RNeasy® 96 BioRobot® 9604 Handbook

For automated RNA isolation from animal and human cells

January 2002

QIAGEN Worldwide

QIAGEN Companies

Australia	**QIAGEN Pty Ltd** ABN 75 072 382 944	PO Box 25 • Clifton Hill • Victoria 3068 Orders 03-9489-3666 • Fax 03-9489-3888 • Technical 1-800-243-066
Canada	**QIAGEN Inc.**	2900 Argentia Road • Unit 23 • Mississauga • Ontario • L5N 7X9 Orders 800-572-9613 • Fax 800-713-5951 • Technical 800-DNA-PREP (800-362-7737)
France	**QIAGEN S.A.**	3 avenue du Canada • LP 809 • 91974 COURTABOEUF CEDEX Orders 01-60-920-920 • Fax 01-60-920-925 • Technical 01-60-920-930
Germany	**QIAGEN GmbH**	Max-Volmer-Straße 4 • 40724 Hilden Orders 02103-29-12000 • Fax 02103-29-22000 • Technical 02103-29-12400
Italy	**QIAGEN S.p.A.**	Via Grosio, 10/10 • 20151 Milano Orders 02-33430411 • Fax 02-33430426 • Technical 02-33430414
Japan www.qiagen.co.jp	**QIAGEN K.K.**	Forefront Tower II • 13-1, Kachidoki 3 Chome • Chuo-ku, Tokyo 104-0054 Telephone 03-5547-0811 • Fax 03-5547-0818 • Technical 03-5547-0811
Switzerland	**QIAGEN AG**	Auf dem Wolf 39 • 4052 Basel Orders 061-319-30-30 • Fax 061-319-30-33 • Technical 061-319-30-31
UK and Ireland	**QIAGEN Ltd.**	Boundary Court • Gatwick Road • Crawley • West Sussex, RH10 9AX Orders 01293-422-911 • Fax 01293-422-922 • Technical 01293-422-999
USA	**QIAGEN Inc.**	28159 Avenue Stanford • Valencia • CA 91355 Orders 800-426-8157 • Fax 800-718-2056 • Technical 800-DNA-PREP (800-362-7737)

www.qiagen.com

QIAGEN Distributors

Please see the last page for contact information for your local QIAGEN distributor.

Contents

Kit Contents

Note: The RNeasy® 96 BioRobot® 9604 Kit (12) consists of one RNeasy 96 Kit (12) box plus one RNeasy 96 BioRobot 9604 Kit (12) buffer set box.

	RNeasy 96 BioRobot 9604 Kit (12)	
Catalog No.	**967142**	
Box	**RNeasy 96 Kit box**	**Buffer set box**
Preparations per kit	12 x 96	
RNeasy 96 Plates	12	–
Register Cards (96-well)	12	–
Square-Well Blocks (2.2 ml)*	2	–
Elution Microtubes (1.2 ml), racked	12 x 96	–
Caps for Elution Microtubes, in strips	165 x 8	–
AirPore™ Tape Sheets (25 sheets per pack)	2	–
Buffer RLT†	2 x 220 ml	–
Buffer RLN	3 x 45 ml	–
Buffer RW1†	4 x 400 ml	2 x 400 ml
Buffer RPE‡	8 x 65 ml	4 x 65 ml
RNase-free water	12 x 30 ml	–
Handbook	1	1

* *Reusable; see page 10 for cleaning instructions.*

† *Not compatible with disinfecting reagents containing bleach. Contains guanidine isothiocyanate, which is an irritant. Take appropriate safety measures, and wear gloves when handling.*

‡ *Buffer RPE is supplied as a concentrate. Add 4 volumes of ethanol (96–100%) before use to obtain a working solution of Buffer RPE.*

Additional Buffer RLT, Square-Well Blocks, Elution Microtubes (1.2 ml), Caps for Elution Microtubes, and AirPore Tape Sheets are available separately. See ordering information (page 47).

Storage Conditions

RNeasy 96 BioRobot 9604 Kits, including all reagents and buffers, should be stored dry, at room temperature (15–25°C), and are stable for at least 9 months under these conditions. Buffer RLN can be precooled for use in the cytoplasmic protocol and can be stored at 2–8°C if desired.

