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RNeasy® 96 BioRobot® 8000 Handbook

For high-throughput RNA purification from animal and human cells, automated on the BioRobot Universal System, BioRobot Gene Expression — Real-Time RT-PCR, or BioRobot 8000

QIAGEN

Contents

Kit Contents

RNeasy 96 BioRobot 8000 Kit	**(12)**
Catalog no.	**967152**
Number of preps	**12 x 96**
RNeasy 96 Plates	12
Register Cards (96-well)	12
S-Blocks*	2
Elution Microtubes CL	12 x 96
Caps for Strips	165 x 8
Buffer RLT†	2 x 220 ml
Buffer RW1†	6 x 350 ml
Buffer RPE‡ (concentrate)	6 x 100 ml
RNase-Free Water	96 x 1.9 ml
Top Elute Fluid	48 x 1.48 ml
Handbook	1

* Reusable; see page 12 for cleaning instructions.

† Contains a guanidine salt. Not compatible with disinfecting reagents containing bleach. See page 6 for safety information.

‡ Add 4 volumes of ethanol (96–100%) before use to obtain a working solution.

The following kit components are also available separately: S-Blocks, Elution Microtubes CL (including caps for strips), Buffer RLT, and Top Elute Fluid. See page 35 for ordering information.

Storage

The RNeasy 96 BioRobot 8000 Kit, including all reagents and buffers, should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of RNeasy 96 BioRobot 8000 Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The RNeasy 96 BioRobot 8000 Kit is intended for research use. No claim or representation is intended to provide information 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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the RNeasy 96 BioRobot 8000 Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Safety Information

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

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

Buffer RLT contains guanidine thiocyanate and Buffer RW1 contains a small amount of guanidine thiocyanate. This chemical can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the BioRobot workstation, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to the components of the RNeasy 96 BioRobot 8000 Kit.

Buffer RLT

Contains guanidine thiocyanate. Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Buffer RW1

Contains ethanol. Risk and safety phrases:* R10

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R32: Contact with acids liberates very toxic gas; S13: Keep away from food, drink and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show this container or label.

Introduction

The RNeasy 96 BioRobot 8000 Kit enables simultaneous purification of total RNA from 96 or 192 samples, each containing up to 5 x 10^5 animal or human cells. The RNeasy 96 BioRobot 8000 Kit provides efficient, high-throughput RNA sample preparation for research use in fields such as drug screening and basic research.

The RNeasy 96 BioRobot 8000 procedure replaces time-consuming and tedious methods involving alcohol-precipitation steps, large numbers of wash steps, or the use of toxic substances such as phenol and/or chloroform. 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

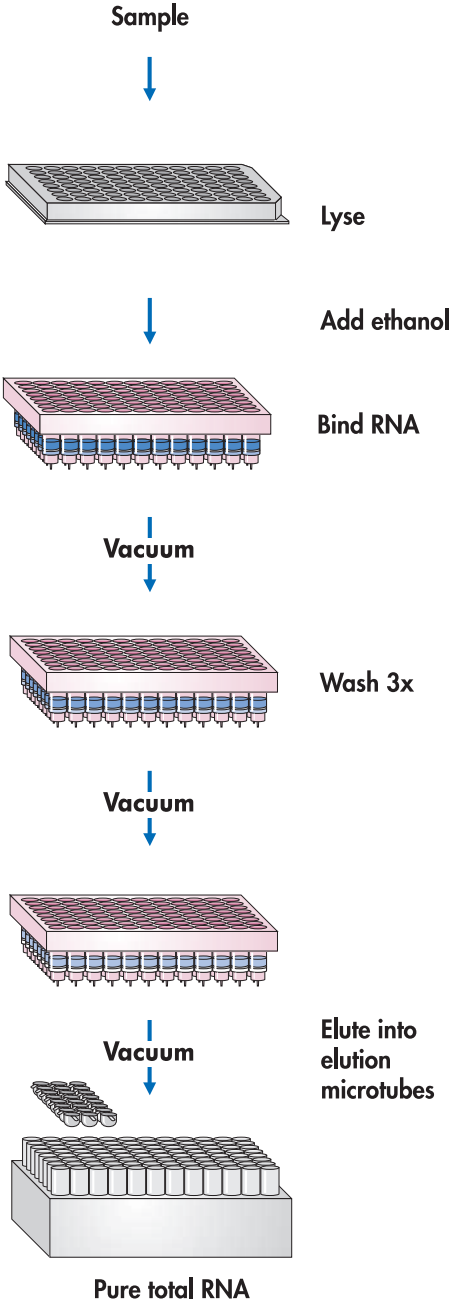
Principle and procedure

The RNeasy 96 BioRobot 8000 Kit uses well-established technology for high-throughput RNA preparation. The kit combines the selective binding properties of a silica-based membrane with the speed of vacuum processing. The BioRobot Universal System, BioRobot Gene Expression — Real-Time RT-PCR, or BioRobot 8000 provide walkaway automation of the RNeasy 96 procedure, for total RNA purification from up to 5 x 10^5 cells per sample.

