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy 96 QIAcube HT Kit	**(5)**
Catalog no.	**74171**
Number of preps	**480**
RNeasy 96 plates	5
Buffer RLT*	2 x 45 ml
Buffer RWT*† (concentrate)	2 x 80 ml
Buffer RPE† (concentrate)	2 x 65 ml
RNase-free water	3 x 30 ml
TopElute Fluid	60 ml
Quick-Start Protocol	1

* **CAUTION:** Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See "Safety Information" on page 6.

† Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

QIAcube HT Plasticware	**(480)**
Catalog no.	**950067**
Number of preps	**480**
S-Blocks	5
Filter-Tips OnCor C	9 x 96
Tape Pad	1
Elution Microtubes RS (EMTR)	5
8-Well Strip Caps for EMTR	120

The following components can also be ordered separately: S-Blocks and Elution Microtubes RS (including caps for strips). See "Ordering Information" on page 70.

Storage

The RNeasy 96 QIAcube HT Kit, including all reagents and buffers, is stable for at least 9 months at room temperature (15–25°C) and under dry conditions if not otherwise stated on the label.

Intended Use

The RNeasy 96 QIAcube HT Kit is intended for the automated extraction of total RNA from human and animal cells and tissue samples using the QIAcube HT instrument. The RNeasy 96 QIAcube HT Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffer RLT and Buffer RWT contain guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.
If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of RNeasy 96 QIAcube HT Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The RNeasy 96 QIAcube HT Kit enables simultaneous purification of total RNA from up to 96 samples, each containing up to 5 x 10^5 animal or human cells or less than 40 mg tissue. The RNeasy 96 QIAcube HT Kit provides efficient, high-throughput RNA sample preparation for research use in fields such as drug screening and basic research. The RNeasy 96 QIAcube HT procedure replaces time-consuming and tedious methods involving alcohol-precipitation steps or large numbers of wash steps. The purified RNA is ready to use in any downstream application, including:

- RT-PCR and real-time RT-PCR
- Differential display
- cDNA synthesis
- Northern, dot and slot blot analyses
- Primer extension
- Poly A+ RNA selection
- RNase/S1 nuclease protection

Principle and procedure

The RNeasy 96 QIAcube HT Kit uses well-established technology for RNA preparation in a 96-well format (see Figure 1 on page 9). The kit combines the selective binding properties of silica-based membrane with the speed of vacuum processing for total RNA purification from up to 5 x 10^5 animal or human cells or less than 40 mg tissue per sample. The QIAcube HT System provides walkaway automation of the RNeasy 96 procedure. Easy operation of the QIAcube HT instrument is allowed by a virtual worktable in the QIAcube HT Prep Manager Software that reflects the worktable on the instrument.

Cell lysis is performed manually under highly denaturing conditions with guanidine thiocyanate to immediately inactivate RNases and ensure purification of intact RNA. After the run is started, ethanol is added to the lysate to provide appropriate binding conditions, and the samples are then applied to the wells of the RNeasy 96 plate. Total RNA binds and contaminants are efficiently washed away. High-quality RNA is then eluted in a small volume of RNase-free water, ready for use in any downstream application.

The automated RNeasy 96 procedure provides two possibilities of purification. The first option purifies all RNA molecules longer than 200 nucleotides. This procedure provides enrichment for mRNA, since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. The size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently.

The second option purifies total RNA including small RNAs from human and animal cells and tissues. For this procedure, we recommend using QIAzol® Lysis Reagent as the lysis buffer.

RNeasy 96 QIAcube HT Procedure

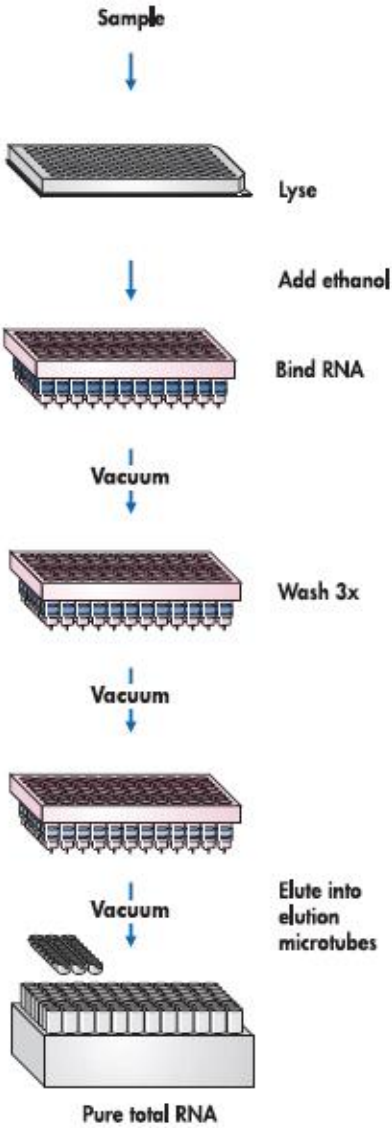


Figure 1: Automated procedure of RNA purification using RNeasy 96 QIAcube HT Kit on the QIAcube HT instrument.

Description of protocols

This handbook describes several different protocols for use with the RNeasy 96 QIAcube HT Kit. The protocols vary depending on the starting material (cells or tissue) and desired size distribution of purified RNA (including or excluding RNA <200 nucleotides).

- RNeasy cell protocol
- RNeasy tissue protocol
- miRNeasy protocol
- RNeasy sample transfer protocol

RNeasy cell protocol

This protocol is for the purification of total RNA (>200 nucleotides) from animal or human cells. For details regarding the optional on-plate DNase digestion, see Appendix D.

RNeasy tissue protocol

This protocol is for the purification of total RNA (>200 nucleotides) from animal or human tissue. For details regarding the optional on-plate DNase digestion, see Appendix D. Tissue samples are processed following sample lysis in QIAzol Lysis Reagent, which must be ordered separately (see "Ordering Information" on page 70).

miRNeasy protocol

This protocol is for the purification of total RNA, including small RNAs (<200 nucleotides) from animal and human tissue and cells. Samples are processed following sample lysis in QIAzol Lysis Reagent, which must be ordered separately (see "Ordering Information" on page 70).

RNeasy sample transfer protocol

This protocol is for the transfer of the upper aqueous phase into a S-block when samples are lysed with the TissueLyser II (using 5 mm beads).

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For all protocols:

- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Ethanol (96–100%)*
- QIAcube HT Instrument
- QIAcube HT Prep Manager Software
- QIAcube HT Reagent Troughs†
- Disposable gloves
- Vortexer

Optional reagents for purification of cell samples with high amounts of RNases

- 14.3 M ß-mercaptoethanol‡ (commercially available solutions are usually 14.3 M)
- RNase-Free DNase Set (cat. no. 79254) for optional on-plate DNase digestion (see Appendix D on page 68, for details)

Optional equipment for purification of tissue samples

- QIAzol Lysis Reagent (200 ml; cat no. 79306)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† For ordering, see "Ordering Information" on page 70.
‡ When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Collection Microtube Racks (cat. no. 19560)
- Collection Microtube Caps (cat. no. 19566)
- Stainless Steel Beads 5 mm (cat. no. 69989)
- Tissue disruption system such as TissueLyser II (cat. no. 85300)
- Centrifuge

TissueLyser II

The TissueLyser II includes a number of different accessories for ease of use with different sample sizes and throughputs. The TissueLyser Adapter Set 2 x 96 allows simultaneous processing of up to 192 samples in collection microtubes. Stainless steel beads with a diameter of 5 mm provide optimal disruption of human and animal tissue. The TissueLyser 5 mm Bead Dispenser, 96-Well, is also available to conveniently deliver 96 beads in parallel into collection microtubes. See "Ordering Information" on page 70.

Centrifuge 4-16KS

For optimal handling, QIAGEN, in cooperation with the centrifuge manufacturer Sigma Laborzentrifugen GmbH, has developed a centrifugation system consisting of the Plate Rotor 2 x 96, and the refrigerated table-top Centrifuge 4-16KS. A temperature of 4°C is necessary during phase separation for optimal removal of genomic DNA.

For further information about the centrifuge and rotor, please contact QIAGEN or your local distributor.

Warning: Do not centrifuge the Plate Rotor 2 x 96 metal holders without the RNeasy 96 plates and S-Blocks, collection microtubes or elution microtubes. If unsupported, the holders will collapse under high g-force. Therefore, remove the holders during test runs.

Standard 96-well microplates may be centrifuged in the holders if a g-force of 500 *x g* is not exceeded.

Important Notes

Starting materials

Amount of cells

The recommended amount of starting material is up to 5 x 10^5 animal or human cells. Direct counting is the most accurate way to quantify the number of cells. A 96-well cell-culture plate with a growth area of 0.32–0.6 cm^2 per well, depending on the supplier, typically contains 4–5 x 10^4 confluent HeLa cells per well. See Table 1 for specifications for the RNeasy 96 plates for cells. Each well of the plate can bind up to 100 µg RNA, but the amount of RNA in up to 5 x 10^5 cells is significantly less than this binding capacity. Expected RNA yields are therefore less than 100 µg RNA, and vary depending on the sample. See Table 2 for expected RNA yields from various cell types.

Table 1. RNeasy 96 plate specifications for cells.