Product Use Limitations

Some QIAGEN® products may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete systems as required by CLIA '88 regulations in the U.S. or equivalents in other countries. 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. We will credit your account or exchange the product — as you wish.

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.

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 9604 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 inside front 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.

Buffers RLT and RW1 contain guanidine thiocyanate, which 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.

The following risk and safety phrases apply to the components of the RNeasy 96 BioRobot 9604 Kit:

Buffer RLT

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

Buffer RW1

Contains ethanol: flammable. Risk and safety phrases:* R10

24-hour emergency information

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.*

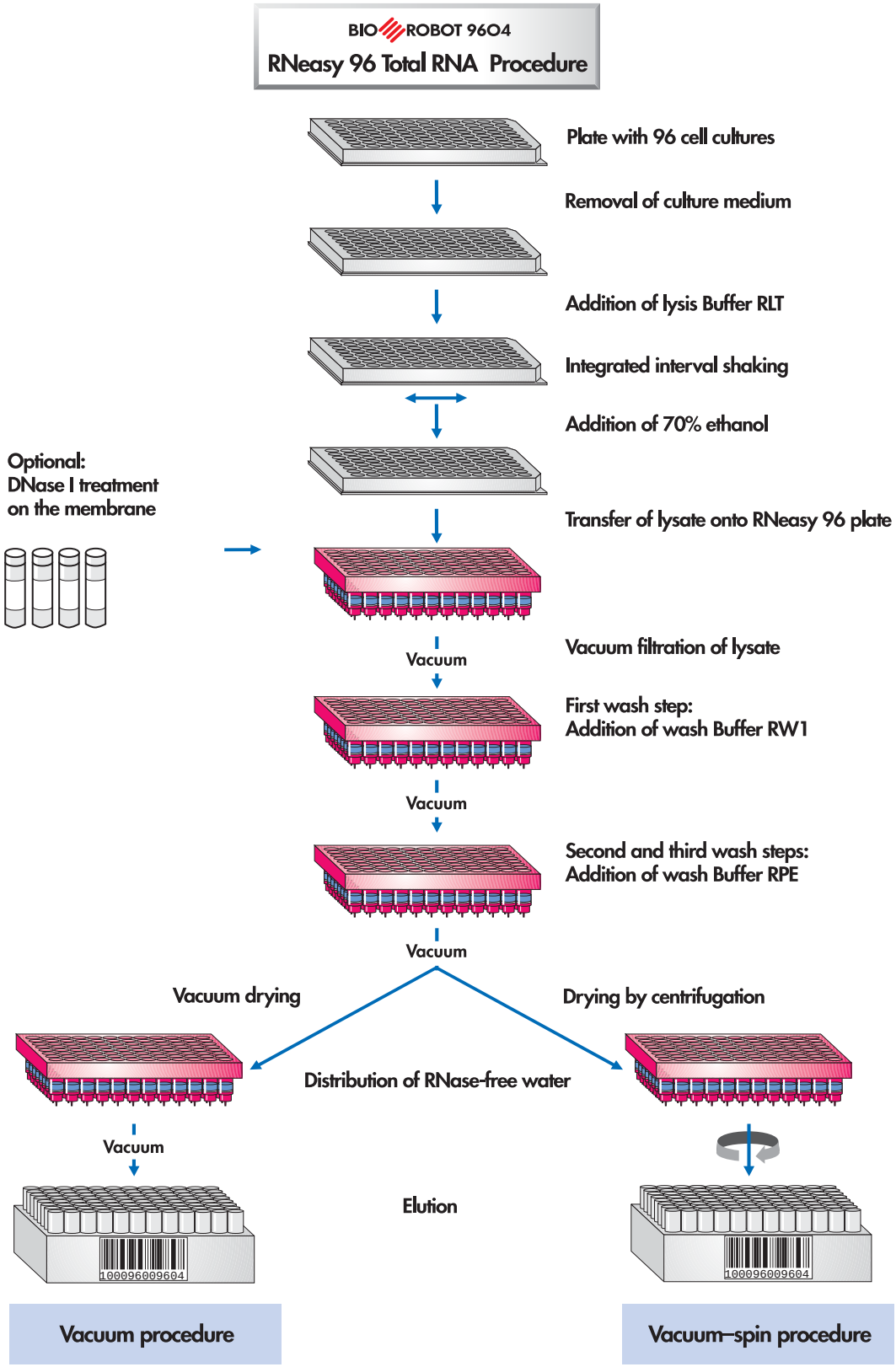

Figure 1. *Total RNA isolation with the RNeasy 96 BioRobot 9604 Kit. Elution steps can be performed directly on the BioRobot 9604 or in a specially designed centrifuge system (see page 9).*