The procedure starts with automated removal of the cell-culture medium. Cells are then lysed directly in the cell-culture plate on the integrated high-speed shaker of the BioRobot workstation. Cell lysis is performed under highly denaturing conditions with guanidine thiocyanate to immediately inactivate RNases and ensure purification of intact RNA. Ethanol is added 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, all RNA molecules longer than 200 nucleotides are purified. The procedure provides an 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 and microRNA from cells and tissues, we recommend using the miRNeasy 96 Kit (see ordering information, page 37).

RNeasy 96 BioRobot 8000 Procedure



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 material safety data sheets (MSDSs), available from the product supplier.

- BioRobot Universal System with Application Pack, Gene Expression; BioRobot Gene Expression — Real-Time RT-PCR (no longer available); or BioRobot 8000 (see ordering information, page 35)
- Disposable gloves
- Disposable Filter-Tips, 1100 µl (cat. no. 9012598)
- Disposable Troughs, 20 ml (cat. no. 9232764)
- Disposable Troughs, 80 ml (cat. no. 9013653)
- S-Blocks (cat. no. 19585)*
- 96–100% ethanol† and 70% ethanol in water†
- Cell-culture plates (see below for recommended suppliers)

Optional reagents

- 14.3 M β-mercaptoethanol (β-ME) (comercially available solutions are usually 14.3 M) (see protocol, page 18, for details)
- RNase-Free DNase Set (cat. no. 79254) for optional on-plate DNase digestion (see Appendix D, page 30, for details)
- Screw-cap tubes (2 ml) for use with the optional DNase treatment (Safe-Lock micro test tubes, Eppendorf, www.eppendorf.com)‡

  Note: Use of other tubes may require modification of the QIAsoft protocol; for assistance, contact QIAGEN. Other tubes may not fit into the reagent holder, 8-tube, 1.5 ml (cat. no. 9011758).

Suppliers of cell-culture plates

- Round-bottom: Greiner (cat. no. 650180) (www.greinerbioone.com)‡
- Flat-bottom: Costar (cat. no. 3599) (www.corning.com)‡

  Note: Use of other cell-culture plates may require modification of the QIAsoft protocol; contact QIAGEN for assistance.

* The kit contains 2 reusable S-Blocks. If processing several RNeasy 96 plates each day, it may be convenient to have extra S-Blocks.

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

‡ This is not a complete list of suppliers and does not include many important vendors of biological supplies.

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. Table 1 gives specifications for the RNeasy 96 plate. 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. Table 2 shows expected RNA yields from various cell types.

Table 1. RNeasy 96 Plate Specifications

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

Table 2. Typical Total RNA Yields with the RNeasy 96 BioRobot 8000 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. Since the RNeasy 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 (lysis buffer), samples can be stored at –70°C for months.

S-Blocks

The kit contains 2 S-Blocks. If processing several RNeasy 96 plates per day, it may be convenient to have extra S-Blocks available (see ordering information, page 35). The S-Blocks are used throughout the RNeasy 96 BioRobot 8000 procedure. Be sure to empty waste from the S-Blocks after use. To reuse the S-Blocks, rinse them thoroughly with tap water, incubate for 2 hours or overnight in 0.1 M NaOH, 1 mM EDTA,* rinse in distilled water, and dry at 50°C.

Preparation of reagents and worktable

Buffer RLT

One bottle of Buffer RLT (220 ml) contains sufficient buffer for 6 runs of 96 samples. Buffer RLT left over after a run should be stored at room temperature (15–25°C) for the next run.

Buffer RW1

One bottle of Buffer RW1 (350 ml) contains sufficient buffer for 2 runs of 96 samples. Buffer RW1 left over after a run should be stored at room temperature (15–25°C) for the next run.

Buffer RPE

Before using a bottle of Buffer RPE for the first time, add 4 volumes of ethanol (96–100%) (i.e., add 400 ml ethanol to 100 ml Buffer RPE). Tick the check box on the label of the bottle to indicate that ethanol has been added. One bottle of reconstituted Buffer RPE (500 ml) contains sufficient buffer for 2 runs of 96 samples. Buffer RPE left over after a run should be stored at room temperature (15–25°C) for the next run.

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

RNase-free water

For a single run of 96 samples, 8 tubes of RNase-free water (1.9 ml each) are required. Be sure to remove the lids before placing the tubes on the BioRobot worktable. RNase-free water left over after a run should be discarded and should not be reused for subsequent runs.

Top Elute Fluid

For a single run of 96 samples, 4 tubes of Top Elute Fluid (1.48 ml each) are required. Be sure to remove the lids before placing the tubes on the BioRobot worktable. Top Elute Fluid left over after a run should be discarded and should not be reused for subsequent runs.

RNase-free DNase I

The RNeasy 96 BioRobot 8000 procedure provides the option of performing 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 30.

