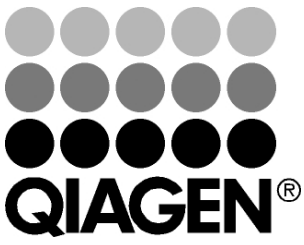
December 2013

RNeasy® 96 QIAcube® HT Handbook

For the automated purification of total RNA
from animal and human cells and tissue using
the QIAcube HT and QIAxtractor® instrument



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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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", 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 kit components are also available separately: S-Blocks, Elution Microtubes RS (including caps for strips), and Top Elute Fluid. See "Ordering Information", page 60.

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

Intended Use

The RNeasy 96 QIAcube HT Kit is intended for automated extraction of total RNA from human and animal cells and tissue samples for molecular biology applications using the QIAcube HT instrument. 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.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:

CHEMTREC

USA & Canada ▪ Tel: 1-800-424-9300

Outside USA & Canada ▪ Tel: +1-703-527-3887 (collect calls accepted)

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the 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 <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 flowchart on the next page). The kit combines the selective binding properties of silica-based membrane with the speed of vacuum processing. The QIAcube HT System provides walkaway automation of the RNeasy 96 procedure, for total RNA purification from up to 5 x 10^5 animal or human cells or <40 mg tissue per sample.

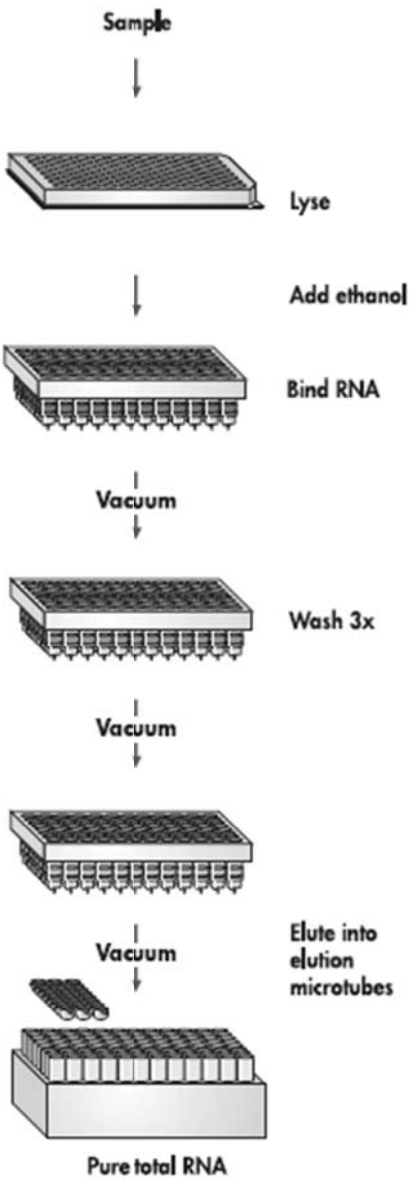
Cell lysis is done manually under highly denaturing conditions with guanidine thiocyanate to immediately inactivate RNases and ensure purification of intact RNA. After the run is started, ethanol is added to the lysate to provide appropriate binding conditions, and the samples are then applied to the 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.

With the automated RNeasy 96 procedure for cells, all RNA molecules longer than 200 nucleotides are purified. 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.

For purification of total RNA from tissue, or of total RNA including small RNAs from cells and tissues, we recommend using QIAzol® Lysis Reagent as the lysis buffer.

RNeasy 96 QIAcube HT Procedure



Description of protocols

There are three protocols in this handbook, depending on the starting material (cells or tissue), and desired size distribution of purified RNA (including or excluding RNA <200 nucleotides).

Cell samples can be purified using the RNeasy 96 QIAcube HT Kit and the protocol "Purification of Total RNA from Animal or Human Cells (with Optional DNase Digestion)", page 23.

Tissue samples (<40mg) and samples for purification of small RNAs are processed following sample lysis in QIAzol Lysis Reagent. This reagent is ordered separately (see "Ordering Information", page 60). These procedures are described in the protocols "Purification of Total RNA from Animal or Human Tissue (with Optional DNase digestion)", page 29, and "Purification of Total RNA, including Small RNAs, from Animal or Human Tissue and Cells", page 36, respectively.

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)
- QIAcube HT instrument
- QIAcube HT Software version 4.17 or higher
- To process dedicated QIAcube HT Kits on the QIAxtractor instrument, QIAcube HT Software version 4.17 or higher is needed together with the Accessories Pack, QXT. See "Ordering Information", page 60.
- Reagent troughs
- Disposable gloves
- Ethanol (96–100%)*

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

- 14.3 M β-mercaptoethanol† (β-ME) (commercially available solutions are usually 14.3 M)
- RNase-Free DNase Set (cat. no. 79254) for optional on-plate DNase digestion (see Appendix D, page 55, for details)

Optional plasticware for purification of tissue samples

- QIAzol Lysis Reagent (200 ml; cat no. 79306)
- Collection Microtube Racks (cat no. 19560) and Collection Microtube Caps (cat no. 19566)

TissueLyser system

The TissueLyser system provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 192 biological samples, including all

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† 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.

types of human and animal tissue. Processing of up to 2 x 96 samples takes as little as 2–5 minutes. Disruption and homogenization using the TissueLyser gives yields comparable or better than those achieved with traditional rotor–stator homogenization methods. With rotor–stator homogenization, the samples must be processed individually, and the rotor–stator homogenizer must be cleaned after each sample to prevent cross-contamination. In contrast, the TissueLyser provides simultaneous disruption for high-throughput processing of a variety of human and animal tissues.

The TissueLyser system 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", page 60.

Centrifuge 4K15C

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 4K15C. A temperature of 4°C is necessary during phase separation for optimal removal of genomic DNA. A wide range of other rotors can be used with the Centrifuge 4K15C in addition to the Plate Rotor 2 x 96.

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

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 RNAs <200 nucleotides, follow the protocol "Purification of Total RNA, including Small RNAs, from Animal or Human Tissue and Cells", page 36.

Table 2. Typical total RNA yields from cells with the RNeasy 96 QIAcube HT Kit

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 liver, 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.