Introduction

The RNeasy 96 principle and procedure

The RNeasy 96 BioRobot 9604 Kit represents a well-established technology for high-throughput RNA preparation. The RNeasy 96 technology combines the selective binding properties of a silica-gel–based membrane with the speed of vacuum or vacuum/spin technology. Automation on the BioRobot 9604 provides state-of-the-art automation technology. After positive identification of individual 96-well cell-culture plates using the Labware Identification System, the procedure starts with automated removal of the cell-culture medium. Cells are then lysed directly in the cell-culture plate on the integrated shaker of the BioRobot 9604 (configuration C). Cell lysis is performed under highly denaturing conditions with guanidine isothiocyanate (GITC) to immediately inactivate RNases and ensure isolation of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is 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 (Figure 1), all RNA molecules longer than 200 nucleotides are isolated. 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 purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently.

Different protocols are provided with different lysis steps and different handling options to pass solutions through the membrane using vacuum or vacuum/spin technology (see below for a detailed description).

Description of protocols

Isolation of Total RNA from Animal Cells

The RNeasy 96 BioRobot 9604 procedure is optimized for processing up to 5 x 10^5 animal cells per sample (see "Sample size" in the *RNeasy 96 Handbook* for more details). In the **RNeasy 96 BioRobot 9604 Protocol for Isolation of Total RNA from Animal Cells** (page 13), the BioRobot 9604 removes the cell-culture medium, and the cells are then lysed in a buffer containing guanidine isothiocyanate (GITC). Ethanol is added to the lysates, creating conditions that promote selective binding of RNA to the RNeasy membrane. The samples are then applied to the wells of the RNeasy 96 plate. Total RNA binds to the membrane at the bottom of each well. Generally, DNase digestion is not required since the RNeasy 96 silica-membrane 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. The optional on-membrane DNase step allows digestion of remaining DNA. DNase and contaminants are efficiently washed away by wash buffers, and the RNeasy membrane is dried. High-quality RNA is then eluted in a small volume of RNase-free water.

Isolation of Cytoplasmic RNA from Animal Cells

The **RNeasy 96 BioRobot 9604 Protocol for Isolation of Cytoplasmic RNA from Animal Cells** (page 27) is optimal for applications where unspliced or partially spliced RNA is not desirable, since the cytoplasm contains RNA in its mature form. The protocol is also advantageous in applications where the absence of DNA contamination is critical, since the intact nuclei are removed (see also "DNA contamination" in Appendix B of the *RNeasy 96 Handbook*). In most cases using the cytoplasmic protocol, DNase digestion is not required: most of the DNA is removed with the nuclei during the procedure, and the RNeasy 96 silica-membrane technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment.

The BioRobot 9604 first removes the cell-culture medium. Cells are then lysed in a buffer containing the non-ionic detergent Nonidet® P-40, which lyses the cell plasma membrane. Nuclei remain intact during the lysis procedure and are removed by centrifugation. GITC-containing lysis buffer and ethanol are added to the supernatant to provide optimal conditions for selectively binding RNA to the RNeasy membrane. Cytoplasmic RNA binds to the membrane at the bottom of each well. The optional on-membrane DNase step allows digestion of remaining DNA. DNase and contaminants are efficiently washed away by wash buffers, and the RNeasy membrane is dried. High-quality RNA is then eluted in a small volume of RNase-free water.

Handling options

Each of the protocols in this handbook is provided with two different handling options, using vacuum technology on the BioRobot 9604 alone or using a combination of vacuum and spin technology. Each handling option provides high yields of high-quality RNA. The requirements of the downstream application determine which option should be used.