Plasticware

One RNeasy 96 plate, one 96-well cell-culture plate, and one S-Block are required for a single run of 96 samples. When placing these items of plasticware on the BioRobot worktable, make sure that position A1 is located at the upper-left corner. Discard the plasticware after use (the S-Block can be reused).

One rack of Elution Microtubes CL is required for a single run of 96 samples. Be sure to keep the lid on and to place the elution microtubes rack on the blue elution microtube adapter. Make sure that the bar code of the elution microtubes rack faces to the right.

Table 3. Loading Buffers and Reagents (BioRobot Universal System)

Item	Position	Volume for one run of 96 samples	Volume for one run of 192 samples
Ethanol (70%)	Reagent holder for 5 x 80 ml troughs (MP Slot 9, Position A)	21 ml	35 ml
Buffer RLT	Reagent holder for 5 x 80 ml troughs (MP Slot 9, Position B)	21 ml	35 ml
Buffer RW1	Reagent carousel (Rotor Slot 2)	165 ml	271 ml
Buffer RPE*	Reagent carousel (Rotor Slot 4)	251 ml	443 ml
Ethanol (96–100%)	Reagent carousel (Rotor Slot 6)	145 ml	190 ml
Distilled water	Reagent carousel (Rotor Slot 8)	700 ml	700 ml
RNase-free water	Reagent holder for 8 x 2 ml tubes (MP Slot 8 – Reagent Holder Tray B)	8 x 1.9 ml	8 x 1.9 ml
	Reagent holder for 8 x 2 ml tubes (MP Slot 8 – Reagent Holder Tray C)	–	8 x 1.9 ml
Top Elute Fluid	Reagent holder for 8 x 1.5 ml tubes (MP Slot 8 – Reagent Holder Tray A)	4 x 1.48 ml	8 x 1.48 ml
Optional: RNase-free DNase I†	Reagent holder for 8 x 1.5 ml tubes (VariTherm Slot – Reagent Holder Tray A)	4 x 1.9 ml	8 x 1.9 ml

* Before using Buffer RPE for the first time, be sure to add 4 volumes of ethanol (96–100%).

† See Appendix D, page 30, for details on preparing RNase-free DNase I.

Table 4. Loading Plasticware (BioRobot Universal System)

Item	Position	Holder/adapter
RNeasy 96 plate	QIAplate Holder silver 11	Silver multiwell-plate holder
96-well cell-culture plate	High-speed shaker system (Shaker back-left)	
S-Block	High-speed shaker system (Shaker front-left)	
Elution Microtubes CL	MP Slot 21	Blue elution microtube adapter
Channeling block	QIAplate Holder black 16	Black multiwell-plate holder
Rack of disposable filter-tips (1100 µl)	Tip-Rack Slot 2, 3, 4, 5, 7, 10, 12, 13, 14, 20, 25	Red tip-tray holders
Additional plasticware if processing 192 samples in one run		
RNeasy 96 plate	QIAplate Holder silver 6	Silver multiwell-plate holder
96-well cell-culture plate	High-speed shaker system (Shaker back-right)	
Elution Microtubes CL	MP Slot 26	Blue elution microtube adapter

Table 5. Loading Plasticware (BioRobot Gene Expression — Real-Time RT-PCR or BioRobot 8000)

Item	Position	Volume for one run of 96 samples	Volume for one run of 192 samples
Ethanol (70%)	Reagent holder for 3 x 20 ml troughs (MP Slot 13, Position 2B)	17 ml	34 ml
Buffer RLT	Reagent holder for 3 x 20 ml troughs (MP Slot 13, Position 2A)	17 ml	34 ml
Buffer RW1	Reagent carousel (Rotor Slot 2)	165 ml	271 ml
Buffer RPE*	Reagent carousel (Rotor Slot 4)	251 ml	443 ml
Ethanol (96–100%)	Reagent carousel (Rotor Slot 6)	145 ml	190 ml
Distilled water	Reagent carousel (Rotor Slot 8)	700 ml	700 ml
RNase-free water	Reagent holder for 8 x 2 ml tubes (MP Slot 12 – Reagent Holder Tray B)	8 x 1.9 ml	8 x 1.9 ml
	Reagent holder for 8 x 2 ml tubes (MP Slot 12 – Reagent Holder Tray C)	–	8 x 1.9 ml
Top Elute Fluid	Reagent holder for 8 x 1.5 ml tubes (MP Slot 12 – Reagent Holder Tray A)	4 x 1.48 ml	8 x 1.48 ml
Optional: RNase-free DNase I†	Reagent holder for 8 x 1.5 ml tubes (VariTherm Slot – Reagent Holder Tray A)	4 x 1.9 ml	8 x 1.9 ml

* Before using Buffer RPE for the first time, be sure to add 4 volumes of ethanol (96–100%).

† See Appendix D, page 30, for details on preparing RNase-free DNase I.