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 "Purification of Total RNA, including Small RNAs, from Animal or Human Tissue and Cells", page 36.

For miRNA isolation, the information given in Tables 3–4 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 samples, we recommend using approximately 5 mg of tissue.

Table 4. Typical total RNA yields from tissues with the RNeasy 96 QIAcube HT Kit

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, age, gender, physiological state, etc. 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 –70°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 –70°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 2 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 system	TissueLyser system	Simultaneously disrupts and homogenizes up to 192 samples in parallel. The TissueLyser 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 simultaneously disrupts and homogenizes up to192 samples in parallel. Results obtained are comparable to those achieved using a rotor–stator homogenizer.

DNase digestion

There are two ways to remove DNA:

- Optional on-plate DNase digestion (see Appendix D, page 55)
- DNase digestion after RNA purification (please contact QIAGEN Technical Services for a protocol).

  Note: Generally, DNase digestion is not required, since 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.

  Note: Please order 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 DNase digestion.

Preparing reagents

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 bottle lid 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 bottle lid 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.

TopElute Fluid

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.

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

TopElute does not evaporate and can be stored in the reagent trough.

RNase-free DNase I

The RNeasy 96 QIAcube HT Kit provides additional protocols which include a 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 a low-abundance target). For further details, see Appendix D, page 55.

Assembling the vacuum chamber

Figure 1 illustrates the assembly of the vacuum chamber when using RNeasy 96 plates.

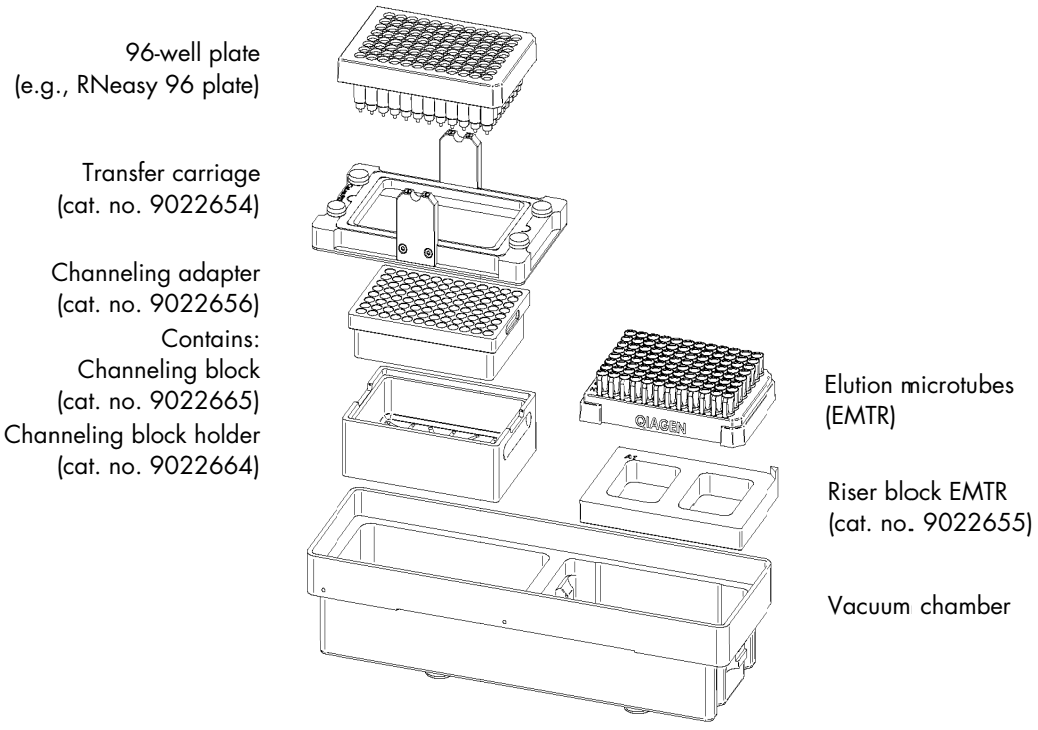


Figure 1. Assembling the vacuum chamber.

All QIAcube HT instruments are delivered with the vacuum chamber components for dedicated QIAcube HT Kits.

IMPORTANT: If you use a QIAxtractor instrument, ensure that only parts from the Accessories Pack, QXT (black parts) are used. See "Ordering Information", page 60.

For further information, please refer to the *QIAcube HT User Manual*.

1. Insert the channeling block holder into the left (waste) compartment 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) compartment 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) compartment 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.

Optional features

Processing of <96 samples per run

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

Note: We recommend using fresh plasticware for every run. In 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 the RNeasy 96 plate with a tape sheet at all times.
- Remove unused Elution Microtubes from the EMTR in rows of eight tubes.

Sample data input, data tracking, and LIMS connection

In the software environment, information about an item can be seen in the right-hand pane (to open the dialog, click on "A1: Reaction").

See Section 5.11 in the *QIAcube HT User Manual* for more information or contact QIAGEN Technical Service for a detailed example.

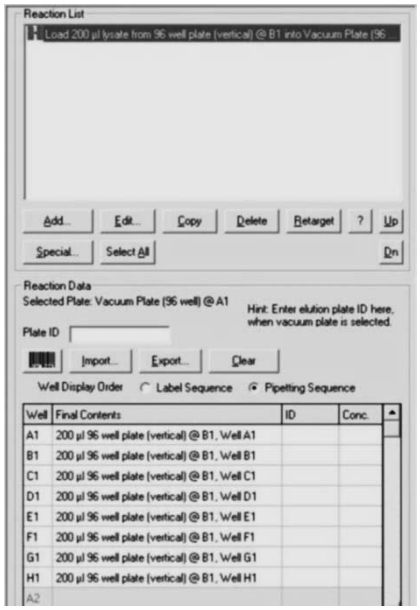


Figure 2: Example for right-hand pane information.

Sample descriptions can be imported, inserted manually, or inserted using a handheld barcode scanner.

The field" Plate ID" can be used for the unique number that is provided on each EMTR RS plate.