Preps per plate	96
Amount of starting material	Up to 5 x 10^5 cells
Maximum binding capacity per well	100 µg RNA
Maximum loading volume per well	1 ml
RNA size distribution	RNA >200 nucleotides*

* For total RNA from cells that includes small RNAs, follow the protocol "miRNeasy protocol" on page 36.

Table 2. Typical total RNA yields from cells.

Cell line	Source	RNA yield (µg per 10^5 cells)*
HeLa	Human cervical carcinoma	1.6
LMH	Chicken hepatoma	1.3
COS-7	Monkey kidney, SV-40 transformed	3.1
Huh7	Human hepatoma	2
Jurkat	Human T-cell leukemia	1.4
K-562	Human chronic myelogenous leukemia in blast crisis	1.9

* Amounts can vary due to factors such as species, developmental stage and growth conditions. If the RNeasy 96 procedure enriches for RNA >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA and other low molecular weight RNAs, which make up 15–20% of total cellular RNA.

Amount of tissue

When working with tissue samples, mechanical or enzymatic disruption of the tissue structure is the prerequisite for liberation of cells, subsequent release of nucleic acids and membrane permeability of the material.

Different tissue types can vary widely with regard to texture and rigidity, cell types and content of host nucleic acids and inhibitory substances.

Using the correct amount of starting material is essential to obtain high yields of pure RNA with the RNeasy 96 QIAcube HT Kit. The maximum amount that can be used is limited by:

- The volume of QIAzol Lysis Reagent required for efficient lysis and the maximum loading volume of the RNeasy 96 plate
- The RNA binding capacity of the RNeasy 96 plate wells (100 µg)
- The type of tissue

The RNeasy 96 QIAcube HT procedure is optimized for use with a maximum of 40 mg human and animal tissue (flash-frozen). With adipose tissue, up to 80 mg can be used. With thymus, spleen or intestine, only 20 mg should be used in order to avoid clogging the RNeasy 96 plate. For RNA*later*® stabilized tissues, half of these amounts should be used.

If processing liver tissue, we recommend starting with less than 5 mg tissue (especially if working with stab tissue). If no clogging issues occur during preparation, it may be possible to increase the amount of tissue in subsequent purifications.

Table 3 gives specifications for the RNeasy 96 plate for tissues. Each well of the plate has a maximum binding capacity of 100 µg of RNA, but actual yields depend on the sample type used. Table 4 gives examples of expected RNA yields from various tissues.

Table 3. RNeasy 96 plate specifications for tissues.

Preps per plate	96
Amount of starting material	40 mg (up to 80 mg adipose tissue; 20 mg flash-frozen liver, thymus, spleen or intestine; half of these amounts for RNA*later* stabilized tissue)
Binding capacity per well	100 µg RNA*
Maximum loading volume per well	1 ml
RNA size distribution	All RNA >200 nucleotides†

* Yields are limited by tissue type and amount. The maximum binding capacity of 100 µg RNA is usually not reached.

† For total RNA from tissue that includes RNAs <200 nucleotides, follow the protocol "RNeasy Tissue Protocol" on page 29.

For miRNA isolation, the information given in Table 3 and Table 4 can generally be applied. Nevertheless, we recommend starting with a lower amount of tissue, for example, 10 mg or even less, because clogging of the membrane may occur with certain tissue types. For liver tissue, we recommend starting with less than 5 mg tissue (especially if working with stab tissue).

Table 4. Typical total RNA yields from tissues.

Tissue	RNA yield (µg per 10 mg tissue)*
Kidney	5–40
Liver	15–80
Lung	5–15
Heart	5–25
Muscle	5–35
Brain	5–20
Adipose tissue	0.5–2.5
Spleen	15–100
Intestine	10–60
Skin	2–5

* Amounts can vary due to factors such as species, developmental stage and growth conditions. If the RNeasy 96 procedure enriches for RNA >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA and other low molecular weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storing starting material

RNA in cells is not protected until the sample is flash-frozen or disrupted in the presence of RNase-inhibiting or denaturing reagents. It is therefore important that cell samples are immediately frozen and stored at –65 to –90°C, or processed immediately after harvesting. Otherwise, unwanted changes in the gene expression profile will occur. The relevant procedures should be carried out as quickly as possible. After disruption in Buffer RLT or QIAzol (lysis buffers), samples can be stored at –65 to –90°C for months.

Disruption and homogenization of starting materials

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA purification procedures. Disruption and homogenization are two distinct steps.

Disruption

Complete disruption of plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced yields.

Homogenization

Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the RNeasy membrane and therefore significantly reduced yields.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 5 gives an overview of different disruption and homogenization methods.

Table 5. Disruption and homogenization methods.

Disruption method	Homogenization method	Comments
TissueLyser II system	TissueLyser II system	Simultaneously disrupts and homogenizes up to 192 samples in parallel. The TissueLyser II system gives results comparable to using a rotor–stator homogenizer.
Rotor–stator homogenization	Rotor–stator homogenization	Simultaneously disrupts and homogenizes individual samples.

Disruption and homogenization method

The TissueLyser II system provides high-throughput processing for simultaneous, rapid and effective disruption of up to 192 biological samples, including all types of human and animal tissue. Processing of up to 2 x 96 samples takes as little as 2–5 minutes. Results obtained are comparable to those achieved using a rotor–stator homogenizer.

We recommend using the TissueLyser II for lysis of tissue samples in a collection microtube rack with 5 mm steel beads. Following phase separation, transfer of the upper aqueous phase into an S-Block can be automated on QIAcube HT instrument.

If automated sample transfer should be used prior to the purification run, select TissueLyser 5 mm in the pre-treatment drop-down box in the **Experiment setup** screen.

Note: Using the RNeasy sample transfer protocol requires an additional experiment setup for the purification of RNA.

Note: Using the RNeasy sample transfer protocol requires calibration of the collection mircotube rack without the 5 mm steel beads.

Processing fewer than 96 samples per run

If processing fewer than 96 samples, reuse of unused portions of RNeasy 96 plates, S-Block and EMTR is possible up to three times.

Note: We recommend using fresh plasticware for every run. If reusing, take extreme care to prevent cross-contamination.

- Store plates in a way that separates the outlet nozzles under the plate, for example, in the S-Block used in the same run or in a fresh 96-well microtiter plate.
- Cover unused wells of the S-Block and RNeasy 96 plate with a tape sheet at all times.

Preparing reagents and instrument

Buffer RLT

Buffer RLT should be stored at room temperature (15–25°C) for the next run.

Buffer RWT

Before using a bottle of Buffer RWT for the first time, add 2 volumes of ethanol (96–100%; i.e., add 160 ml ethanol to 80 ml Buffer RWT). Tick the check box on the label on the lid of the bottle to indicate that ethanol has been added. Buffer RWT should be stored at room temperature (15–25°C).

Buffer RPE

Before using a bottle of Buffer RPE for the first time, add 4 volumes of ethanol (96–100%; i.e., add 260 ml ethanol to 65 ml Buffer RPE). Tick the check box on the label on the lid of the bottle to indicate that ethanol has been added. Buffer RPE should be stored at room temperature (15–25°C).

RNase-free water

RNase-free water left over after a run should be discarded and should not be reused for subsequent runs.

RNase-free DNase I

The RNeasy 96 QIAcube HT Kit provides optional steps for DNase digestion during RNA purification. Generally, DNase digestion is not required, since the procedure efficiently removes most of the DNA without the use of DNase. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., real-time RT-PCR analysis with low-abundance targets). For further details, see Appendix D on page 68.

Assembling the vacuum chamber

Figure 2 illustrates the assembly of the vacuum chamber. For further information, please refer to the *QIAcube HT User Manual*.

1. Insert the channeling block holder into the left (waste) chamber of the vacuum chamber.
2. Press firmly on the sides of the channeling block holder to seat it in the chamber, sealing the O-ring on the spigot into the drain.
3. Then, place the channeling block into the channeling block holder.
4. Place the RNeasy 96 plate in the transfer carriage. Load the carriage with the RNeasy 96 plate into the left (waste) chamber of the vacuum chamber.
5. Ensure that the carriage is positioned to the left inside the vacuum chamber. Place the riser block EMTR in the right (elution) chamber of the vacuum chamber with the pin of the riser block EMTR in the top right position.
6. Load an elution microtubes rack (EMTR) into the elution chamber.

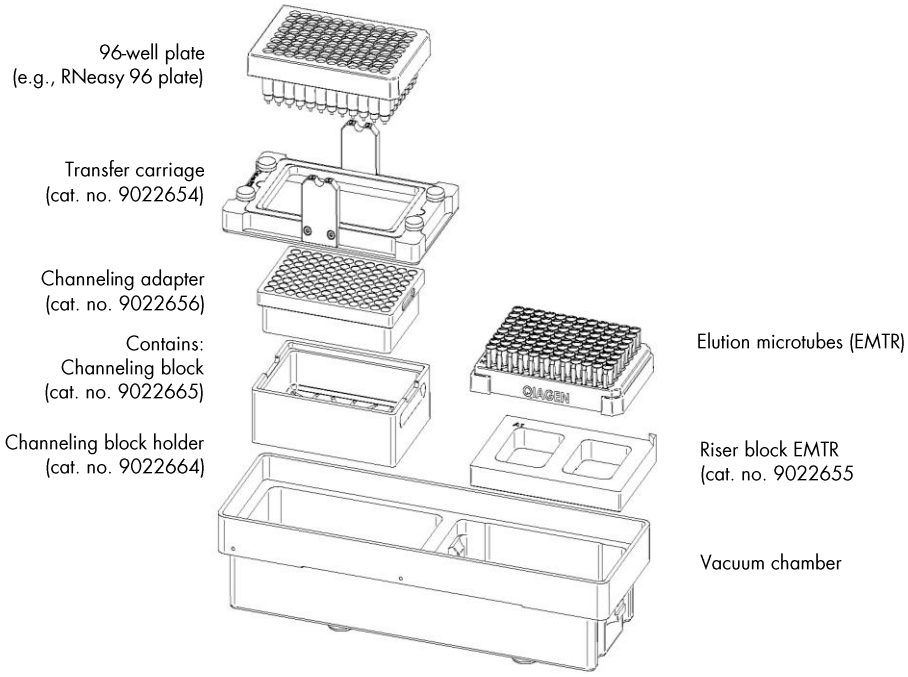


Figure 2. Assembling the vacuum chamber.