I. Vacuum technology

Using the integrated vacuum manifold on the BioRobot 9604 is the most convenient way to carry out the RNeasy 96 RNA isolation. Up to 96 RNA samples can be processed in 75 minutes. RNA isolated using vacuum technology can be used in any non-enzymatic application (e.g., northern, dot, and slot blot analysis). The RNA may also be used in enzymatic applications. However, because RNA samples prepared using vacuum technology may still contain trace amounts of salt, we recommend preliminary experiments with the application required. If RNA performance is unsatisfactory, the RNeasy 96 BioRobot 9604 vacuum/spin option should be used.

II. Vacuum/spin technology

Using vacuum/spin technology, all protocol steps up to the final post-wash drying step are performed on the integrated vacuum manifold on the BioRobot 9604. The final steps, including membrane drying and all the elution steps, are performed in the Centrifuge 4-15C or Centrifuge 4K15C with the Plate Rotor 2 x 96. The special centrifuge adapter (96-well-plate) fits between the RNeasy 96 plate and elution microtube rack to ensure correct orientation during centrifugation. (Use the special centrifuge adapter: do not centrifuge elution microtube racks with the elution microtube adapter, which is for use on the vacuum manifold only.) RNA isolated using vacuum/spin technology can be used for any non-enzymatic or enzymatic downstream application including quantitative RT-PCR analysis by TaqMan® technology.

Table 1. Approximate run times for RNeasy 96 BioRobot 9604 protocols

RNeasy 96 BioRobot 9604 Protocol for Isolation of:	DNase digestion	Time
Total RNA	No	1 h 15 min
Total RNA	Yes	1 h 35 min
Cytoplasmic RNA	No	1 h 40 min
Cytoplasmic RNA	Yes	2 h 00 min
Includes:		
Hands-on time (all protocols)		5 min
Turnover time between runs (all protocols)		10 min

There is no significant differences in run times with the different handling options.

Important Notes before Using the RNeasy 96 BioRobot 9604 Kit

Table 2. RNeasy 96 plate specifications

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

* *The RNeasy 96 BioRobot 9604 procedure is optimized for processing up to 5 x 10^5 animal cells. Depending on the cells used it may be possible to increase the maximum amount of starting cells up to 1 x 10^6 cells (see the* RNeasy 96 Handbook *for more details). Please call QIAGEN Technical Services for guidelines to purify RNA from 10–100 cells.*

† *Yields are limited by cell type and number. The maximum binding capacity of 100 µg RNA is usually not reached (see the* RNeasy 96 Handbook *for more details).*

Square-Well Blocks

Two Square-Well Blocks are supplied per kit. If several RNeasy 96 plates are processed per day, it may be convenient to keep extra Square-Well Blocks on hand. See ordering information on page 47.

The Square-Well Blocks are used throughout the RNeasy 96 BioRobot 9604 procedure. Be sure to empty waste from the Square-Well Block after use.

To reuse the Square-Well Blocks, rinse them thoroughly with tap water, incubate for 2 h or overnight in 0.1 M NaOH/1 mM EDTA, rinse in distilled water, and dry at 50°C.

Note: Do not use bleach. Bleach may react with residual amounts of Buffers RLT and RW1 on the Square-Well Blocks.

Reagents and equipment to be supplied by user

For all protocols

- Disposable gloves
- Disposable Filter Tips (cat. no. 990252 and 990255)
- Disposable Troughs, 20 ml (available from QIAGEN, please inquire)
- Square-Well Blocks (cat. no. 19573)*
- 96–100% ethanol
- 14.5 M β-mercaptoethanol (β-ME, optional)†
- Cell-culture plates (see appendix, page 46, for recommended suppliers and ordering information)
- Elution Microtube Adapter (available from QIAGEN Technical Services)

Using vacuum/spin technology

- Centrifuge 4-15C or Centrifuge 4K15C
- Plate Rotor 2 x 96
- Centrifuge Adapter (available from QIAGEN Technical Services; use only the centrifuge adapter designed for use with the RNeasy 96 BioRobot 9604 Kit)

For protocol to isolate total RNA

- 70% ethanol in water

For protocol to isolate cytoplasmic RNA

- Centrifuge 4-15C, Centrifuge 4K15C, or other centrifuge and rotor for 96-well microplates
- RNase inhibitor (optional)‡
- DTT (optional)‡
- Ice (optional)§
- Multichannel pipet with tips¶ (see appendix, page 46, for recommended suppliers and ordering information)