Table 6. Loading Plasticware (BioRobot Gene Expression — Real-Time RT-PCR or BioRobot 8000)

Item	Position	Holder/adapter
RNeasy 96 plate	QIAplate Slot 6	QIAGEN multiwell-plate Holder
96-well cell-culture plate	High-speed shaker system (Shaker back-left)	
S-Block	High-speed shaker system (Shaker front-left)	
Elution Microtubes CL	MP Slot 21	Blue elution microtube adapter
Channeling block	QIAplate Slot 16	Black multiwell-plate holder
Rack of disposable filter-tips (1100 µl)	Tip Rack Slot 3, 4, 5, 8, 9, 10, 20	Red tip-tray holders
Additional plasticware if processing 192 samples in one run		
RNeasy 96 plate	QIAplate Slot 7	QIAGEN multiwell-plate holder
96-well cell-culture plate	High-speed shaker system (Shaker back-right)	
Elution Microtubes CL	MP Slot 26	Blue elution microtube adapter

Protocol: Purification of Total RNA from Animal or Human Cells

Important points before starting

- If preparing RNA for the first time, read Appendix A (page 24).
- If using the RNeasy 96 BioRobot 8000 Kit for the first time, read "Important Notes" (page 11).
- 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. In these cases, small residual amounts of DNA can be removed by optional on-plate DNase digestion (see Appendix D, page 30) or by DNase digestion after RNA purification (please contact QIAGEN Technical Services for a protocol).
- Buffer RLT may form a precipitate upon storage. If necessary, warm to 37°C to redissolve.
- When purifying RNA from cells containing high amounts of RNases, it may be necessary to add β-mercaptoethanol (β-ME) to Buffer RLT to avoid degradation of RNA. β-ME supports the inactivation of RNases by guanidine thiocyanate. Add 10 µl β-ME per 1 ml Buffer RLT. 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. In most cases, it will not be necessary to add β-ME to Buffer RLT.
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- 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.
- Check that all buffers are at 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 30.

Procedure

1. **If cells have been stored at a lower temperature, equilibrate them to room temperature (15–25°C).**
2. **Make sure that the BioRobot workstation is switched on.**
3. **Switch on the computer and monitor.**
4. **Launch the QIAsoft Operating System.**

   If using the BioRobot Universal System, start the QIAsoft 5 Operating System from the Microsoft Windows "Start" menu, where it is located under Programs/QIAsoft 5/QIAsoft 5. Enter your user name and password in the "Login" dialog box, and click "OK" to access QIAsoft 5.

   If using the BioRobot Gene Expression — Real-Time RT-PCR or the BioRobot 8000, the QIAsoft 4.2 Operating System is required. Start the software from the Microsoft Windows "Start" menu, where it is located under Programs/QIAsoft 4.2/QIAsoft 4.2.
5. **Select "RNeasy 96 Total RNA" from the protocol selection box.**

   This protocol is for RNA purification only. If you want to perform RNA purification and RT-PCR setup in the same run on the BioRobot Gene Expression — Real-Time RT-PCR or BioRobot 8000, select "RNeasy 96 RxSetup" from the protocol selection box.
6. **Click RUN to start the protocol.**

   The QIAsoft Operating System will now guide you through the remaining steps required to set up the BioRobot workstation for the RNeasy 96 BioRobot 8000 protocol. Follow the steps detailed in each protocol message before proceeding to the next protocol message.

   You will be prompted to enter information for the following options:

   - **Layout configuration**: Select the type of 96-well cell-culture plate used.
   - **Number of samples**: Select 96 samples (1-plate protocol) or 192 samples (2-plate protocol).
   - **DNase treatment**: Enter "yes" to perform DNase digestion on the RNeasy 96 plate (see page 29).
   - **Change tips**: Indicate if you want to change the tips during removal of culture supernatant. For many applications, changing tips is not necessary. The cells remain intact during removal of the culture supernatant, and cross-contamination of RNA between samples is minimal. For sensitive applications, the tips can be changed so as to eliminate the possibility of cross-contamination.
   - **Elution volume**: Choose the elution volume. For most applications, we recommend the default elution volume.

- **Automatic clog detection (BioRobot Universal System only)**: Enter "yes" to perform clog detection. The BioRobot workstation will then check for clogged membranes on the RNeasy 96 plate. Any wells with clogged membranes will not be processed further in the RNA purification procedure. Note that this option requires the use of more disposable tips and increases run time.

If using the BioRobot Universal System, a load check will be automatically performed after you have set up the workstation to check that the volumes of the reagents and the positions of the plasticware are correct.

7. **At the end of the protocol, follow the protocol messages which guide you through the steps to clean up the BioRobot workstation. Tasks include removing reagents and plasticware, cleaning the channeling block, and cleaning the vacuum manifold. If reusing Buffer RW1 and RPE in a subsequent run, be sure to close the bottles.**

   Use the elution microtube caps (caps for strips) provided to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

   If using the BioRobot Universal System, a protocol is available for real-time RT-PCR setup: select "RT-PCR Reaction Setup" from the protocol selection box.