A post-run report is generated for each run and can be used for quality management purposes. It is shown after each run and is automatically saved in the "Reports" subdirectory of the "Data" directory (default location is **C:\Program Files\QIAcubeHT\Data**

Protocol: Purification of Total RNA from Animal or Human Cells (with Optional DNase Digestion)

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

Important points before starting

- Read Appendix A, if preparing RNA for the first time (page 48).
- Read "Important Notes" on page 13.
- Check Buffer RLT for precipitate upon storage. If it contains precipitates, warm to 37°C to dissolve precipitates.
- Add 10 µl β-ME* per 1 ml Buffer RLT, when purifying RNA from cells containing high amounts of RNases.

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

  IMPORTANT: Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-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", page 6.
- All steps of the procedure should be performed at room temperature (20–25°C). Avoid any interruptions.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- Buffer RWT is supplied as a concentrate. Before using for the first time, add 2 volumes of ethanol (96–100%) to obtain a working solution.
- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).

* 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.

- Ensure that the relevant version of the **RNeasy 96 QIAcube HT total RNA Cell.QSP** run file is installed.

  Optional: Select the relevant version of the protocol **RNeasy 96 QIAcube HT total RNA Cell with DNase.QSP** for optional on-plate DNase digestion. (See Appendix D, page 55, for more information).

  QIAcube HT protocol files (file extension ***.QSP**), which contain all the information required to perform a protocol run on the QIAcube HT instrument, are available from www.qiagen.com/p/QIAcubeHT, under the "Resources" tab.
- Ensure that Software version 4.17 or higher is installed. This is mandatory to process RNeasy 96 plates on the QIAcube HT and QIAxtractor.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Procedure

1. **Place the tip discard chute on the worktable so that the chute is over the tip disposal box.**

   Ensure that the tip discard 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 discard chute.
2. **Switch on the instrument. The switch is located at the back of the instrument, on the lower left.**
3. **Launch the QIAcube HT Software.**

   Note: If the QIAcube HT Software is already open, click in the toolbar.
4. **The following screen appears.**

5. **Select the "QProtocols" tab.**

All Q Protocols that are saved in the appropriate "QProtocols" folder will be listed.

6. **To open the run file, select the Q Protocol and then click "Open". Alternatively, double left-click on the Q Protocol.**
7. **A "Protocol Description" of the selected Q Protocol will be displayed and the QIAGEN Protocol icon will appear in the toolbar.**
8. **Check that the Q Protocol meets your requirements, and then click "Close".**

   Note: To view the Q Protocol information box again, click on the icon in the toolbar.

9. **Click in the toolbar.**

   The "Configuration (1)" step of the "Vacuum extraction" wizard opens. This wizard displays protocol parameters. For information about adjusting the parameters, see the *QIAcube HT User Manual*.

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

    Reagent and consumable lot numbers can be entered in the "Configuration (1)" window for tracking.

    The "Jump to End" button is located at the bottom left of the "Configuration (1)" window.

    The "Wizard Summary" window opens. The information in this window can be printed for documentation purposes.

11. **Confirm the protocol by clicking "Finish". The wizard closes.**

    The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the *QIAcube HT User Manual*.

12. **Ensure that there are sufficient numbers of tips for all steps, at least up to and including lysate transfer. Ensure that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes.**

    Check that the number and position of available and unused tips is the same on the instrument worktable and in the software workspace.

    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 pre-run check. For more information, see the *QIAcube HT User Manual*.

In the software environment, click on a tip in any tip position to open the "tip info" preview.

13. **Prepare the vacuum chamber as described in "Assembling the vacuum chamber", page 20. See the *QIAcube HT User Manual* for more information.**

    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.

    Note: Trim any excess tape.

    Note: When reusing 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.

Harvesting cells

14. **[A] Cells grown in a monolayer: Cells grown in a monolayer in multiwell cell-culture plates can be lysed directly in the wells. Completely remove medium by pipetting, and continue with step 15.**

    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.

    [B] 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 15.

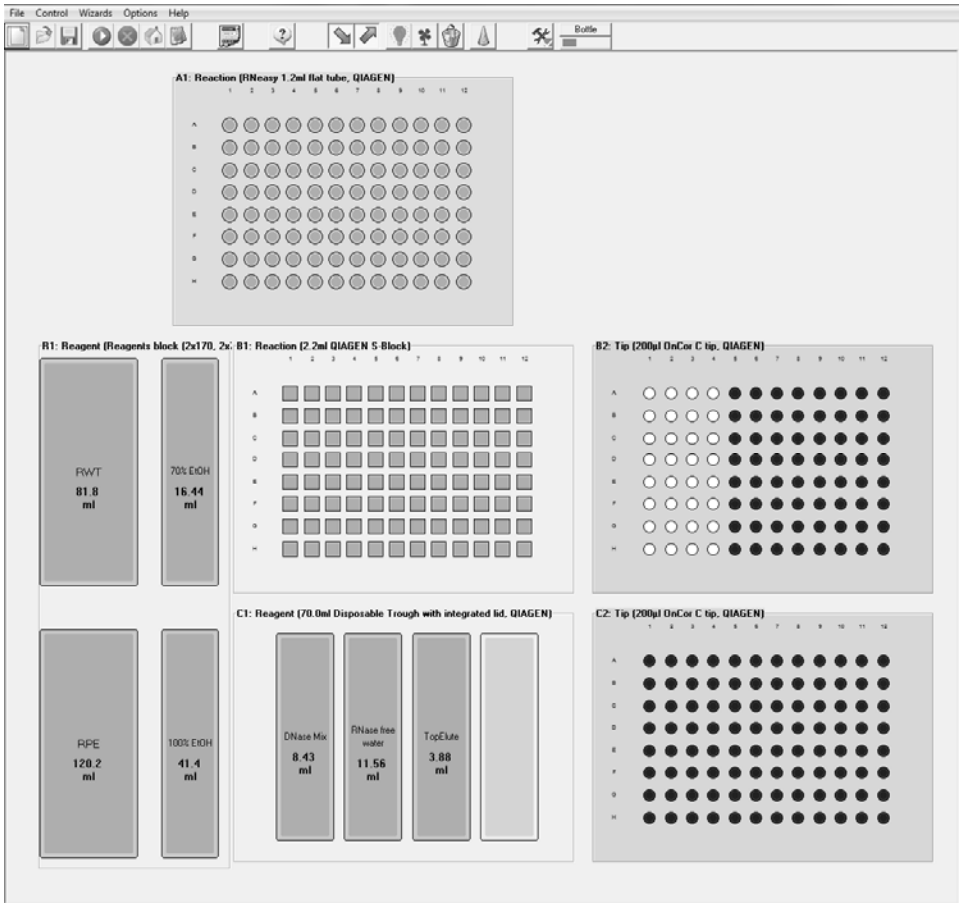
    Note: Incomplete removal of the supernatant will dilute Buffer RLT in subsequent steps, inhibiting lysis and the binding of RNA to the RNeasy membrane. This will lead to reduced yield.