* *Two Square-Well Blocks are supplied with the kit. They can be reused. If several plates are processed per day it may be convenient to have extra Square-Well Blocks available.*

† *Addition of β-ME to Buffer RLT is optional for the RNeasy 96 BioRobot 9604 protocols (see protocols for detailed information).*

‡ *Addition of RNase inhibitor and DTT to Buffer RLN is optional (see RNeasy 96 BioRobot 9604 Protocol for Isolation of Cytoplasmic RNA from Animal Cells, page 27).*

§ *For most preparations, all steps of the RNeasy 96 BioRobot 9604 protocols should be performed at room temperature. In rare cases, when analyzing transcripts from RNase-rich cells or when analyzing exceptionally labile transcripts, it may be advantageous to perform cell lysis on ice.*

¶ *If using round-bottom cell-culture plates, transfer of the lysed cytoplasm is carried out by the BioRobot 9604, and a multichannel pipet is not required. When using flat-bottom cell-culture plates, the transfer must be carried out by the user with a multichannel pipet.*

For optional DNase treatment on RNeasy 96 plates

Generally, DNase digestion is not required since the RNeasy 96 silica-membrane 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 (e.g., TaqMan RT-PCR analysis with a low-abundant target). In these cases, an optional on-membrane DNase step allows digestion of the small residual amounts of DNA remaining. The DNase is efficiently removed in the following wash step of the protocol. The on-membrane DNase treatment is included as an optional step in all RNeasy 96 BioRobot 9604 protocols.

- DNase I, **RNase-free**, ≥1.8 Kunitz units/µl

  The amount of DNase I used in the protocols is given in Kunitz units. A Kunitz unit is a commonly used unit for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. (1950) J. Gen. Physiol. **33**, 349 and 363).

  Note: Some suppliers have different unit definitions for DNase I. For example, 1 unit of DNase I from Pharmacia will degrade 1 µg of pBR322 in 10 minutes at 37°C at pH 7.5. One Pharmacia unit is equal to approximately 0.3 Kunitz units. **Make sure to use the appropriate conversion factor for DNase I preparations that are not quantified in Kunitz units.**

- Buffer RDD from QIAGEN, optimized for DNase I digestion on the RNeasy membrane (available from QIAGEN upon request)

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

- RNase-free water

- 2 ml Sarstedt screw-cap tubes (cat. no. 72.6940.06)*

Preparation of DNase I incubation mix

Prepare DNase I incubation mix immediately before starting the RNeasy 96 protocol. Prepare the mix according to the table below. Vortex briefly. Aliquot into four 2 ml Sarstedt screw-cap tubes* with 2.0 ml DNase I incubation mix per tube, and keep on ice until use.

Component	Amount/96-well plate
DNase I, RNase-free	1800 Kunitz units (maximum 1.0 ml)†
Buffer RDD‡	7.0 ml
RNase-free water	Add RNase-free water to 8.0 ml if necessary

† *Make sure to use a DNase I solution with ≥1.8 Kunitz units/µl. Use of more than 1.0 ml will not provide optimal reaction conditions due to high concentrations of glycerol, which is commonly used in DNase I storage buffers. Use the appropriate conversion factor for DNase I preparations that are not quantified in Kunitz units (see above).*

‡ *Available from QIAGEN upon request. Standard DNase buffers are not compatible with on-membrane DNase digestion. Use of other buffers may affect the binding of the RNA to the RNeasy membrane, reducing the yield and integrity of the RNA.*

* ***Note:*** *Use of other tubes may require adjusting the QIAsoft programming on the BioRobot 9604. Other tubes also may not fit in the BioRobot 9604 thermoblock. See appendix, page 46, for ordering information.*