8. **Be sure to perform daily, weekly, monthly, and annual maintenance of the BioRobot workstation.**

   If using the BioRobot Universal System, enter the "Maintenance" environment to find out which maintenance procedures need to be carried out. For details on how to use the "Maintenance" environment, refer to the *QIAsoft 5 Operating System User Manual*.

   If using the BioRobot Gene Expression — Real-Time RT-PCR or BioRobot 8000, refer to the *BioRobot 8000 User Manual* for details about maintenance procedures.

   For all BioRobot workstations, it is particularly important to prevent RNase contamination by cleaning the tubing of the workstation with 0.1 M NaOH, 1 mM EDTA solution. This is done during the monthly maintenance. For details, refer to the "Maintenance" environment or *BioRobot 8000 User Manual*.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (see back cover for contact information).

	Comments and suggestions
Clogged plate wells	
Too much starting material	Reduce the amount of starting material. It is essential to use the correct amount of starting material (see "Amount of cells", page 11).
Little or no RNA eluted	
a) Too much starting material	Overloading significantly reduces RNA yield. Reduce the amount of starting material (see "Amount of cells", page 11).
b) Buffer temperatures too low	All buffers must be at room temperature (15–25°C) throughout the procedure.
c) Residual liquid in cell-culture plate after removal of medium	Make sure the correct layout configuration (flat-bottom or round-bottom) is entered in the "Run Protocol: Layout Configuration" dialog box. Use of plates from some suppliers may result in incomplete removal of cell-culture medium. See "Equipment and Reagents to Be Supplied By User", page 10, for recommended suppliers.
Low A_{260}/A_{280} value	
Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the RNA sample before measuring purity (see Appendix B, page 26).

	Comments and suggestions
RNA degraded	
a) Inappropriate handling of starting material	Ensure that cells have been properly handled and that the protocol has been performed without interruptions, especially the initial steps involving cell lysis. See Appendix A (page 24), "Handling and storing starting material" (page 12), and "Important points before starting" (page 18).
b) RNase contamination	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 24). Carry out periodic maintenance as described in the "Maintenance" environment (BioRobot Universal System) or *BioRobot 8000 User Manual* (BioRobot Gene Expression — Real-Time RT-PCR or BioRobot 8000) to prevent RNase contamination of the BioRobot workstation.
DNA contamination in downstream experiments	
a) No DNase treatment	Carry out the optional on-plate DNase digestion (see Appendix D, page 30). Alternatively, DNase digest the RNA eluates. After heat-inactivating the DNase, the RNA samples can be used directly in downstream applications without further treatment or can be repurified (see the cleanup protocols in the *RNeasy MinElute® Cleanup Handbook* or *RNeasy 96 Handbook*).
b) Improper setup of DNase solutions	Make sure that the tubes of DNase I incubation mix each contain 1.99 ml of DNase I mix. Use 2 ml Safe-Lock tubes from Eppendorf. Use of other tubes may require modification of the QIAsoft protocol. Other tubes also may not fit in the reagent holder, 8-tube, 1.5 ml.

RNA does not perform well in downstream experiments	
Salt carryover during elution	Ensure that Buffer RPE is at room temperature (15–25°C).

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. In order 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.

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.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 25). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), 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). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allowed to dry.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. 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 and water. 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, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: RNeasy buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

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

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

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 water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA should 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 be difficult to determine amounts photometrically. Small amounts of RNA can be accurately quantified using an 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 milliliter (A_{260}=1 → 44 µg/ml). This relation 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 27), 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 25). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 20 µl of RNA sample + 180 µl of 10 mM Tris·Cl,* pH 7.0 (1/10 dilution)

Measure absorbance of diluted sample in a 0.2 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 10
= 88 µg/ml

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

Total amount = concentration x volume of sample in milliliters

= 88 µg/ml x 0.1 ml

= 8.8 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, 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 26).

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. To prevent any interference by DNA in RT-PCR applications, such as Applied Biosystems® and LightCycler® RT-PCR analyses, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. Alternatively, DNA contamination can be detected on agarose gels following RT-PCR by performing control experiments in which no reverse transcriptase is added prior to the PCR step or by using intro-spanning primers. For sensitive applications, such as differential display, or if it is not practical to use splice-junction primers, DNase digestion of the purified RNA with RNase-free DNase is recommended.

* 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.

A protocol for optional on-plate DNase digestion using the RNase-Free DNase Set is provided in Appendix D (page 30). The DNase is efficiently washed away in the subsequent wash steps. Alternatively, after the RNeasy procedure, the eluate containing the RNA can be treated with DNase (please contact QIAGEN Technical Services for a protocol). The RNA can then be repurified using an RNeasy RNA cleanup protocol (see the *RNeasy 96 Handbook* or the *RNeasy MinElute Cleanup Handbook*), or after heat inactivation of the DNase, the RNA can be used directly in downstream applications.

Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide* staining or using an Agilent 2100 bioanalyzer. 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. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

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, J. et al. [1989] *Molecular cloning — a laboratory manual*. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

FA gel preparation

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

1.2 g agarose*

10 ml 10x FA gel buffer (see composition below)

Add RNase-free water to 100 ml

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

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

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

RNA sample preparation for FA gel electrophoresis

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

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

Gel running conditions

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

Composition of FA gel buffers

10x FA gel buffer

200 mM 3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)*

50 mM sodium acetate*

10 mM EDTA*

pH to 7.0 with NaOH*

1x FA gel running buffer

100 ml 10x FA gel buffer

20 ml 37% (12.3 M) formaldehyde

880 ml RNase-free water

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

RNase-free water to 10 ml

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 material safety data sheets (MSDSs), 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 RW1, the RNA is treated with DNase I while bound to the silica membrane. The DNase is removed by a second wash with Buffer RW1. 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 RNeasy 96 technology efficiently removes 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., real-time 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 BioRobot 8000 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.

Procedure

D1. Add 670 µl DNase I stock solution (see above) to 7.3 ml Buffer RDD. 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. Aliquot into four 2 ml Safe-Lock tubes with 1.99 ml DNase I incubation mix per tube, and keep on ice* until ready to load on the BioRobot workstation.

Note: Use of other tubes may require modification of the QIAsoft protocol; for assistance, contact QIAGEN. Other tubes also may not fit in the reagent holder, 8-tube, 1.5 ml (cat. no. 9011758).

Appendix E: RT-PCR and Real-Time RT-PCR

RT-PCR

To perform PCR using RNA as a starting template, the RNA must first be reverse transcribed into cDNA in a reverse transcription (RT) reaction. RT and PCR can be carried out either sequentially in the same tube (one-step RT-PCR) or separately (two-step RT-PCR).

One-step RT-PCR requires gene-specific primers. For this application, QIAGEN offers the QIAGEN OneStep RT-PCR Kit, which enables one-step RT-PCR of any RNA template without optimization.

Two-step RT-PCR is generally carried out using oligo-dT primers in the RT step and gene-specific primers in the PCR step. For the RT step, QIAGEN offers two kits for efficient and sensitive reverse transcription:

- Omniscript® RT Kit — for cDNA synthesis using 50 ng – 2 µg RNA per reaction
- Sensiscript® RT Kit — for cDNA synthesis using less than 50 ng RNA per reaction

For the PCR step, QIAGEN offers enzymes that minimize PCR optimization:

- *Taq* DNA Polymerase — for PCR without a hot start
- HotStarTaq® DNA Polymerase — for PCR with a hot start
- HotStarTaq *Plus* DNA Polymerase — for PCR with a hot start and a fast 5-minute enzyme activation time

For more information on QIAGEN products for one-step RT-PCR and two-step RT-PCR, visit www.qiagen.com/products/pcr .

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

Real-time RT-PCR

The range of QuantiTect® Kits guarantee highly specific and sensitive results in real-time RT-PCR on any real-time cycler and require no optimization of reaction and cycling conditions. QuantiTect Kits are available for two-step and one-step RT-PCR and are compatible with detection by SYBR® Green I dye or by sequence-specific probes (e.g., TaqMan® and FRET probes). Multiplex RT-PCR of up to 5 targets is also possible. Predesigned QuantiTect Primer Assays are primer sets for use with SYBR Green detection and are easily ordered online at www.qiagen.com/GeneGlobe . For more information on QuantiTect Kits and Assays, visit www.qiagen.com/geneXpression .

Quantification on real-time cyclers

Quantification is based on the threshold cycle, where the amplification plot crosses a defined fluorescence threshold. Comparison of the threshold cycles provides a highly sensitive measure of relative template concentration in different samples. Figure 1 shows an example of real-time analysis using dual-labeled probes in TaqMan analysis. Monitoring during the early cycles, when PCR efficiency is at its highest, provides precise data for accurate quantification.

High-Quality RNA for Sensitive Analysis of a Low-Copy Transcript

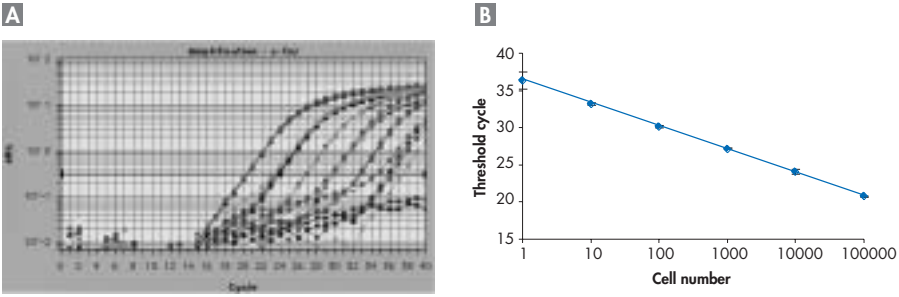


Figure 1 RNA was purified from 1 to 1 x 10^5 HeLa cells using the RNeasy 96 BioRobot 8000 procedure. Total RNA was eluted in 100 µl RNase-free water, and 5 µl was used for RT-PCR. Quantitative, real-time, one-step RT-PCR analysis was carried out on an ABI PRISM® Sequence Detection System using the QuantiTect Probe RT-PCR Kit with primers and probe specific for the low-copy c-fos transcript. A Amplification plot B C_T values. Error bars represent standard deviation from 8 different samples for each cell number.