RNeasy Cell Protocol

This protocol is for the purification of total RNA (>200 nucleotides) from animal or human cells.

Important points before starting

- Read "Important Notes" on page 14.
- Read Appendix A on page 58, if preparing RNA for the first time.
- Check Buffer RLT for precipitates upon storage. If it contains precipitates, warm to 37°C to dissolve precipitates.
- If purifying RNA from cells containing high amounts of RNase, add 10 µl ß-mercaptoethanol* (ß-ME) per 1 ml Buffer RLT.

  Note: ß-ME aids the inactivation of RNases by guanidine thiocyanate. In most cases, it will not be necessary to add ß-ME to Buffer RLT.

  Important: Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing ß-ME can be stored at room temperature (15–25°C) for up to 1 month.
- Do not use bleach. Buffer RLT and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information" on page 6.
- All steps of the procedure should be performed at room temperature (15–25°C). Avoid any interruptions.
- Do not overload the RNeasy membrane as this can lead to impaired RNA extraction and/or performance in downstream assays.
- Avoid repeated freezing and thawing of samples as this may reduce RNA yield and quality.

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Check for precipitates in reagents. If a reagent contains precipitates, incubate at 37°C with gentle shaking to dissolve precipitates. Avoid vigorous shaking, which causes foaming.
- Check that Buffer RPE and Buffer RWT concentrates have been diluted in ethanol (96–100%) according to the instructions on the buffer bottles.
- Ensure that the relevant version of the **RNeasy 96** protocol is available on the computer.

  QIAcube HT Prep Manager protocol files (file extension ***.qpmx**), which contain all the information required to perform a run on the QIAcube HT instrument, are available from **www.qiagen.com/p/QIAcubeHT**, under the **Resources** tab.
- Ensure that QIAcube HT Prep Manager Software is installed.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Harvesting cells

1. Cells grown in a monolayer can be lysed directly in the wells of the multiwell cell culture plate. Completely remove medium by pipetting, and continue with step 2.

   For cells grown in suspension, transfer aliquots of up to 5 x 10^5 cells into the wells of an S-Block. Spin cells for 5 min at 300 *x g*, and completely remove the supernatant by pipetting. Proceed with step 2.

   Note: Incomplete removal of the supernatant will dilute Buffer RLT in subsequent steps, inhibiting lysis and the binding of RNA to the RNeasy membrane. This will lead to reduced yield.
2. Add 140 µl Buffer RLT to each well. Keeping the microplate or S-Block flat on the bench, shake it vigorously back and forth for 10 s. While continuing to keep the plate flat on the bench, rotate the plate/S-Block by 90° and shake it for an additional 10 s.

Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting for subsequent reuse of the S-Block

Note: If less than 8 samples per column are to be processed, unused wells in the column must contain water or buffer. The volume added to unused wells should be the same as the sample volume to avoid foaming

3. If cells are still in multiwell cell culture plates, transfer the lysed samples from the microplate in an S-Block. Place the S-Block on the indicated position of the instrument worktable.
4. Proceed with "RNeasy cell protocol on the QIAcube HT" described below.

RNeasy cell protocol on the QIAcube HT

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all times.
2. Launch the QIAcube HT Prep Manager Software.
3. On the **Home** screen, select **RNeasy 96** experiment.
4. Enter the kit information.
5. Select the type of sample to be processed. Sample types are combined in categories. Select **Cell** or **Any** when running the RNeasy cell protocol.
6. Select the pre-treatment from the drop-down menu.

   Note: The selected sample type will determine which pre-treatment options appear. When running the RNeasy cell protocol, it is not possible to select **TissueLyser** as the pre-treatment.
7. Select the protocol: RNeasy cell protocol.
8. Optional: Set optional steps for checking vacuum performance.

   Note: The run script provided by QIAGEN includes all necessary steps to guarantee best performance. Nevertheless, there is the possibility to check the vacuum performance during the run. If this option is selected, the instrument will pause after the binding step,

so you can check if any wells may be clogged. For more information, see section "Vacuum performance check" on page 49.

9. Set the optional step for on-plate DNase digestion. For more information, see "Optional steps" on page 49 or Appendix D on page 68.
10. Click **Next**.
11. Optionally, click **Save**. The QIAcube HT Prep Manager Software allows to save an experiment at any step during the run setup procedure.
12. In the **Labware Selection** screen, select **Use sample ID or existing sample input file** and define the samples.

    To enter sample IDs manually or to automatically generate sample IDs, select **Enter sample IDs**. Then, start naming the samples by selecting **Define Samples**. For information about how to automatically create sample IDs, refer to the *QIAcube HT User Manual*. Alternatively, to load an existing sample file select **Load existing sample files** and then either click **Load sample file** or use the **Scan** button to import the data.

    Note: Changing the input labware is not recommended by QIAGEN.
13. In the **Plate Assignment** screen, select the samples from the input and assign them to the output plate. Then, click **Next**.

    The input plate is set according to your definition in the **Labware selection** screen. Select the sample columns on the input labware and then click **Assign** to assign them to the output plate.

    It is possible to change the assignment, if required. Select the columns on the output labware and click **Remove assignment**. Press and hold the left mouse button to mark the columns and rearrange them as needed.
14. In the **Worktable setup** screen, select any virtual worktable position marked by yellow color and follow the instructions to set up the instrument worktable.

    Important: All positions to be used on the instrument worktable must be calibrated. If the **Calibrate Labware** button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.

15. Ensure that the liquid level in the waste bottle matches the indicated level on the virtual desktop. The level is displayed by a color code: green indicates that no action is necessary; yellow indicates that the bottle is almost full, but the run can be started; red indicates to empty the bottle before starting the run.
16. Place the tip chute on the worktable so that the chute is over the tip disposal box. Ensure that the tip chute is open and unblocked. Remove the UV protective cap from the tip chute. Ensure that the tip disposal box is empty and that the opening is aligned with the tip chute.
17. Prepare the vacuum chamber as shown in Figure 2 on page 22. This assembly is also described in the *QIAcube HT User Manual*.

    Important: If fewer than 12 columns (96 wells) are to be processed, seal the unused columns of the RNeasy 96 plate with adhesive tape (supplied). Unused wells must be sealed to ensure proper vacuum operation. Please process a minimum of 24 samples per run.

    Note: Trim any excess tape.

    Note: When reusing a RNeasy 96 plate, S-Block or elution plate, take care to avoid cross-contamination.

    Note: Make sure the RNeasy 96 plate is aligned to the left in the carriage and that the carriage is positioned to the left inside the vacuum chamber.
18. Load the tips in the indicated positions and remove the lids from the tip boxes. Ensure that there is a sufficient number of tips to follow the protocol at least up to completion of lysate transfer.
    Check that the number and position of available and unused tips on the instrument worktable is the same as the displayed tips on the virtual worktable.
    If more tips are required, you will be prompted to replace empty tip racks with new tip racks during the run. Information about the approximate time for refill will be given in the run screen. For more information, see the *QIAcube HT User Manual*.
19. Transfer the indicated volumes of all reagents into the corresponding reagent troughs. Close the lids and place the troughs in the indicated positions on the worktable

20. Place the S-Block containing the samples on the indicated position of the QIAcube HT worktable.

    Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting so that unused S-Block wells can be used in subsequent runs.

    Note: If fewer than 8 samples in a column are to be processed, add water or buffer to the unused wells in the column. To avoid foaming, the volume of water or buffer added to the unused wells should be the same as the sample volume.

21. Close the instrument hood and start the run by clicking **Start run**.

22. The protocol run begins.

    Important: At the beginning of each run, an open circuit test and a plate detection test are performed automatically. If the RNeasy 96 plate in the transfer carriage is improperly aligned to the left compartment of the vacuum chamber, you will be prompted to position it correctly. After adjusting the position, click **Retry** to initiate the tests again.

    For more information about the system tests, see the *QIAcube HT User Manual*.

23. Optional: If you selected to use a vacuum performance check, the protocol stops for a manual interaction after the binding step.

24. After the protocol is finished, create a report if necessary.

25. Cover the elution plate (EMTR) with the lid and remove from the elution chamber.

26. Two liquid phases might be found in the elution microtubes. If this is the case, TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

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

27. Proceed to "Cleaning the instrument after completing a run" on page 48.

RNeasy Tissue Protocol

This protocol is for the purification of total RNA (>200 nucleotides) from animal or human tissues.