RNeasy 96 BioRobot 9604 Protocol for Isolation of Total RNA from Animal Cells

Important notes before starting

- If preparing RNA for the first time, please read Appendix A in the *RNeasy 96 Handbook*. If using the RNeasy 96 BioRobot 9604 Kit for the first time, please read ''Important Notes before Using the RNeasy 96 BioRobot 9604 Kit'' (page 10).
- All centrifugation steps in the vacuum–spin protocol are performed in a Centrifuge 4-15C or Centrifuge 4K15C.
- Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to the right-hand side. For safety reasons, do not use plates that have been damaged in any way.
- In step 26 of all RNeasy 96 BioRobot 9604 protocols, place the elution microtube rack on top of the new elution microtube adapter before placing the assembly into MP-Slot 4.
- Buffer RLT may form a precipitate upon storage. If necessary, warm to 37°C to redissolve.
- When isolating 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 GITC. Add 10 µl of 14.5 M β-ME per 1 ml of Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME. In most cases it will not be necessary to add β-ME to Buffer RLT.
- 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.
- One bottle of RNase-free water in the RNeasy 96 BioRobot 9604 Kit contains sufficient buffer for two runs of 96 samples each.
- All steps of the RNeasy 96 BioRobot 9604 protocol for isolation of total RNA should be performed at room temperature. Avoid interruptions during the procedure.
- Generally, DNase digestion is not required since the RNeasy 96 silica-membrane 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. See "DNA contamination" in Appendix B of the *RNeasy 96 Handbook* for more information. See page 12 for details to prepare the DNase I incubation mix for the optional on-membrane DNase step included in the protocol.

RNeasy 96 BioRobot 9604 Protocol for Isolation of Total RNA from Animal Cells

I. Using vacuum technology

This protocol is a brief description of the steps performed by the BioRobot 9604 for preparation of total RNA using the RNeasy 96 BioRobot 9604 Kit and vacuum technology. Refer to the *BioRobot 9604 User Manual* and the context-sensitive help in the QIAsoft™ Operating System for further information.

1. **Prepare all reagents required for the protocol.**
   If cells have been stored at a lower temperature, equilibrate them to room temperature (15–25°C). Check that Buffer RLT and Buffer RPE have been prepared according to the instructions on page 13. If using the optional on-membrane DNase digestion, see page 12 for details to prepare the DNase I incubation mix.
2. **Make sure that the High-Speed Pipetting System is switched on.**
   Note: Always switch on the High-Speed Pipetting System before switching on the BioRobot 9604.
3. **Make sure that the BioRobot 9604 is switched on (switch is on the rear panel, lower right, looking from the front).**
   Note: The BioRobot 9604 should be left on at all times.
4. **Switch on the computer and monitor.**
5. **Launch the QIAsoft Operating System if necessary.**
6. **Select "RNeasy 96 VacTotal RNA" from the protocol field.**
7. **Click "RUN" on the toolbar.**
   The "Run Protocol: Slot Configuration" dialog box appears.
8. **Select the type of 96-well cell-culture plate used. Click "OK".**
   The "Run Protocol: No. of Samples" dialog box appears.
9. **Enter the number of samples to be processed (4 to 96) or the well positions of the first and last samples.**
   Note: Only multiples of 4 samples can be selected.
10. **Click "OK".**
11. **The first Enter Variable Value box appears.**

Enter Variable Value
Enter a text into this variable…
report file name report =

Enter a name as required with the suffix ".txt".

12. **Click "Continue".**
    The second Enter Variable Value box appears.

Enter Variable Value
Enter a text into this variable…
Operator name Operator =

Enter a name or identification code for the operator as required.

13. Click "Continue".

The third Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
Plate Identification barcode	PlateID =

Enter the identification number for the 96-well plate as required. The identification number can be entered manually or using the Labware Identification System hand scanner.

14. Click "Continue".

A protocol message box appears showing the identification number entered for the plate in the Enter Variable Value box.

Protocol Message
Plate Identification Number:

15. Click "Continue" to confirm the identification number and continue the protocol.

The fourth Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
DNase Treatment?	dnase = no

If the optional on-membrane DNase digestion is desired, enter "yes" for this variable. The default value is "no". Generally, DNase digestion is not required since the RNeasy 96 silica-membrane 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. See page 12 for details to prepare the DNase I incubation mix.

16. Click "Continue".

The fifth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Number of elution cycles ecycl = 2.00
(Value range: 1.00 … 2.00)

Enter a value of either 1.00 or 2.00 for the number of elution cycles desired. The default value is 2.00.