15. **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 <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.

16. **If cells are still in multiwell cell-culture plates, transfer the lysed samples from the microplate in an S-Block. Place the S-Block in the B1 position of the instrument worktable.**
17. **Transfer the indicated volumes of all reagents into the corresponding reagent troughs, close the lids, and place them on the indicated positions on the worktable.**



18. **Optional: Prepare the DNase I incubation mix as described in Appendix D, page 55.**

    Note: Generally, DNase digestion is not required.

19. **Start the run immediately by clicking**  **.**

    The pre-run checklist appears.

20. **Perform the pre-run check.**

    Check the state of the worktable items. Confirm that worktable is set up correctly (instrument does not perform checks for all items). Check the box to the left of the items.

    A pre-run report can be saved for documentation purposes by clicking  .

21. **After completing the pre-run check, close the instrument hood and click "OK".**

The "OK" button is disabled until all pre-run check items have been checked.

22. **Click "Cancel" when the "Save as" dialog box appears.**

    Optional: Save the run file with a unique file name. See the *QIAcube HT User Manual* for more details.

23. **The protocol run begins.**

    IMPORTANT: At the beginning of each run, an open circuit test and a plate detection test is 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 place it correctly. After adjusting the position, click "Retry" to initiate the tests again.

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

    See the *QIAcube HT User Manual* for detailed instructions.

    Two liquid phases may 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.

Cleaning the instrument after completing a run

1. **Discard racks containing only used tips.**
2. **Discard leftover reagents.**

   We recommend not reusing reagents in multiple runs. Reagents provided are sufficient for at least 5 runs of 96 samples.

   Do not clean the trough containing TopElute Fluid with water. Clean with a dry, lint-free cloth only.

3. **Discard the S-Block or keep partially used blocks for reuse.**
4. **Remove the transfer carriage and discard the RNeasy 96 plate or keep partially used RNeasy 96 plates for reuse.**
5. **Clean the carriage, channeling-block, channeling-block holder, and tip chute.**
6. **With a damp cloth, clean any spilt reagent on the instrument worktable or vacuum chamber.**

   For all further cleaning and maintenance operations, see Section 7 of the *QIAcube HT User Manual*.

7. **Turn on the UV lamp to decontaminate the worktable by clicking  .**

   See the *QIAcube HT User Manual* for detailed instructions.

Protocol: Purification of Total RNA from Animal or Human Tissue (with Optional DNase Digestion)

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

Important points before starting

- Read Appendix A, if preparing RNA for the first time (page 48).
- Read "Important Notes", page 13.
- 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", page 6.
- For this protocol, QIAzol Lysis Reagent (200 ml; cat no. 79306) and Collection Microtube Racks (cat no. 19560) need to be ordered separately.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- Buffer RWT is supplied as a concentrate. Before using for the first time, add 2 volumes of ethanol (96–100%) to obtain a working solution.
- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- If carrying out optional on-plate DNase digestion, prepare the DNase I incubation mix as described in Appendix D, page 55.
- Ensure that the relevant version of the **RNeasy 96 QIAcube HT total RNA Tissue.QSP** run file is installed on the instrument.

  Optional: Select the relevant version of the **RNeasy 96 QIAcube HT total RNA Tissue with DNase.QSP** for optional on-plate DNase digestion. See Appendix D, page 55, for more information.

  Optional: For samples lysed using the TissueLyser, phase separation and transfer of the upper aqueous phase into an S-Block can be automated. Install the relevant version of the **RNeasy 96 QIAcube HT pre-treatment.QSP** run file on the instrument.

  QIAcube HT protocol files (file extension ***.QSP**), which contain all the information required to perform a protocol run on the QIAcube HT

instrument, are available from www.qiagen.com/p/QIAcubeHT, under the "Resources" tab.

- Ensure that Software version 4.17 or higher is installed. This is mandatory to process RNeasy 96 plates on the QIAcube HT and QIAxtractor.
- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Procedure

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: We recommend using the TissueLyser for disruption and homogenization of up 192 samples in parallel. Alternatively a rotor–stator homogenizer can be used for tissue disruption and homogenization. This protocol describes RNA purification from flash-frozen tissue using the TissueLyser. 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. Do not use more than 40 mg flash-frozen tissue, 20 mg liver, thymus, spleen, intestine, or 80 mg adipose tissue. Use half of these amounts when working with RNA*later* stabilized tissues. Transfer tissue immediately to a cooled collection microtube. Repeat this until all required pieces of tissues are placed in the collection microtubes.**

   Weighing tissue is the most accurate way to determine the amount. See page 17 for guidelines to determine the amount of starting material. 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 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.**

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. 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.**

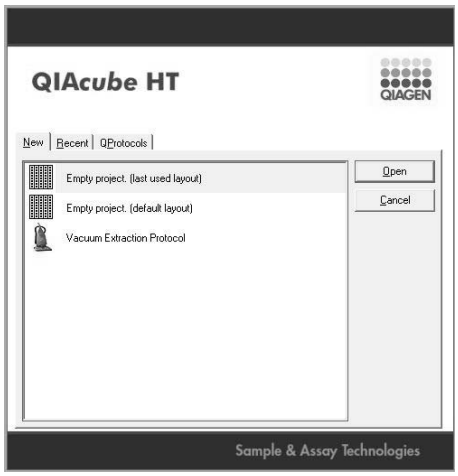
   Thorough mixing is important for subsequent phase separation.
10. **Place the collection microtube rack on the benchtop at room temperature for 2–3 min.**
11. **Centrifuge at 6000 x *g* for 15 min at 4°C.**

    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. **Place the tip discard chute on the worktable so that the chute is over the tip disposal box.**

    Ensure that the tip discard 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 discard chute.
13. **Switch on the instrument. The switch is located at the back of the instrument, on the lower left.**
14. **Launch the QIAcube HT Software.**

    Note: If the QIAcube HT Software is already open, click  in the toolbar.
15. **The following screen appears.**



16. Select the "QProtocols" tab.

All Q Protocols that are saved in the appropriate "QProtocols" folder will be listed.