Important points before starting

- Read "Important Notes" on page 14.
- Read Appendix A on page 58, if preparing RNA for the first time.
- Check Buffer RLT for precipitates upon storage. If it contains precipitates, warm to 37°C to dissolve precipitates.
- Do not use bleach. Buffer RLT and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information" on page 6.
- For this protocol, you will also need to order QIAzol Lysis Reagent (200 ml; cat. no. 79306) and Collection Microtube Racks (cat. no. 19560).
- All steps of the procedure should be performed at room temperature (15–25°C). Avoid any interruptions.
- Do not overload the RNeasy membrane as this can lead to impaired RNA extraction and/or performance in downstream assays.
- Avoid repeated freezing and thawing of samples as this may reduce RNA yield and quality.

Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).

- Check for precipitates in reagents. If a reagent contains precipitates, incubate at 37°C with gentle shaking to dissolve precipitates. Avoid vigorous shaking, which causes foaming.
- Check that Buffer RPE and Buffer RWT concentrates have been diluted in ethanol (96–100%) according to the instructions on the buffer bottles.
- Ensure that the relevant version of the **RNeasy 96** protocol is available on the computer.

  QIAcube HT Prep Manager protocol files (file extension ***.qpmx**), which contain all the information required to perform a run on the QIAcube HT instrument, are available from **www.qiagen.com/p/QIAcubeHT**, under the **Resources** tab.
- Ensure that QIAcube HT Prep Manager Software is installed.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Preparation of tissue samples

This procedure describes RNA purification from flash-frozen tissue or tissue stabilized in RNA*later* RNA Stabilization Reagent using the TissueLyser II. Alternatively, a rotor–stator homogenizer can be used for tissue disruption and homogenization.

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.

   Note: For RNA*later* stabilized tissue, cooling the collection microtube rack on dry ice is not necessary.
2. Remove the tissue sample from RNA*later* RNA Stabilization Reagent, or from cold storage. Do not allow the tissue to thaw before it is placed in QIAzol Lysis Reagent.
3. Determine the amount of tissue per sample by weighing the samples. Immediately transfer each sample to a cooled collection microtube. Repeat this until all tissue samples are placed into collection microtubes.

Note: If processing flash-frozen samples, do not use more than 40 mg of tissue, 80 mg of adipose tissue, or 20 mg of spleen, thymus or intestine per sample. If processing tissues stabilized in RNA*later*, use only half of these amounts.

Important: RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen or disrupted and homogenized. Frozen human and animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

4. Remove the collection microtube rack from the dry ice, and immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube.
5. Close the collection microtube rack and homogenize on the TissueLyser II for 5 min at 25 Hz.
6. Rotate the TissueLyser rack to allow even homogenization, and homogenize for another 5 min at 25 Hz.
7. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.

   Note: Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.
8. Place the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 *x g* for 1 min to collect residual liquid from the caps of the tubes.
9. Add 150 µl chloroform to each microtube. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps, and shake it vigorously while inverting the rack for 15 s.

   Note: Thorough mixing is important for subsequent phase separation.
10. Place the collection microtube rack on the benchtop at room temperature for 2–3 min.
11. Centrifuge at 6000 *x g* for 15 min at 4°C.

    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues

with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µl.

12. Transfer the upper aqueous phase into a S-Block, either manually or automated using the QIAcube HT.

    For manual transfer, carefully pipet 350 µl of the upper aqueous phase into a new S-Block. Proceed with "RNeasy tissue protocol on the QIAcube HT" on page 32.

    For automated transfer, proceed to the "RNeasy Sample Transfer Protocol" on page 45. Then proceed with "RNeasy tissue protocol on the QIAcube HT" on page 32.

RNeasy tissue protocol on the QIAcube HT

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all times.
2. Launch the QIAcube HT Prep Manager Software.
3. On the **Home** screen, select **RNeasy 96** experiment.
4. Enter the kit information.
5. Select the type of sample to be processed. Sample types are combined in categories. Select the category that best fits your sample type.
6. Select the pre-treatment from the drop-down menu.

   Note: The selected sample type will determine which pre-treatment options appear.
7. Select the protocol: RNeasy tissue protocol.
8. Optional: Set optional steps for checking vacuum performance.

   Note: The run script provided by QIAGEN includes all necessary steps to guarantee best performance. Nevertheless, there is the possibility to check the vacuum performance during the run. If this option is selected, the instrument will pause after the binding step, so you can check if any wells may be clogged. For more information, see section "Vacuum performance check" on page 49.

9. Set the optional step for on-plate DNase digestion. For more information, see "Optional steps" on page 49 or Appendix D on page 68.
10. Click **Next**.
11. Optionally, click **Save**. The QIAcube HT Prep Manager Software allows to save an experiment at any step during the run setup procedure.
12. In the **Labware Selection** screen, select **Use sample ID or existing sample input file** and define the samples.
    To enter sample IDs manually or to automatically generate sample IDs, select **Enter sample IDs**. Then, start naming the samples by clicking **Define Samples**. For information about how to automatically create sample IDs, refer to the *QIAcube HT User Manual*. Alternatively, to load an existing sample file select **Load existing sample files** and then either click **Load sample file** or use the **Scan** button to import the data.

    Note: Changing the input labware is not recommended by QIAGEN.
13. In the **Plate Assignment** screen, select the samples from the input and assign them to the output plate. Then, click **Next**.
    The input plate is set according to your definition in the **Labware selection** screen. Select the sample columns on the input labware and then click **Assign** to assign them to the output plate.
    It is possible to change the assignment, if required. Select the columns on the output labware and click **Remove assignment**. Click and hold the left mouse button to mark the columns and rearrange them as needed.
14. In the **Worktable setup** screen, select any virtual worktable position marked by yellow color and follow the instructions to set up the instrument worktable.

    Important: All positions to be used on the instrument worktable must be calibrated. If the **Calibrate Labware** button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.
15. Ensure that the liquid level in the waste bottle matches the indicated level on the virtual desktop. The level is displayed by a color code: green indicates that no action is

necessary; yellow indicates that the bottle is almost full, but the run can be started; red indicates to empty the bottle before starting the run.

16. Place the tip chute on the worktable so that the chute is over the tip disposal box. Ensure that the tip chute is open and unblocked. Remove the UV protective cap from the tip chute. Ensure that the tip disposal box is empty and that the opening is aligned with the tip chute.

17. Prepare the vacuum chamber as shown in Figure 2 on page 22. This assembly is also described in the *QIAcube HT User Manual*.

    Important: If fewer than 12 columns (96 wells) are to be processed, seal the unused columns of the RNeasy 96 plate with adhesive tape (supplied). Unused wells must be sealed to ensure proper vacuum operation. Please process a minimum of 24 samples per run.

    Note: Trim any excess tape.

    Note: When reusing a RNeasy 96 plate, S-Block or elution plate, take care to avoid cross-contamination.

    Note: Make sure the RNeasy 96 plate is aligned to the left in the carriage and that the carriage is positioned to the left inside the vacuum chamber.

18. Load the tips in the indicated positions and remove the lids from the tip boxes. Ensure that there is a sufficient number of tips to follow the protocol at least up to completion of lysate transfer.
    Check that the number and position of available and unused tips on the instrument worktable is the same as the displayed tips on the virtual worktable.
    If more tips are required, you will be prompted to replace empty tip racks with new tip racks during the run. Information about the approximate time for refill will be given in the run screen. For more information, see the *QIAcube HT User Manual*.

19. Transfer the indicated volumes of all reagents into the corresponding reagent troughs. Close the lids and place the troughs in the indicated positions on the worktable

20. Place the S-Block containing the aqueous phase samples on the indicated position of the QIAcube HT worktable.

Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting so that unused S-Block wells can be used in subsequent runs.

Note: If fewer than 8 samples in a column are to be processed, add water or buffer to the unused wells in the column. To avoid foaming, the volume of water or buffer added to the unused wells should be the same as the sample volume.

21. Close the instrument hood and start the run by clicking **Start run**.
22. The protocol run begins.

    Important: At the beginning of each run, an open circuit test and a plate detection test are performed automatically. If the RNeasy 96 plate in the transfer carriage is improperly aligned to the left compartment of the vacuum chamber, you will be prompted to position it correctly. After adjusting the position, click **Retry** to initiate the tests again.

    For more information about the system tests, see the *QIAcube HT User Manual*.
23. Optional: If you selected to use a vacuum performance check, the protocol stops for a manual interaction after the binding step.
24. After the protocol is finished, create a report if necessary.
25. Cover the elution plate (EMTR) with the lid and remove from the elution chamber.
26. Two liquid phases might be found in the Elution microtubes. If this is the case, TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

    Important: Please ensure that you only take the eluate from below the top layer.
27. Proceed to "Cleaning the instrument after completing a run" on page 48.

miRNeasy Protocol

This protocol is for the purification of total RNA, including small RNAs (<200 nucleotides), from animal or human tissue and cells.

Important points before starting

- Read "Important Notes" on page 14.
- Read Appendix A on page 58, if preparing RNA for the first time.
- Check Buffer RLT for precipitates upon storage. If it contains precipitates, warm to 37°C to dissolve precipitates.
- Do not use bleach. Buffer RLT and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information" on page 6.
- For this protocol, you will also need to order QIAzol Lysis Reagent (200 ml; cat. no. 79306) and Collection Microtube Racks (cat. no. 19560), and Collection Microtube Caps (cat. no. 19566).
- All steps of the procedure should be performed at room temperature (15–25°C). Avoid any interruptions.
- Do not overload the RNeasy membrane as this can lead to impaired RNA extraction and/or performance in downstream assays.
- Avoid repeated freezing and thawing of samples as this may reduce RNA yield and quality.

Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).

- Check for precipitates in reagents. If a reagent contains precipitates, incubate at 37°C with gentle shaking to dissolve precipitates. Avoid vigorous shaking, which causes foaming.
- Check that Buffer RPE and Buffer RWT concentrates have been diluted in ethanol (96–100%) according to the instructions on the buffer bottles.
- Ensure that the relevant version of the **RNeasy 96** protocol is available on the computer.

  QIAcube HT Prep Manager protocol files (file extension ***.qpmx**), which contain all the information required to perform a run on the QIAcube HT instrument, are available from **www.qiagen.com/p/QIAcubeHT**, under the **Resources** tab.
- Ensure that QIAcube HT Prep Manager Software is installed.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Preparation of cell samples

1. Cells grown in a monolayer can be lysed directly in the wells of the multiwell cell culture plate. Completely remove medium by pipetting, and continue with step 2.

   For cells grown in suspension, transfer aliquots of up to 5 x 10^5 cells into the wells of an S-Block. Spin cells for 5 min at 300 *x g*, and completely remove supernatant by pipetting. Proceed with step 2.

   Note: Incomplete removal of the supernatant will dilute Buffer RLT in subsequent steps, inhibiting lysis and the binding of RNA to the RNeasy membrane. This will lead to reduced yield.

   Note: If you want to use the QIAcube HT for the transfer of the upper aqueous phase, place 5 mm stainless steel beads into the collection microtubes (1 bead per tube).
2. Disrupt the cells by adding QIAzol Lysis Reagent.

   For pelleted cells, first loosen the cell pellet thoroughly by flicking the tube. Add 750 µl QIAzol Lysis Reagent to each plate well or collection microtube. Pipet up and down 3 times.

Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting so that unused S-Block wells can be used in subsequent runs.

Note: If fewer than 8 samples in a column are to be processed, add water or buffer to the unused wells in the column. To avoid foaming, the volume of water or buffer added to the unused wells should be the same as the sample volume.

3. If the lysates are in plate wells, transfer to collection microtubes (not supplied).
4. Close collection microtubes using collection microtube caps. Vortex for 1 min at maximum speed.

   Note: If vortexing for 1 min is not sufficient for homogenization of cells, the TissueLyser II system should be used.

   Note: Homogenized cell lysates can be stored at –65 to –90°C for several months.
5. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.
6. Place the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 *x g* for 1 min to collect residual liquid from the caps of the tubes.
7. Add 150 µl chloroform to each sample. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps, and shake it vigorously while inverting the rack for 15 s.

   Note: Thorough mixing is important for subsequent phase separation.
8. Place the collection microtube rack on the benchtop at room temperature for 2–3 min.
9. Centrifuge at 6000 *x g* for 15 min at 4°C.

   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µl.
10. Transfer the upper aqueous phase into a S-Block, either manually or automated using the QIAcube HT.

For manual transfer, carefully pipet 350 µl of the upper aqueous phase into a new S-Block. Proceed with "miRNeasy protocol on the QIAcube HT" on page 41.

For automated transfer, proceed to the "RNeasy Sample Transfer Protocol" on page 45. Then proceed with "miRNeasy protocol on the QIAcube HT" on page 41.

Preparation of tissue samples

This procedure describes RNA purification from flash-frozen tissue or tissue stabilized in RNA*later* RNA Stabilization Reagent using the TissueLyser II. Alternatively, a rotor–stator homogenizer can be used for tissue disruption and homogenization.

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.

   Note: For RNA*later* stabilized tissue, cooling the collection microtube rack on dry ice is not necessary.

2. Remove the tissue sample from RNA*later* RNA Stabilization Reagent, or from cold storage. Do not allow the tissue to thaw before it is placed in QIAzol Lysis Reagent.

3. Determine the amount of tissue per sample by weighing the samples. Immediately transfer each sample to a cooled collection microtube. Repeat this until all tissue samples are placed into collection microtubes.

   Note: If processing flash-frozen samples, do not use more than 40 mg of tissue, 80 mg of adipose tissue, or 20 mg of spleen, thymus or intestine per sample. If processing tissues stabilized in RNA*later*, use only half of these amounts.

   Note: If processing liver tissue, we recommend starting with less than 5 mg, especially if working with stab tissue. If no clogging issues occur during preparation, it may be possible to increase the amount of tissue in subsequent runs.

   Important: RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen or disrupted and homogenized. Frozen human and animal tissue should not

be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

4. Remove the collection microtube rack from the dry ice, and immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube.
5. Close the collection microtube rack and homogenize on the TissueLyser II for 5 min at 25 Hz.
6. Rotate the TissueLyser rack to allow even homogenization, and homogenize for another 5 min at 25 Hz.
7. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.

   Note: Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.
8. Place the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 *x g* for 1 min to collect residual liquid from the caps of the tubes.
9. Add 150 µl chloroform to each microtube. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps, and shake it vigorously while inverting the rack for 15 s.

   Note: Thorough mixing is important for subsequent phase separation.
10. Place the collection microtube rack on the benchtop at room temperature for 2–3 min.
11. Centrifuge at 6000 *x g* for 15 min at 4°C.

    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µl.
12. Transfer the upper aqueous phase into a S-Block, either manually or automated using the QIAcube HT.

For manual transfer, carefully pipet 350 µl of the upper aqueous phase into a new S-Block. Proceed with "miRNeasy protocol on the QIAcube HT" on page 41.

For automated transfer, proceed to the "RNeasy Sample Transfer Protocol" on page 45. Then proceed with "miRNeasy protocol on the QIAcube HT" on page 41.

Note: In case you disrupted the samples by means other than using the 5 mm steel beads, the upper aqueous phase can only be transferred manually.

miRNeasy protocol on the QIAcube HT

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all times.
2. Launch the QIAcube HT Prep Manager Software.
3. On the **Home** screen, select **RNeasy 96** experiment.
4. Enter the kit information.
5. Select the type of sample to be processed. Sample types are combined in categories. Select the category that best fits your sample type.
6. Select the pre-treatment from the drop-down menu.

   Note: The selected sample type will determine which pre-treatment options appear.
7. Select the protocol: miRNeasy protocol.
8. Optional: Set optional steps for checking vacuum performance.

   Note: The run script provided by QIAGEN includes all necessary steps to guarantee best performance. Nevertheless, there is the possibility to check the vacuum performance during the run. If this option is selected, the instrument will pause after the binding step, so you can check if any wells may be clogged. For more information, see section "Vacuum performance check" on page 49.
9. Click **Next**.

10. Optionally, click **Save**. The QIAcube HT Prep Manager Software allows to save an experiment at any step during the run setup procedure.

11. In the **Labware Selection** screen, select **Use sample ID or existing sample input file** and define the samples.
    To enter sample IDs manually or to automatically generate sample IDs, select **Enter sample IDs**. Then, start naming the samples by clicking **Define Samples**. For information about how to automatically create sample IDs, refer to the *QIAcube HT User Manual*.
    Alternatively, to load an existing sample file select **Load existing sample files** and then either click **Load sample file** or use the **Scan** button to import the data.

    Note: Changing the input labware is not recommended by QIAGEN.

12. In the **Plate Assignment** screen, select the samples from the input and assign them to the output plate. Then, click **Next**.
    The input plate is set according to your definition in the **Labware selection** screen. Select the sample columns on the input labware and then click **Assign** to assign them to the output plate.
    It is possible to change the assignment, if required. Select the columns on the output labware and click **Remove assignment**. Click and hold the left mouse button to mark the columns and rearrange them as needed.

13. In the **Worktable setup** screen, select any virtual worktable position marked by yellow color and follow the instructions to set up the instrument worktable.

    Important: All positions to be used on the instrument worktable must be calibrated. If the **Calibrate Labware** button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.

14. Ensure that the liquid level in the waste bottle matches the indicated level on the virtual desktop. The level is displayed by a color code: green indicates that no action is necessary; yellow indicates that the bottle is almost full, but the run can be started; red indicates to empty the bottle before starting the run.

15. Place the tip chute on the worktable so that the chute is over the tip disposal box. Ensure that the tip chute is open and unblocked. Remove the UV protective cap from the

tip chute. Ensure that the tip disposal box is empty and that the opening is aligned with the tip chute.

16. Prepare the vacuum chamber as shown in Figure 2 on page 22. This assembly is also described in the *QIAcube HT User Manual*.

    Important: If fewer than 12 columns (96 wells) are to be processed, seal the unused columns of the RNeasy 96 plate with adhesive tape (supplied). Unused wells must be sealed to ensure proper vacuum operation. Please process a minimum of 24 samples per run.

    Note: Trim any excess tape.

    Note: When reusing a RNeasy 96 plate, S-Block or elution plate, take care to avoid cross-contamination.

    Note: Make sure the RNeasy 96 plate is aligned to the left in the carriage and that the carriage is positioned to the left inside the vacuum chamber.