Note: We recommend initially testing the RNeasy 96 procedure both with one elution cycle and with two elution cycles in order to determine which gives optimal results for a particular sample type.

17. Click "Continue".

The sixth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 1 Evol1 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 70.00 µl.

Or, if only one elution cycle was selected:

Enter Variable Value
Enter a number for this variable…
Elution volume Evol = 100.00 µl
(Value range: 80.00 … 150.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 100.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

18. Click "Continue."

If two elution cycles were selected, the seventh Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 2 Evol2 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the second elution cycle. The default value is 70.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

19. Click "Continue".

20. Wait for the BioRobot 9604 to initialize and calibrate itself.

21. A series of protocol message boxes appear detailing the preparation steps required before the BioRobot 9604 protocol can continue. Follow the instructions in each protocol message box before proceeding.

A protocol message box appears.

Protocol Message
Fill system liquid container with distilled water.
Empty waste container.
Empty vacuum trap.
Empty Tip Waste Bag.

22. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place buffer bottles in the external holder: make sure that buffer bottle RW1 is connected to the green and Buffer RPE to the red adapter.

23. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place the bottle containing RNase-free water into buffer slot B6.

Use only the RNase-free water supplied in the RNeasy 96 BioRobot 9604 Kit. Do not aliquot the RNase-free water: use it on the BioRobot 9604 in the bottle supplied.

24. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place a Square-Well Block into MP-Slot 5.

25. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Fill a buffer trough with 20 ml of Buffer RLT, and place it into MP-Slot 2A. Fill a buffer trough with 20 ml of 70% Ethanol, and place it into MP-Slot 2C.

26. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place an elution microtube rack, assembled on top of the elution microtube adapter, into MP-Slot 4.

27. **Click "Continue".**

If the on-membrane DNase digestion is included, the next protocol message box appears.

Protocol Message
Place 4 x 2-ml microcentrifuge tubes, each containing 2 ml of Buffer RDD, RNase-free DNase, and water, into positions A1–A4 in the thermoblock.

28. Click "Continue".

The next protocol message box appears.

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the beveled edges pointing to the right-hand side.

Or, when preparing less then 96 samples:

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the beveled edges pointing to the right-hand side. Tape unused wells throughout the procedure with adhesive tape.

Do not use the AirPore Tape Sheets supplied in the RNeasy 96 BioRobot 9604 Kit. Use either adhesive tape or Tape Pads (cat. no. 19570) from QIAGEN.

29. Click "Continue".

The next protocol message box appears.

Protocol Message
Place sufficient tips into the tip racks. A run of 96 samples requires 3 x 96 tips. Make sure the tip racks are oriented and seated correctly on the holders.

30. Click "Continue".

The next protocol message box appears.

Protocol Message
Make sure that cassettes of the peristaltic pump are fitted.

31. Click "Continue".

The next protocol message box appears.

Protocol Message
Place cell-culture plate containing samples onto the MP-Slot in the 96-Well Shaker back/right position. Press "Continue" to start the protocol.

32. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol now starts.

A beeper sounds to let you know it is time for the first user interaction.

User interaction 1

33. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Tap the RNeasy 96 plate firmly on a stack of paper towels. Repeat several times until no further liquid is released. Place the elution microtube rack, assembled on top of the elution microtube adapter, into the vacuum manifold base.

Residual Buffer RPE from the collars and nozzles of each well will be absorbed by the paper towels. Use a stack of paper towels approximately 4 cm high. Droplets adhering to the nozzles and collars should be removed with a tissue.

34. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place the RNeasy 96 plate into the vacuum manifold top plate with the beveled edges pointing to the right-hand side. Open bottle with RNase-free water.

35. **Click "Continue".**

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know it is time for the next user interaction.

User interaction 2

36. **Click the speaker icon to stop the beeper.**

The next protocol message box appears.

Protocol Message
Discard the RNeasy 96 plate, and close the elution microtubes with the caps provided in the kit. Store at –20°C. Press "Continue".

37. **Click "Continue".**

The Report File appears, in which comments can be inserted.

38. **Click "Continue".**

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know the main protocol has finished.

39. **Click the speaker icon to stop the beeper.**

Wash procedure

40. **Click "OK".**

The next protocol message box appears.