Note: For samples lysed in a collection microtube rack using the TissueLyser and a 5 mm steel bead, phase separation and transfer of the upper aqueous phase into an S-Block can be automated by following these steps.

Note: For samples prepared differently, transfer only the upper, aqueous phase containing RNA into the S-Block manually and place the S-Block in the B1 position of the instrument worktable then proceed with step 17.

16a.Select the RNeasy 96 QIAcube HT pre-treatment.QSP run file from the "QProtocols" tab.

IMPORTANT: The position of the collection microtube rack in position C1 must be calibrated. For further information, see Appendix E, page 57.

16b.If processing <96 samples, follow the steps below. Otherwise proceed to step 16c.

(i) Unlock the opened protocol.

(ii). Select the appropriate wells on position C1 on the worktable setup.

(iii) Right-click, then select "Add selected wells to sample bank".

(iv) Give the bank a name, then click "Add Selection and Close".

(v) Click on reaction plate on position B1, then double-click the reaction list in the right-hand panel to open the reaction configuration. Select the appropriate sample bank set and click "OK".

16c.Load tips. Place the collection microtube rack containing your sample in position C1. Load an empty S-Block to position B1.

16d. Start the run by clicking .

16e. After the run, remove the collection microtube rack containing the organic phase and dispose of according to laboratory guidelines.

16f. If continuing directly with RNA purification, leave the S-Block on position B1 and proceed with step 17.

If not, remove the S-Block from the instrument and seal it with tape. Samples should be frozen if RNA isolation is not planned within the next 8 h.

17. To open the run file, select "New file" in the Toolbar of QIAcube HT software and choose the desired Q Protocol (RNeasy 96 QIAcube HT total RNA Tissue.QSP or RNeasy 96 QIAcube HT total RNA Tissue with DNase.QSP) and then click "Open".

18. A "Protocol Description" of the selected Q Protocol will be displayed and the QIAGEN Protocol icon will appear in the toolbar.

19. Check that the Q Protocol meets your requirements, and then click "Close".

Note: To view the Q Protocol information box again, click on the icon in the toolbar.

20. Click in the toolbar.

The "Configuration (1)" step of the "Vacuum extraction" wizard opens. This wizard displays protocol parameters. For information about adjusting the parameters, see the *QIAcube HT User Manual*.

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

Reagent and consumable lot numbers can be entered in the "Configuration (1)" window for tracking.

The "Jump to End" button is located at the bottom left of the "Configuration (1)" window.

The "Wizard Summary" window opens. The information in this window can be printed for documentation purposes.

22. Confirm the protocol by clicking "Finish". The wizard closes.

The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the *QIAcube HT User Manual*.

23. Ensure that there are sufficient numbers of tips for all steps, at least up to and including lysate transfer. Ensure that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes.

Check that the number and position of available and unused tips is the same on the instrument worktable and in the software workspace.

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 pre run check. For more information, see the *QIAcube HT User Manual*.

In the software environment, click on a tip in any tip position to open the "tip info" preview.

24. **Prepare the vacuum chamber as described in "Assembling the vacuum chamber", page 20. See the *QIAcube HT User Manual* for more information.**

    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.

    Note: Trim any excess tape.

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

    Note: Make sure that 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.

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

    Optional: Prepare the DNase I incubation mix as described in Appendix D, page 55.

    Note: Generally, DNase digestion is not required.

26. **Start the run immediately by clicking**  .

    The pre-run checklist appears.

27. **Perform the pre-run check.**

    Check the state of the worktable items.

    Confirm that worktable is set up correctly (instrument does not perform checks for all items). Check the box to the left of the items.

    A pre-run report can be saved for documentation purposes by clicking  .

28. **After completing the pre-run check, close the instrument hood and click "OK".**

    The "OK" button is disabled until all pre-run checklist items have been checked.

29. **Click "Cancel" when the "Save as" dialog box appears.**

    Optional: Save the run file with a unique file name. See the *QIAcube HT User Manual* for more details.

30. **The protocol run begins.**

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

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

    See the *QIAcube HT User Manual* for detailed instructions.

    Two liquid phases may be found in the Elution Microtubes. TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

Cleaning the instrument after completing a run

1. **Discard racks containing only used tips.**
2. **Discard leftover reagents.**

   We recommend not reusing reagents in multiple runs. Reagents provided are sufficient for at least 5 runs of 96 samples. Do not clean the trough containing TopElute Fluid with water. Clean with a dry, lint-free cloth only.

3. **Discard the S-Block or keep partially used blocks for reuse.**
4. **Remove the transfer carriage and discard the RNeasy 96 plate or keep partially used RNeasy 96 plates for reuse.**
5. **Clean the carriage, channeling-block, channeling-block holder, and tip chute.**
6. **With a damp cloth, clean any spilt reagent on the instrument worktable or vacuum chamber.**

   For all further cleaning and maintenance operations, see Section 7 of the *QIAcube HT User Manual*.

7. **Turn on the UV lamp to decontaminate the worktable by clicking .**

   See the *QIAcube HT User Manual* for detailed instructions.

Protocol: Purification of Total RNA, including Small RNAs, from Animal or Human Tissue and Cells

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 Appendix A, if preparing RNA for the first time (page 48).
- Read "Important Notes", page 14.
- 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", page 6.
- For this protocol, QIAzol Lysis Reagent (200 ml; cat no. 79306), Collection Microtube Racks (cat no. 19560), and Collection Microtube Caps (cat.no. 19566) need to be ordered separately.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- Buffer RWT is supplied as a concentrate. Before using for the first time, add 2 volumes of ethanol (96–100%) to obtain a working solution.
- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Ensure that the relevant version of the **miRNeasy 96 QIAcube HT.QSP** run file is installed on the instrument.