17. Load the tips in the indicated positions and remove the lids from the tip boxes. Ensure that there is a sufficient number of tips to follow the protocol at least up to completion of lysate transfer.
    Check that the number and position of available and unused tips on the instrument worktable is the same as the displayed tips on the virtual worktable.
    If more tips are required, you will be prompted to replace empty tip racks with new tip racks during the run. Information about the approximate time for refill will be given in the run screen. For more information, see the *QIAcube HT User Manual*.

18. Transfer the indicated volumes of all reagents into the corresponding reagent troughs. Close the lids and place the troughs in the indicated positions on the worktable.

19. Place the S-Block containing the aqueous phase samples on the indicated position of the QIAcube HT worktable.

    Note: Samples are processed in columns of 8 wells. We recommend covering unused wells during pipetting so that unused S-Block wells can be used in subsequent runs.

Note: If fewer than 8 samples in a column are to be processed, add water or buffer to the unused wells in the column. To avoid foaming, the volume of water or buffer added to the unused wells should be the same as the sample volume.

20. Close the instrument hood and start the run by clicking **Start run**.
21. The protocol run begins.

    Important: At the beginning of each run, an open circuit test and a plate detection test are performed automatically. If the RNeasy 96 plate in the transfer carriage is improperly aligned to the left compartment of the vacuum chamber, you will be prompted to position it correctly. After adjusting the position, click **Retry** to initiate the tests again.

    For more information about the system tests, see the *QIAcube HT User Manual*.
22. Optional: If you selected to use a vacuum performance check, the protocol stops for a manual interaction after the binding step.
23. After the protocol is finished, create a report if necessary.
24. Cover the elution plate (EMTR) with the lid and remove from the elution chamber.
25. Two liquid phases might be found in the elution microtubes. If this is the case, TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

    Important: Please ensure that you only take the eluate from below the top layer.
26. Proceed to "Cleaning the instrument after completing a run" on page 48.

RNeasy Sample Transfer Protocol

This protocol is for the transfer of the upper aqueous phase into a S-block when samples are lysed with the TissueLyser (using 5 mm beads).

Important points before starting

- Read "Important Notes" on page 14.
- Read Appendix A on page 58, if preparing RNA for the first time.
- For this protocol, you will also need to order QIAzol Lysis Reagent (200 ml; cat. no. 79306) and Collection Microtube Racks (cat. no. 19560), and Collection Microtube Caps (cat. no. 19566).

Things to do before starting

- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Ensure that the relevant version of the **RNeasy 96** protocol is available on the computer.

  QIAcube HT Prep Manager protocol files (file extension ***.qpmx**), which contain all the information required to perform a run on the QIAcube HT instrument, are available from **www.qiagen.com/p/QIAcubeHT**, under the **Resources** tab.
- Ensure that QIAcube HT Prep Manager Software is installed.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

RNeasy sample transfer protocol on the QIAcube HT

1. Switch on the QIAcube HT instrument and the connected laptop computer. We recommend leaving the vacuum station switched on at all times.
2. Launch the QIAcube HT Prep Manager Software.

3. On the **Home** screen, select **RNeasy 96** experiment.
4. Enter the kit information.
5. Select the type of sample to be processed. Sample types are combined in categories. Select the category that best fits your sample type.
6. Select the pre-treatment from the drop-down menu.

   Note: The selected sample type will determine which pre-treatment options appear.
7. Select the protocol: RNeasy sample transfer protocol.

   Note: If you observe any carryover in the samples after using the RNeasy sample transfer protocol for upper phase transfer, contact QIAGEN Technical Services or your QIAGEN sales representative to request a customized protocol.
8. Click **Next**.
9. Optionally, click **Save**. The QIAcube HT Prep Manager Software allows to save an experiment at any step during the run setup procedure.
10. In the **Labware Selection** screen, select **Use sample ID or existing sample input file** and define the samples.

    To enter sample IDs manually or to automatically generate sample IDs, select **Enter sample IDs**. Then, start naming the samples by clicking **Define Samples**. For information about how to automatically create sample IDs, refer to the *QIAcube HT User Manual*. Alternatively, to load an existing sample file select **Load existing sample files** and then either click **Load sample file** or use the **Scan** button to import the data.

    Note: Changing the input labware is not recommended by QIAGEN.
11. In the **Plate Assignment** screen, select the samples from the input and assign them to the output plate. Then, click **Next**.

    The input plate is set according to your definition in the **Labware selection** screen. Select the sample columns on the input labware and then click **Assign** to assign them to the output plate.

    It is possible to change the assignment, if required. Select the columns on the output labware and click **Remove assignment**. Click and hold the left mouse button to mark the columns and rearrange them as needed.

12. In the **Worktable setup** screen, select any virtual worktable position marked by yellow color and follow the instructions to set up the instrument worktable.

    Important: All positions to be used on the instrument worktable must be calibrated. If the **Calibrate Labware** button appears yellow, calibration is required. For further information, see the *QIAcube HT User Manual*.

13. Ensure that the liquid level in the waste bottle matches the indicated level on the virtual desktop. The level is displayed by a color code: green indicates that no action is necessary; yellow indicates that the bottle is almost full, but the run can be started; red indicates to empty the bottle before starting the run.

14. Place the tip chute on the worktable so that the chute is over the tip disposal box. Ensure that the tip chute is open and unblocked. Remove the UV protective cap from the tip chute. Ensure that the tip disposal box is empty and that the opening is aligned with the tip chute.

15. Load the tips in the indicated positions and remove the lids from the tip boxes. Ensure that there is a sufficient number of tips to follow the protocol at least up to completion of lysate transfer.

16. Place the collection microtube rack containing the aqueous phase samples on the indicated position of the QIAcube HT worktable.

17. Close the instrument hood and start the run by clicking **Start run**.

18. The protocol run begins.

19. After the run, remove the collection miroctube rack containing the organic phase and dispose of it according to laboratory guidelines.

    If continuing directly with RNA purification, proceed to "Cleaning the instrument after completing a run" on page 48 and then to the "RNeasy Tissue Protocol" on page 29 or the "miRNeasy Protocol" on page 36.

    If the RNA purification protocol will not be performed within the next 8 h, remove the S-Block from the instrument and seal it with tape. Freeze the samples. Proceed to "Cleaning the instrument after completing a run" on page 48.

Cleaning the instrument after completing a run

1. Follow the instructions in the QIAcube HT Prep Manager Software for cleaning the instrument after a run.
2. Cover the output plate with the lid and remove it from the worktable.
3. Cover tip racks that contain only unused tips with the lid and remove them from the worktable.
4. Cover fractions of partly used tip racks with an adhesive tape. Then cover the tip racks with the lid and remove from the worktable. Discard empty tip racks.
5. If the run has been stopped and the instrument did not remove all used tips, remove them now and discard them.
6. Remove all reagent troughs and discard them.

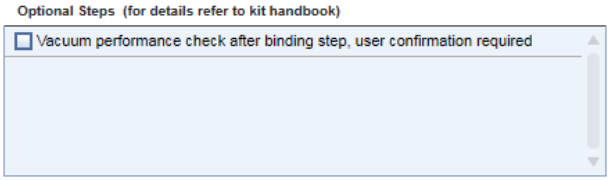
   Note: We recommend not reusing reagents for multiple runs.
7. Remove the input plate.
8. Discard the RNeasy 96 plate or keep partially used RNeasy 96 plates for subsequent reuse. In this case cover used fractions with an adhesive tape.
9. Remove the tip chute and all adapters from the worktable. Remove the carriage, channeling adapter and riser block from the vacuum chamber. Clean all parts as described in the *QIAcube HT User Manual*.
10. Discard the tip disposal box.
11. Clean any reagents that may have spilled on the instrument worktable or vacuum chamber with a damp cloth.
12. Discard all waste according to local safety regulations.

Note: For all further cleaning and maintenance operations, see the *QIAcube HT User Manual* for detailed instructions.

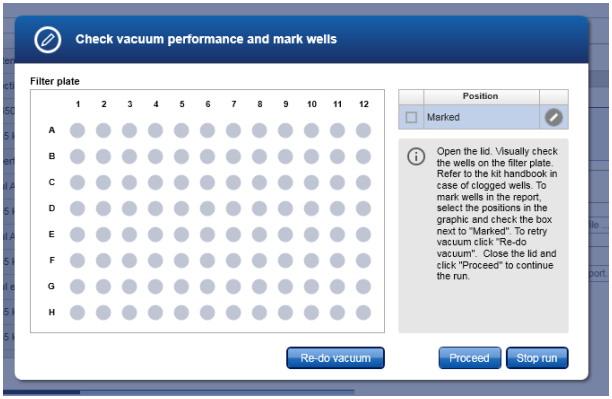
Optional steps

Vacuum performance check

Using the vacuum performance check option results in one manual intermediate step after the binding process. This optional setting allows the user to check whether all the liquid has passed through the membranes. By default, this step is not activated.



If the vacuum performance check step is activated, the instrument will pause after the binding step. The intermediate step allows to evaluate if all liquid has passed through the membranes. If liquid remains on the surface, the user can re-apply vacuum (**Re-do vacuum**), if not, continue with the procedure (**Proceed**).



1. Open the instrument lid.

   Note: The lid sensor is disabled during the vacuum performance check, allowing to the user to observe the wells.

2. Check the wells on the RNeasy 96 plate for any remaining liquid.

   If no liquid is visible in the wells after the vacuum step, click **Proceed** to continue the run.