For transcription analysis and quantification, quantitative RT-PCR assays require the highest-quality RNA. TaqMan technology was used in the development and evaluation of RNeasy 96 Kits, and RNA purified with RNeasy 96 Kits continues to be thoroughly tested by TaqMan and other real-time analyses. RNeasy 96 Kits are the only high-throughput total cellular RNA purification system providing RNA that meets stringent TaqMan standards.

Guidelines for quantitative RT-PCR analysis

Quantitative, real-time RT-PCR analysis can be carried out in a two-step or one-step format. In two-step RT-PCR analysis, reverse transcription and PCR quantification are performed sequentially in 2 separate reactions. This can be carried out using the QuantiTect Reverse Transcription Kit for reverse transcription, followed by PCR using a QuantiTect PCR Kit. For one-step RT-PCR analysis using a QuantiTect RT-PCR Kit, both reactions are performed in the same tube on a real-time cycler. Generally, one-step RT-PCR is more commonly used. Some guidelines for setting up quantitative, real-time RT-PCR analysis and determining the linear range of the system are given below.

1. Purify RNA from cells using the RNeasy 96 BioRobot 8000 Kit. For the elution step, elute with 80–140 µl RNase-free water.
2. For quantitative results, the amount of input RNA must be within the linear response range of the real-time assay, which may vary with the primers used and the transcripts assayed. In order to determine the optimal linear range of input RNA for a specific system, run a series of trial assays with 1, 2, 4, 6, 8, and 10 µl of an RNeasy 96 eluate in a 25 µl reaction volume.* For statistical significance, we recommend assaying each volume in triplicate and repeating the (triplicated) series of assays at least once.
3. Plot the resulting threshold cycle against the logarithm of the eluate volume. Figure 2 shows an example of such an experiment, with a linear response over the entire range. Note that, for some systems, the linear response will not cover the full range. Volumes outside the linear range will not yield quantitative results.

* The free sample volume in a 25 µl one-tube, real-time RT-PCR analysis is typically 9–10 µl. For a 50 µl assay, with approximately twice the free sample volume, we recommend using 1, 3, 6, 9, 12, 15, 18, and 20 µl.

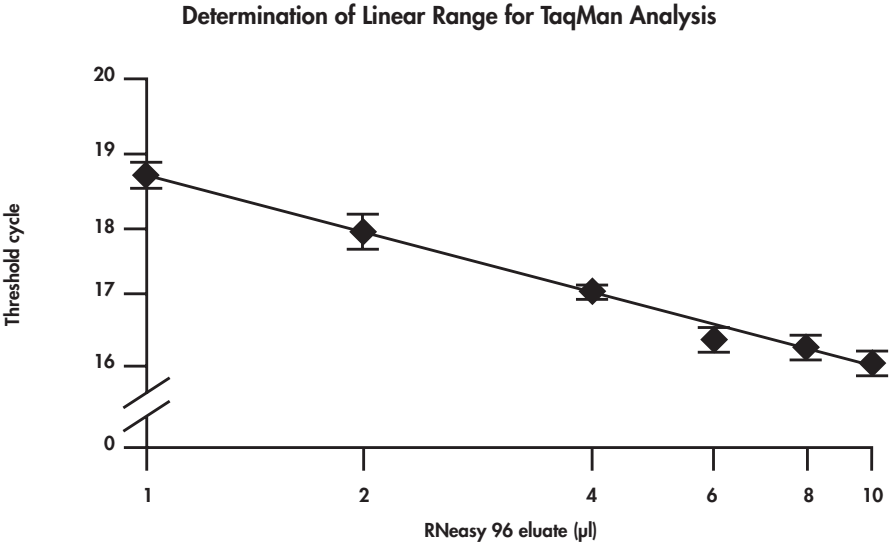


Figure 2 Linearity of RNeasy 96 RNA purification for one-tube β-actin RT-PCR analysis using dual-labeled (TaqMan) probes. Total RNA was purified from 5 x 10^4 HeLa cells with the RNeasy 96 Kit. RNA was eluted in 2 x 60 µl RNase-free water. RT-PCR TaqMan analysis of β-actin mRNA was performed in triplicate using 1, 2, 4, 6, 8, and 10 µl of the RNeasy 96 eluate in a 25 µl reaction volume, and the entire triplicated series was repeated three times. The mean of the threshold cycle for each volume is presented here, plotted against the logarithm of the volume. Error bars represent the σ_{n-1} standard deviation. The linear response range covers all volumes from 1 to 10 µl.