  Optional: For tissue samples lysed using the TissueLyser, phase separation and transfer of the upper aqueous phase into an S-Block can be automated. Install the relevant version of the **RNeasy 96 QIAcube HT pre-treatment.QSP** run file on the instrument.

  QIAcube HT protocol files (file extension ***.QSP**), 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 Software version 4.17 or higher is installed. This is mandatory to process RNeasy 96 plates on the QIAcube HT and QIAxtractor.

- Ensure that you are familiar with operating the instrument. Refer to the *QIAcube HT User Manual* for operating instructions.

Procedure — Cells

1. **[A] Cells grown in a monolayer: Cells grown in a monolayer in multiwell cell-culture plates can be lysed directly in the wells. Completely remove medium by pipetting, and continue with step 2.**

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

   [B] 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 the QIAzol Lysis Reagent in subsequent steps, inhibiting lysis and the binding of RNA to the RNeasy membrane. This will lead to reduced yield.

2. **Disrupt the cells by adding QIAzol Lysis Reagent. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 750 µl QIAzol Lysis Reagent to each plate well/collection microtube. Pipet up and down 3 times. If the lysates are in plate wells, transfer to collection microtubes (not supplied).**

   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 <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. **Close collection microtubes using collection microtube caps. Vortex for 1 min at maximum speed.**

   Note: If vortexing for 1 min is insufficient for homogenization of cells, the TissueLyser system should be used. See page 38.

   Note: Homogenized cell lysates can be stored at –70°C for several months.

4. **Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.**
5. **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.**

6. **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.**

   Thorough mixing is important for subsequent phase separation.

7. **Place the collection microtube rack on the benchtop at room temperature for 2–3 min.**
8. **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.

Procedure — Tissue

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: We recommend using the TissueLyser for disruption and homogenization of up 192 samples in parallel. Alternatively a rotor–stator homogenizer can be used for tissue disruption and homogenization. This protocol describes RNA purification from flash-frozen tissue using the TissueLyser. 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. Do not use more than 40 mg flash-frozen tissue, 20 mg thymus, spleen, intestine, or 80 mg adipose tissue. If processing liver, use less than 10 mg. Use half of these amounts when working with RNA*later* stabilized tissues. Transfer tissue immediately to a cooled collection microtube. Repeat this until all required pieces of tissues are placed in the collection microtubes.**

   Weighing tissue is the most accurate way to determine the amount. See page 15 for guidelines to determine the amount of starting material. RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen, or disrupted and homogenized. Frozen human or 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 using the supplied strips of collection microtube caps and homogenize on the TissueLyser 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.**

   Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol 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. 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.**

   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.

Procedure for cell and tissue samples

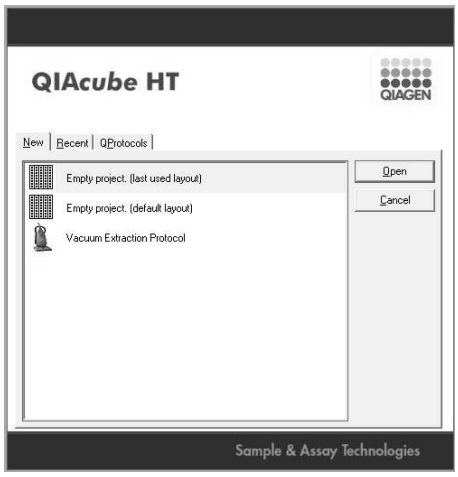
1. **Place the tip discard chute on the worktable so that the chute is over the tip disposal box.**

   Ensure that the tip discard chute is open and unblocked. Remove the UV protective cap from the tip chute.

   Make sure that the tip disposal box is empty and that the opening is aligned with the tip discard chute.
2. **Switch on the instrument. The switch is located at the back of the instrument, on the lower left.**
3. **Launch the QIAcube HT Software.**

   Note: If the QIAcube HT Software is already open, click in the toolbar.

4. **The following screen appears.**



5. **Select the "QProtocols" tab.**

   All Q Protocols that are saved in the appropriate "QProtocols" folder will be listed.

   Note: For samples lysed in a collection microtube rack using the TissueLyser and a 5 mm steel bead, phase separation and transfer of the upper aqueous phase into an S-Block can be automated by following these steps.

   Note: For cell culture samples or samples prepared differently transfer only the upper, aqueous phase containing RNA into the S-Block manually and place the S-Block in the B1 position of the instrument worktable then proceed with step 6.

5a. Select the RNeasy 96 QIAcube HT pre-treatment .QSP run file from the "QProtocols" tab.

IMPORTANT: The position of the collection microtube rack in position C1 must be calibrated. For further information, see Appendix E, page 57.

5b. If processing <96 samples, follow the steps below. Otherwise proceed to step 5c.

(i) Unlock the opened protocol.

(ii). Select the appropriate wells on position C1 on the worktable setup.

(iii) Right-click, then select "Add selected wells to sample bank".

(iv) Give the bank a name, then click "Add Selection and Close".

(v) Click on reaction plate on position B1, then double-click the reaction list in the right-hand panel to open the reaction configuration. Select the appropriate sample bank set and click "OK".

5c. Load tips. Place the collection microtube rack containing your sample in position C1. Load an empty S-Block to position B1.

5d. Start the run by clicking  .

5e. After the run, remove the collection microtube rack containing the organic phase and dispose of according to laboratory guidelines.

5f. If continuing directly with RNA purification, leave the S-Block on position B1 and proceed with step 6.

If not, remove the S-Block from the instrument and seal it with tape. Samples should be frozen if RNA isolation is not planned within the next 8 h.

6. To open the miRNeasy 96 QIAcube HT.QSP run file, select "New file" in the Toolbar of QIAcube HT software and then click "Open".

**7. A "Protocol Description" of the selected Q Protocol will be displayed and the 

icon will appear in the toolbar.**

8. Check that the Q Protocol meets your requirements, and then click "Close".

Note: To view the Q Protocol information box again, click on the  icon in the toolbar.