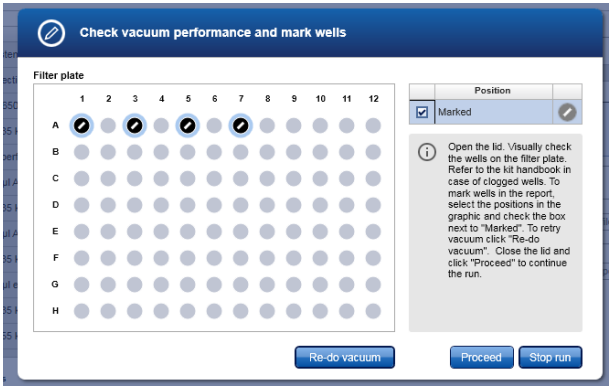
   If liquid remains in the wells, click the **Re-do vacuum** button to apply the same vacuum pressure again. The vacuum will be activated for a certain time or until you press the **Stop vacuum** button.

3. Mark any well that is clogged or not empty in the dialog that appears. This information will be included in the run report. To mark a well, select the position in the dialog.

   To select multiple positions, press the **Shift** key and left-click with the mouse to select adjacent positions or press the **CTRL** key and left-click with the mouse to select multiple, nonadjacent positions. Alternatively, drag the mouse to select adjacent positions in a rectangle.

   In the position table at the right, check the box next to **Marked**. The selected position on the RNeasy 96 plate will be displayed as marked.

   Note: To unmark a position, select the position and uncheck the box next to **Marked**.



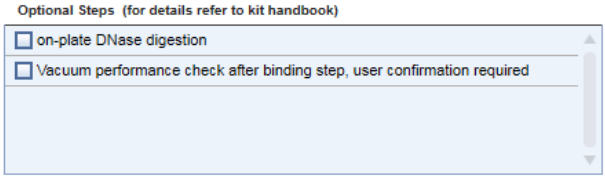
4. If liquid still remains in any well, manually remove the liquid using a pipet.

5. After the instrument has added additional reagents, open the hood to pause the run. Check to see whether the affected well is still blocked. If so, manually remove the liquid from the affected well using a pipet.
6. Either click **Proceed** to continue the run, or click **Stop run** to stop the run.

DNase digestion

Generally, DNase digestion is not required, since the RNeasy 96 technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA.

If additional DNase digestion should be carried out during the run, check the option **on-plate DNase digestion**. See Appendix D on page 688 for more details.



Note: Please order the RNase-Free DNase Set (50) (cat. no. 79254) from QIAGEN. Two RNase-Free DNase Sets are required for a single run with 96 samples, if performing the optional DNase digestion step.

Advanced options

Important: QIAGEN does not recommend modifying any of the parameters found in the **Advanced options** section.

These parameters have been optimized for each QIAcube HT Kit to guarantee accurate and valid experiment results. Please note that any changes to these options are carried out at your own risk.

Note: A warning icon and a corresponding warning message will be displayed if you change any parameter. The warning text contains the recommended value. If you return to the recommended value, the warning message will disappear.

Vacuum parameter

In the **Vacuum parameter** section, it is possible to change two parameters: vacuum intensity and vacuum time. The default settings are 25 kPa for the vacuum intensity and between 60–180 sec for the vacuum time, depending on the selected protocol.

The vacuum intensity can be changed from 25 kPa to 70 kPa. Changing the vacuum intensity parameter only affects the vacuum intensity following the binding step. All other vacuum steps will be unaffected.

The vacuum time can be changed as described in Table 6 below. Changing the vacuum time parameter only affects the vacuum time following the binding step. All other vacuum steps will be unaffected.

Table 6. Possible vacuum time settings.

Protocol	Default vacuum time (sec)	Range (sec)
RNeasy cell protocol	60	60–360
RNeasy tissue protocol	180	60–360
miRNeasy protocol	120	60–360

Elution parameter

In the **Elution parameter** section, it is possible to change the total elution volume and the elution step. The recommended values for these parameters are shown in the QIAcube HT Prep Manager Software. The total elution volume can be changed to another value within the defined range.

Changing the number of elution steps will affect the elution buffer distribution, incubation pause and vacuum step(s) without influencing the total amount of elution volume.

TopElute

TopElute Fluid is used during elution of nucleic acids from the RNeasy membrane. It enables application of a stable and high vacuum and results in equal eluate volumes. In addition, TopElute Fluid eliminates the formation of drops of elution buffer at the outlet nozzles of the

RNeasy 96 plates. By default, the Top Elute option is checked. In case TopElute Fluid should not be used during the run, uncheck the **TopElute** option under **Advanced** options.

Important: Changing the usage of TopElute Fluid is not recommended or tested by QIAGEN.

Note: TopElute Fluid might be found as a top layer over the elution buffer. It is inert and has no effects on downstream applications.

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

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Phases do not separate completely	
a) No chloroform added or chloroform not pure	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
b) Homogenate not sufficiently mixed before centrifugation	After addition of chloroform, the homogenate must be vigorously shaken. If the phases are not well separated, shake the rack vigorously while inverting it for at least 15 s, and repeat the incubation and centrifugation.
c) Organic solvents in samples used for purification	Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers or alkaline reagents. These can interfere with the phase separation.
Clogged plate wells	
a) Inefficient disruption and/or homogenization	See "Disruption and homogenization of starting materials" (page 17) for a detailed description of disruption and homogenization methods.
b) Too much starting material	Reduce amount of starting material. It is essential not to exceed the maximum amount of starting material (see page 16).
Little or no RNA eluted	
a) Too much starting material	Overloading significantly reduces yield. Reduce the amount of starting material (see page 16).
b) Buffer temperatures too low	All buffers must be at room temperature (15–25°C) throughout the procedure.

	Comments and suggestions
Low A_{260}/A_{280} value	
a) Not enough QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.
b) Sample not incubated for 5 min after homogenization	Place the sample at room temperature (15–25°C) for 5 min after homogenization, as indicated in the protocol.
c) Water used to dilute RNA for A260/A260 measurement	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see "Purity of RNA", Appendix B, page 62).
RNA degraded	
a) Inappropriate handling of starting material	Ensure that tissues have been properly handled and that the protocol has been performed without interruptions, especially the initial steps involving tissue lysis and homogenization. Some tissues (e.g., pancreas or intestine) contain high amounts of RNases. Care must be taken to excise these tissues from animals as fast as possible and to stabilize them either by freezing in liquid nitrogen or by immersion in RNA*later* RNA Stabilization Reagent immediately after excision.
b) RNase contamination	Check for RNase contamination of buffers. Although all buffers have been tested and are guaranteed RNase free, RNases can be introduced during use. Be sure not to introduce any RNases during the procedure or later handling. See Appendix A (page 58) for general remarks on handling RNA.
DNA contamination in downstream experiments	
a) Phase separation performed at too high a temperature	The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
b) Interphase contamination of aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the eluate. Make sure to transfer the aqueous phase without interphase contamination.
c) No DNase treatment	Follow the optional on-plate DNase digestion using the RNase-Free DNase Set (Appendix D, page 68) at the point indicated in the protocol. Alternatively, after the RNeasy 96 procedure, DNase digest the eluate containing the RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment, or repurified using an RNeasy RNA cleanup protocol (see the *RNeasy 96 Handbook* or the *RNeasy MinElute Cleanup Handbook*).
RNA does not perform well in downstream applications	
Salt carryover during elution	Ensure that Buffer RPE is at room temperature (15–25°C).

	Comments and suggestions
Low well-to-well reproducibility	
a) Incomplete homogenization	Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample.
b) Variability between samples	RNA yields from tissue samples can vary more than, for example, cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability.
Instrument issues	
Recovery in case of user interruption	The QIAcube HT interrupts a run upon opening of the hood. The run will proceed normally once the hood is closed. To ensure process safety, this incident is reported in the post-run report.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks) can be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 59), or rinse with chloroform‡ if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5%

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

SDS)[§], rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant) and allow to dry.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in "Solutions", page 59.

Solutions

Solutions should be purchased RNase-free or treated with 0.1% DEPC[§]. We recommend purchasing RNase-free water. This is because, if trace amounts of DEPC remain after autoclaving buffer, purine residues in RNA might be modified by carbethoxylation and performance of enzymatic reactions such as PCR may be negatively affected. Therefore, residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for at least 15 minutes.

DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react

with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, use RNase-free water to dissolve Tris to make the appropriate buffer.

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Appendix B: Storage, Quantification and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –20°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may not be possible to accurately determine amounts photometrically. Small amounts of RNA can be quantified using the QIAxcel® system (**www.qiagen.com/QIAxcel**) or Agilent® 2100 Bioanalyzer®, quantitative RT-PCR, or fluorometric quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml. This relation (A_{260}=1 → 44 µg/ml) is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH*. As discussed below (see "Purity of RNA," page 62), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 59). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)

A_{260} = 0.2

Concentration of RNA sample	= 44 µg/ml x A_{260} x dilution factor
	= 44 µg/ml x 0.2 x 50
	= 440 µg/ml
Total amount	= concentration x volume in milliliters
	= 440 µg/ml x 0.1 ml
	= 44 µg of RNA

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination[†]. For accurate values, we recommend measuring

[†] Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1‡ in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA," page 61).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While the RNeasy 96 QIAcube HT procedure will remove the vast majority of cellular DNA, trace amounts may still remain, depending on the amount and nature of the sample.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect® Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see www.qiagen.com/GeneGlobe). For two-step, real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit (cat. no. 205311) provides fast cDNA synthesis with removal of genomic DNA contamination. For one-step, real-

‡ Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

time RT-PCR, the QuantiFast® Probe RT-PCR Plus Kit (cat. no. 204482) integrates reverse transcription and removal of genomic DNA contamination.