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 BioRobot 8000 Kit (12)	For 12 x 96 total RNA preps on the BioRobot Universal System, BioRobot Gene Expression — Real-Time RT-PCR, or BioRobot 8000: 12 RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, RNase-Free Reagents and Buffers	967152
BioRobot Universal System — for fully automated high-throughput applications in systems biology, in 96-well format		
BioRobot Universal System	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft 5 Operating System, installation, 1-year warranty on parts and labor*	9001094
Application Pack, Gene Expression	Protocols and application-specific accessories for RNA purification and RT-PCR setup	9016754
BioRobot 8000 — for flexible automation for purification of DNA or RNA, reaction setup, and reaction cleanup in 96-well format		
BioRobot 8000	Robotic workstation with selected system components, computer, QIAsoft 4.2 Operating System, installation, training, and 1-year warranty on parts and labor†	900500
Accessories		
Disposable Filter-Tips, 1100 µl (960)	Conducting disposable filter-tips; pack of 960	9012598
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585

* Warranty PLUS 2 (cat. no. 9239573) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

† Warranty PLUS 2 (cat. no. 9236465) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

Ordering Information

Product	Contents	Cat. no.
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
Buffer RLT (220 ml)	220 ml RNeasy Lysis Buffer	79216
Top Elute Fluid (48 x 1.48 ml)	48 x 1.48 ml Top Elute Fluid	1020460
RNase-Free DNase Set (50)	1500 units RNase-Free DNase I, RNase-Free Buffer RDD, and RNase-Free Water	79254
Related products for RNA purification*		
RNeasy 96 Universal Tissue 8000 Kit — for automated, high-throughput RNA purification from any type of tissue		
RNeasy 96 Universal Tissue 8000 Kit (12)†	For 12 x 96 total RNA preps on the BioRobot Universal System, BioRobot Gene Expression — Real-Time RT-PCR, or BioRobot 8000: 12 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	967852
RNeasy 96 Kit — for high-throughput RNA minipreps from cells		
RNeasy 96 Kit (4)‡	For 4 x 96 total and cytoplasmic RNA preps: 4 RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, RNase-Free Reagents and Buffers	74181

* QIAGEN offers a wide range range of RNA purification kits for different sample types, sizes, and throughputs. These include the BioRobot EZ1 and BioRobot M48 for automated RNA purification from 1–6 and 6–48 cell or tissue samples, respectively. For details, visit www.qiagen.com/RNA .

† Requires use of the Plate Rotor 2 x 96 and Centrifuge 4K15C (TissueLyser recommended for disruption and homogenization).

‡ Larger kit size available; please inquire. Requires use of QIAvac 96 or the QIAGEN 96-Well-Plate Centrifugation system.

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 Universal Tissue Kit — for high-throughput RNA purification from any type of animal tissue		
RNeasy 96 Universal Tissue Kit (4)*†	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	74881
miRNeasy 96 Kit — for purification of microRNA and total RNA from a wide range of animal tissues and cells		
miRNeasy 96 Kit (4)†	For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
Related products for one-step RT-PCR and real-time, one-step RT-PCR‡		
QIAGEN OneStep RT-PCR Kit — for fast and successful one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit (25)*	For 25 x 50 µl reactions: Enzyme Mix, 5x PCR Buffer, dNTP Mix, 5x Q-Solution, RNase-Free Water	210210
QuantiTect SYBR Green RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green RT-PCR Kit (200)*§	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204243

* Larger kit size available; please inquire.

† Requires use of the Plate Rotor 2 x 96 and Centrifuge 4K15C (TissueLyser recommended for disruption and homogenization; QIAvac 96 optional).

‡ QIAGEN also offers kits for reverse transcription, two-step RT-PCR, and real-time, two-step RT-PCR. For details, visit www.qiagen.com/products/pcr .

§ Visit www.qiagen.com/GeneGlobe to search for and order QuantiTect Primer Assays, which are gene-specific primer sets for use with this kit.

Ordering Information

Product	Contents	Cat. no.
QuantiTect Probe RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Probe RT-PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204443
QuantiTect Multiplex RT-PCR Kits — for quantitative, multiplex, real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex RT-PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204643
QuantiTect Multiplex RT-PCR NR Kit (200)*‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains no ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204843

The BioRobot Universal System and BioRobot 8000 workstations are intended for research applications. No claim or representation is intended for their use to provide information for the diagnosis, prevention, or treatment of a disease.

The RNeasy 96 Kit, RNeasy 96 Universal Tissue Kit, RNeasy 96 Universal Tissue 8000 Kit, miRNeasy 96 Kit, QIAGEN OneStep RT-PCR Kit, QuantiTect SYBR Green RT-PCR Kit, QuantiTect Primer Assays, QuantiTect Probe RT-PCR Kit, and QuantiTect Multiplex RT-PCR Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

* Larger kit size available; please inquire.

† Recommended for instruments from Applied Biosystems.

‡ Recommended for instruments from other suppliers.

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