9. Click  in the toolbar.

The "Configuration (1)" step of the "Vacuum extraction" wizard opens. This wizard displays protocol parameters. For information about adjusting the parameters, see the *QIAcube HT User Manual*.

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

Reagent and consumable lot numbers can be entered in the "Configuration (1)" window for tracking.

The "Jump to End" button is located at the bottom left of the "Configuration (1)" window.

The "Wizard Summary" window opens. The information in this window can be printed for documentation purposes.

11. Confirm the protocol by clicking "Finish". The wizard closes.

The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the *QIAcube HT User Manual*.

12. Ensure that there are sufficient numbers of tips for all steps, at least up to and including lysate transfer. Ensure that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes.

Check that the number and position of available and unused tips is the same on the instrument worktable and in the software workspace.

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 pre run check. For more information, see the *QIAcube HT User Manual*.

In the software environment, click on a tip in any tip position to open the "tip info" preview.

13. **Prepare the vacuum chamber as described in "Assembling the vacuum chamber", page 20. See the *QIAcube HT User Manual* for more information.**

    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.

    Note: Trim any excess tape.

    Note: When reusing 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.

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

15. **Start the run immediately by clicking  .**

    The pre-run checklist appears.

16. **Perform the pre-run check.**

    Check the state of the worktable items.

    Confirm that worktable is setup correctly (instrument does not perform checks for all items). Check the box to the left of the items.

    A pre-run report can be saved for documentation purposes by clicking  .

17. **After completing the pre-run check, close the instrument hood and click "OK".**

    The "OK" button is disabled until all pre-run checklist items have been checked.

18. **Click "Cancel" when the "Save as" dialog box appears.**

    Optional: Save the run file with a unique file name. See the *QIAcube HT User Manual* for more information.

19. **The protocol run begins.**

    IMPORTANT: At the beginning of each run, an open circuit test and a plate detection test is performed automatically. If the RNeasy 96 plate in the transfer carriage is improperly aligned to the left side of the vacuum compartment, you will be prompted to place it correctly. Click "Retry" to initiate the tests again.

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

    See the *QIAcube HT User Manual* for detailed instructions.

    Two liquid phases may be found in the Elution Microtubes. TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

Cleaning the instrument after completing a run

1. **Discard racks containing only used tips.**
2. **Discard leftover reagents.**

   We recommend not reusing reagents in multiple runs. Reagents provided are sufficient for at least 5 runs of 96 samples.

   Do not clean the trough containing TopElute Fluid with water. Clean with a dry, lint-free cloth only.

3. **Discard the S-Block or keep partially used blocks for reuse.**
4. **Remove the transfer carriage and discard the RNeasy 96 plate or keep partially used RNeasy 96 plates for reuse.**
5. **Clean the carriage, channeling-block, channeling-block holder, and tip chute.**
6. **With a damp cloth, clean any spilt reagent on the instrument worktable or vacuum chamber.**

   For all further cleaning and maintenance operations, see Section 7 of the *QIAcube HT User Manual*.

7. **Turn on the UV lamp to decontaminate the worktable by clicking  .**

   See the *QIAcube HT User Manual* for detailed instructions.

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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or 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 18) 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 14).
Little or no RNA eluted	
a) Too much starting material	Overloading significantly reduces yield. Reduce the amount of starting material (see page 14).
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 A_{260}/A_{260} 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 50).
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 48) 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.

	Comments and suggestions
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 55) 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).
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	
a) Recovery in case of instrument failure or 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.

	Comments and suggestions
b) Instrument failure/cancelled run	It is possible to restart the protocol from the last successful step. The post-run report indicates the step where the error occurred. It is often possible to delete all steps before the indicated step in the right-hand pane and to restart the run from this point. Be sure that all parts and buffers are in the correct position.

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.

To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), use of RNaseKiller (cat. no. 2500080) from 5 PRIME (www.5prime.com) is recommended. RNase contamination can alternatively 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 49), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% 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.

*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.

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" below.

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

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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 51), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

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 49). 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:

* 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.

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 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 50).

* 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.

† 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.

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

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. 205311) provides fast cDNA synthesis with removal of genomic DNA contamination. For one-step, real-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.

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.

* 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.

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

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 4°C

* 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.

† 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 –20°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 6. Preparation of DNase-mix

Samples	**24**	**32**	**40**	**48**	**56**
Buffer RDD (ml)	1.96	2.52	3.08	3.64	4.2
DNase I stock (µl)	280	360	440	520	600
Samples	**64**	**72**	**80**	**88**	**96**
Buffer RDD (ml)	4.76	5.32	5.88	6.44	7
DNase I stock (µl)	680	760	840	920	1000

Procedure

D1. 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.

D2. Add DNase mix to a 70 ml trough just before starting the QIAcube HT run.

Appendix E: Calibration of Collection Microtube Rack Position for Automated Aqueous Phase Separation

For samples lysed using the TissueLyser, phase separation and transfer of the upper aqueous phase from the collection microtube plate into an S-Block can be automated. Automated pretreatment requires installation of the relevant version of the **RNeasy 96 QIAcube HT pre-treatment.QSP** run file on the instrument.

The position and height of the collection plate must be calibrated properly to assure a complete and clean separation of aqueous and organic phases with the RNeasy pretreatment protocol. For further information about plate calibration see the *QIAcube HT User Manual*, Section 5.13.

Calibrating plate position

Plate position calibration defines the positions of wells or tubes in a plate to be used on the QIAcube HT worktable. Calibration ensures that the robotic arm moves to the correct location for every well on the plate.

For information about plate position calibration, see the *QIAcube HT User Manual*, Section 5.13.

Calibrating plate height

Plate height calibration defines the base position of the wells of a plate to be used on the QIAcube HT worktable. Calibration allows software to correctly calculate the height at which tips must be placed for optimal aspirate and eject pipetting operations. If the tip is positioned too low, the tip may pierce the intermediate phase and contaminate the sample with organic liquid.

Note: As tips are used during height calibration, ensure that the workspace configuration correctly describes the worktable setup of tip numbers and tip rack positions before calibrating.

Performing plate height calibration

Perform calibration with an unused collection microtube rack.