Integrity of RNA

The integrity and size distribution of total RNA purified with the RNeasy 96 QIAcube HT Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel system or Agilent 2100 Bioanalyzer. For intact RNA, the respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1.

*When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Appendix C: Formaldehyde Agarose Gel Electrophoresis

The following protocol for formaldehyde agarose (FA) gel electrophoresis is routinely used at QIAGEN and gives enhanced sensitivity for gel and subsequent analysis (e.g., northern blotting). A key feature is the concentrated RNA loading buffer that allows a larger volume of RNA sample to be loaded onto the gel than conventional protocols (e.g., Sambrook et al. (1989] Molecular cloning – a laboratory manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

FA gel preparation

To prepare FA gel (1.2% agarose) of size 10 x 14 x 0.7 cm, mix:

- 1.2 g agarose*
- 10 ml 10x FA gel buffer (see composition below)
- Add RNase-free water to 100 ml

If smaller or larger gels are needed, adjust the quantities of components proportionately.

Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde* and 1 µl of a 10 mg/ml ethidium bromide* stock solution. Mix thoroughly and pour onto gel support. Prior to running the gel, equilibrate in 1x FA gel running buffer (see composition below) for at least 30 minutes.

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x RNA loading buffer (see composition below) to 4 volumes of RNA sample (for example 10 µl of loading buffer and 40 µl of RNA) and mix.

Incubate for 3–5 minutes at 65°C, chill on ice,* and load onto the equilibrated FA gel.

Gel running conditions

Run gel at 5–7 V/cm in 1x FA gel running buffer.

Composition of FA gel buffers

10x FA gel buffer

- 200 mM 3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)*
- 50 mM sodium acetate*
- 10 mM EDTA*

pH to 7.0 with NaOH*

1x FA gel running buffer

- 100 ml 10x FA gel buffer
- 20 ml 37% (12.3 M) formaldehyde*
- 880 ml RNase-free water

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

5x RNA loading buffer

- 16 µl saturated aqueous bromophenol blue solution†
- 80 µl 500 mM EDTA*, pH 8.0
- 720 µl 37% (12.3 M) formaldehyde*
- 2 ml 100% glycerol*
- 3.084 ml formamide*
- 4 ml 10x FA gel buffer
- 10 ml RNase-free water

Stability: approximately 3 months at 2–8°C

† To make a saturated solution, add solid bromophenol blue to distilled water. Mix and continue to add more bromophenol blue until no more will dissolve. Centrifuge to pellet the undissolved powder, and carefully pipet the saturated supernatant.

Appendix D: Optional On-Plate DNase Digestion with the RNase-Free DNase Set

The RNase-Free DNase Set (cat. no. 79254) provides efficient on-plate digestion of DNA during RNA purification. The DNase is efficiently removed in subsequent wash steps.

Note: Standard DNase buffers are not compatible with on-plate DNase digestion. Use of other buffers may affect the binding of the RNA to the RNeasy silica membrane, reducing the yield and integrity of the RNA.

Lysis and homogenization of the sample and binding of RNA to the silica membrane are performed according to the standard protocol. After washing with a reduced volume of Buffer RWT, the RNA is treated with DNase I while bound to the silica membrane. The DNase is removed by a second wash with Buffer RWT. Washing with Buffer RPE and elution are then performed according to the standard protocol.

Important points before starting

Generally, DNase digestion is not required, since integrated QIAzol and RNeasy 96 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., QuantiTect RT-PCR analysis with a low-abundance target). DNA can also be removed by a DNase digestion following RNA purification.

Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

Things to do before starting

Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. The RNeasy 96 QIAcube HT procedure requires 2 RNase-Free DNase Sets per 96-well plate. Dissolve 2 vials of solid DNase I (2 x 1500 Kunitz units) in 2 x 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vials. Inject RNase-free water into the vials using an RNase-free needle and syringe. Mix gently by inverting the vials. Do not vortex.

Unused DNase I stock solution can be stored at –15 to –30°C for up to 9 months. Thawed stock solution can be stored at 2–8°C for up to 6 weeks. Do not refreeze the DNase I stock solution after thawing.

Table 7. Preparation of DNase mix.

	Number of samples									
	24	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**
Buffer RDD (ml)	2.24	2.8	3.36	3.99	4.55	5.18	5.81	6.44	7	7.63
DNase I stock (µl)	320	400	480	570	650	740	830	840	1000	1090

Procedure

1. To prepare the right amount of DNase-mix, add the appropriate amount of Buffer RDD and DNase I stock solution into a suitable vessel. Mix by gently inverting the tube.

   Buffer RDD is supplied with the RNase-Free DNase Set.

   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
2. Add DNase mix to a trough and place on the indicated worktable position just before starting the QIAcube HT run.

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 QIAcube HT Kit	For 480 preps: RNeasy 96 plates, RNase-Free Water, Buffers	74141
TissueLyser		
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96*	85300
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser LT	Compact bead mill, 100-240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube†	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989
QIAcube HT instrument		
QIAcube HT System	Robotic workstation with UV lamp, HEPA filter, laptop, QIAcube HT operating software, start-up pack, installation and training, 1-year warranty on parts and labor	9001793

* TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.
† TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

Product	Contents	Cat. no.
Consumables		
QIAcube HT Plasticware	For 480 preps: 5 S-Blocks, 5 EMTR RS, 2 x 50 Caps for EMTR, 9 x 96 Filter-Tips OnCor C, TapePad	950067
Reagent Trough (with Lid), 170 ml	Box of 20 plus lid; liquid reservoirs	990556
Reagent Trough (with Lid), 70 ml	Box of 10 plus lid; liquid reservoirs	990554
Elution Microtubes RS	24 x 96 Elution Microtubes, racks of 96; includes cap strips	120008
S-Blocks	24 x 96-well blocks with 2.2 ml wells	19585
Collection Microtubes (racked)	(10 x 96) nonsterile polypropylene tubes; 960 in racks of 96	19560
Collection Microtube Caps	For capping collection microtubes and round-well blocks; 960 in strips of 8	19566
QIAzol Lysis Reagent (200 ml)	200 ml QIAzol Lysis Reagent	79306
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA preps	79254

For a complete list of consumables, visit **www.qiagen.com/p/QIAcubeHT**.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Quick-Start Protocol

This protocol is for use with QIAcube HT Prep Manager Software. If you are using QIAcube HT 4.17 Software, download the corresponding protocol at **www.qiagen.com/HB-1569**.

Further information

- *RNeasy 96 QIAcube HT Handbook:* **www.qiagen.com/HB-2164**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This kit includes four different protocols for RNA purification using the RNeasy 96 QIAcube HT Kit and QIAcube HT Prep Manager Software:
  a. RNeasy cell protocol: for purification of total RNA from cells
  b. RNeasy tissue protocol: for purification of total RNA from animal tissues
  c. RNeasy miRNA protocol: for purification of total RNA, including miRNA from animal tissues and cells
  d. RNeasy sample transfer protocol: for transferring the aqueous phase from sample pretreatment to the S-block
- For the RNeasy tissue protocol or the RNeasy miRNA protocol, you also need QIAzol® Lysis Reagent (cat. no. 79306), Stainless Steel Beads 5 mm (cat. no. 69989), Collection Microtube Racks (cat. no. 19560) and Collection Microtube Caps (cat. no. 19566).
- See the kit handbook for information on handling various sample types.
- If carrying out optional on-plate digestion, use the optional step. For further information, see the kit handbook. The RNase-free DNA Set (cat. no. 79254) is also required.
- Prepare Buffers RWT and RPE according to the instructions in the kit handbook.

1. Start the QIAcube HT Prep Manager Software. Click on the **Home** icon in the main toolbar to access the Home screen.
2. Select RNeasy 96 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: a. RNeasy cell protocol, b. RNeasy Tissue protocol, c. RNeasy miRNA protocol, or d. RNeasy sample transfer protocol. For information about optional steps and advanced options see the kit handbook.
5. Define samples in the **Labware selection** step.
6. Arrange samples to the output plate in the **Assignment** step. 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 instrument worktable.
8. If running protocols a, b or c, add the appropriate volume of lysate to the selected S-Blocks wells. If running protocol d, load a Collection Microtube Rack containing separated organic and aqueous phase onto the worktable.
9. Save the experiment by clicking the **Save** button in the button bar.
10. Click the **Start** run button to start the run.
    Important: If the optional Vacuum performance check has been selected, the software will show a dialog that needs to be confirmed after defined vacuum steps.
11. When the protocol is complete, cover the elution plate (EMTR) with the lid and remove it from the elution chamber.
    Note: If using Top Elute fluid, there may be 2 liquid phases in the elution microtubes. Top Elute fluid will be the top layer over the elution buffer.
12. Create a report (if required).
13. Follow the cleaning procedure.

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Document revision history	
R2/2017	Spelling corrected: "RNeasy", addition of note for transfer of upper phase, a picture exchanged: the range of elution volume was adapted, updated page numbers, updated standard temperature ranges, updated ordering information

Notes

Notes

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