E1. Ensure that the collection microtube rack to be calibrated for height has been calibrated for position (see Section 5.13 of the *QIAcube HT User Manual*).

E2. Open the instrument hood.

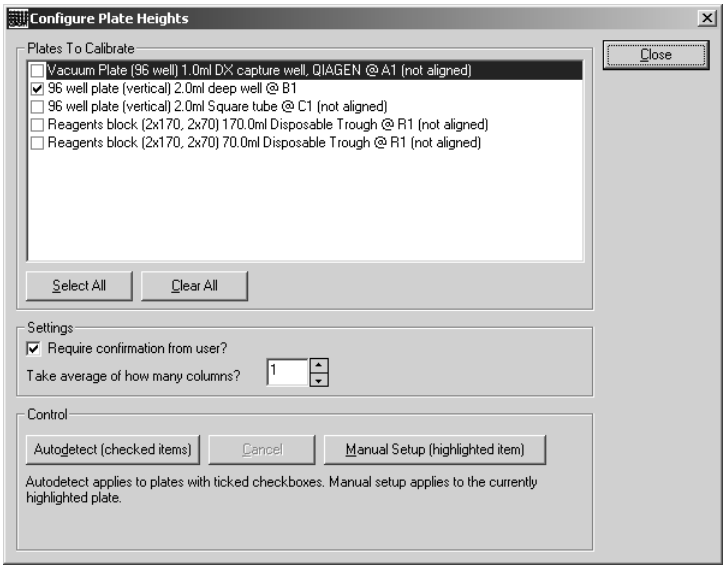
E3. Place the collection microtube rack in the C1 position on the instrument worktable.

E4. Ensure that 200 µl tips in an appropriate, calibrated tip rack are placed properly on the worktable and that the tip availability is set correctly.

E5. Select "Options/Robot Setup/Calibrate plate height".

E6. Read the warning about using the height calibration function (shown in the previous section), and click "Yes".

E7. The "Height Calibration" window appears.

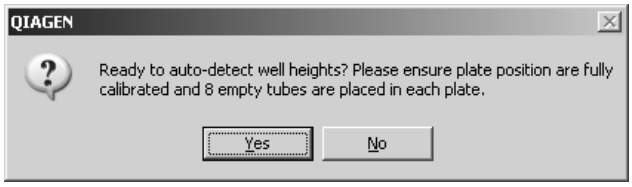


E8. From the "Plate Selection" list, select the 96 well plate (vertical) 1.2 ml Collection Microtube (supernatant) by checking the corresponding boxes.

E9. Enter the number of columns (8 wells per column) to be measured for calculating the average in the "Take average of how many columns?" field. We recommend a minimum of 2 columns.

E10. Click "Autodetect (checked items)" at the bottom of the window.

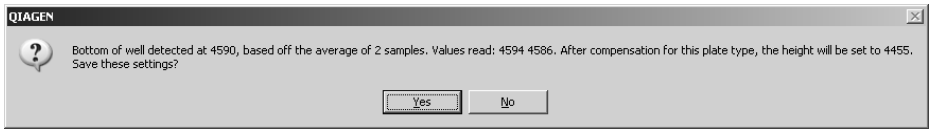
E11. Read the warning about the number of empty wells or tubes required to take the number of measurements for averaging, and click "Yes".



E12. A final notification appears regarding the plate type and position that is to be automatically height calibrated. Acknowledge by clicking "OK" to proceed.

The instrument collects required tips and proceeds with automatic detection of heights in the wells or tubes to be measured.

On completion, a notification about the height values determined is displayed.



E13. Accept the reported average and record it.

If the averages for a plate are within 135 steps of one another, the numbers can be accepted.

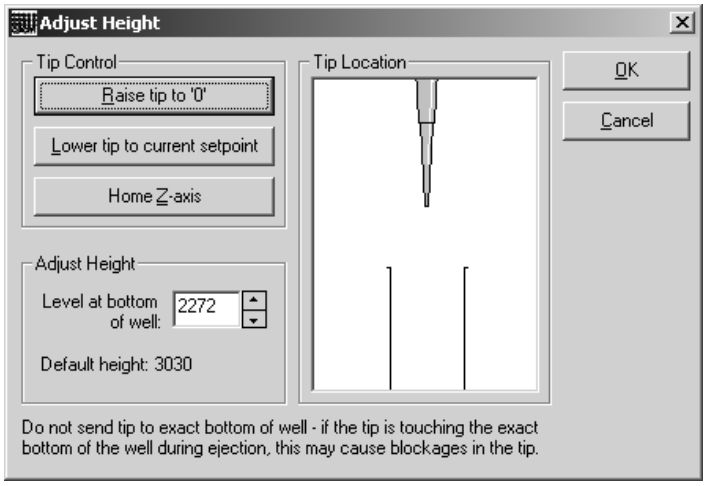
If the numbers are not within 135 steps of one another, check that the plate is sitting correctly and that tips are not bent. If a calibration is rejected, it must be repeated.

E14. Click "Manual Setup" [make sure that the 1.2 ml Collection Microtube (supernatant) plate is highlighted].

E15. Approve the next message to manually calibrate 1.2 ml Collection Microtube (supernatant).

E16. A message opens showing the current plate height and a suggested safety plate height. Click "No".

E17. Subtract 1479 steps from the automatically calibrated heights and enter this value in the box "Level at bottom of well".



E18. Click "OK" to confirm the adjustment.

E19. When calibration is completed, click "Close" to exit the window.

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 QIAcube HT Kit	For 480 preps: RNeasy 96 plates, RNase-Free Water, Buffers	74171
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
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
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 (available separately)*	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 (available separately)†	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980

*The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

† The TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

Product	Contents	Cat. no.
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
Accessories Pack, QXT‡	Upgrade kit for QIAxtractor instrument; Adapter set to use dedicated QIAcube HT kits on the QIAxtractor Contains: Transfer Carriage (9022654), Riser Block EMTR (9022655) and Channeling Adapter (9022656)	9022649
Accessories*		
Collection Microtubes (racked)	(10 x 96) Nonsterile polypropylene tubes ,960 in racks of 96	19560
Collection Microtube Caps	960 in strips of 8, For capping collection microtubes and round-well blocks	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 accessories, 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.

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