Protocol Message
Connect the buffer bottle connectors to the adapters on the wash bottle filled with distilled water. Press "Continue" to start the wash procedure.

If you wish to perform another run immediately after the current run has finished, this wash step can be omitted; enter "no", and click "Continue".

41. **Click "Continue".**

After the wash procedure is finished, a protocol message box appears.

Protocol Message
Process done! Close all buffer bottles. Release cassettes of the peristaltic pump.

RNeasy 96 BioRobot 9604 Protocol for Isolation of Total RNA from Animal Cells

II. Using vacuum/spin technology

This protocol is a brief description of the steps performed by the BioRobot 9604 for preparation of total RNA using the RNeasy 96 BioRobot 9604 Kit and vacuum/spin technology. The protocol involves centrifugation steps at the end of the procedure and must be used in connection with the QIAGEN Centrifuge 4-15C or Centrifuge 4K15C. Refer to the *BioRobot 9604 User Manual* and the context-sensitive help in the QIAsoft Operating System for further information.

1. **Prepare all reagents required for the protocol.**

   If cells have been stored at a lower temperature, equilibrate them to room temperature (15–25°C). Check that Buffer RLT and Buffer RPE have been prepared according to the instructions on page 13. If using the optional on-membrane DNase digestion, see page 12 for details to prepare the DNase I incubation mix.

2. **Make sure that the High-Speed Pipetting System is switched on.**

   Note: Always switch on the High-Speed Pipetting System before switching on the BioRobot 9604.

3. **Make sure that the BioRobot 9604 is switched on (switch is on the rear panel, lower right, looking from the front).**

   Note: The BioRobot 9604 should be left on at all times.

4. **Switch on the computer and monitor.**

5. **Launch the QIAsoft Operating System if necessary.**

6. **Select "RNeasy 96 VS Total RNA" from the protocol field.**

7. **Click "RUN" on the toolbar.**

   The "Run Protocol: Slot Configuration" dialog box appears.

8. **Select the type of 96-well cell-culture plate used. Click "OK".**

   The "Run Protocol: No. of Samples" dialog box appears.

9. **Enter the number of samples to be processed (4 to 96) or the well positions of the first and last samples.**

   Note: Only multiples of 4 samples can be selected.

10. **Click "OK".**

11. **The first Enter Variable Value box appears.**

    Enter Variable Value
    Enter a text into this variable…
    report file name report =

    Enter a name as required with the suffix ".txt".

12. **Click "Continue".**

    The second Enter Variable Value box appears.

    Enter Variable Value
    Enter a text into this variable…
    Operator name Operator =

    Enter a name or identification code for the operator as required.

13. Click "Continue".

The third Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
Plate Identification barcode	PlateID =

Enter the identification number for the 96-well plate as required. The identification number can be entered manually or using the Labware Identification System hand scanner.

14. Click "Continue".

A protocol message box appears showing the identification number entered for the plate in the Enter Variable Value box.

Protocol Message
Plate Identification Number:

15. Click "Continue" to confirm the identification number and continue the protocol.

The fourth Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
DNase Treatment?	dnase = no

If the optional on-membrane DNase digestion is desired, enter "yes" for this variable. The default value is "no". Generally, DNase digestion is not required since the RNeasy 96 silica-membrane 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. See page 12 for details to prepare the DNase I incubation mix.

16. Click "Continue".

The fifth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Number of elution cycles ecycl = 2.00
(Value range: 1.00 … 2.00)

Enter a value of either 1.00 or 2.00 for the number of elution cycles desired. The default value is 2.00.

Note: We recommend initially testing the RNeasy 96 procedure both with one elution cycle and with two elution cycles in order to determine which gives optimal results for a particular sample type.

17. Click "Continue".

The sixth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 1 Evol1 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 70.00 µl.

Or, if only one elution cycle was selected:

Enter Variable Value
Enter a number for this variable…
Elution volume Evol = 100.00 µl
(Value range: 80.00 … 150.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 100.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

18. Click "Continue."

If two elution cycles were selected, the seventh Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 2 Evol2 = 70.00 ul
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the second elution cycle. The default value is 70.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

19. Click "Continue".

20. Wait for the BioRobot 9604 to initialize and calibrate itself.

21. A series of protocol message boxes appear detailing the preparation steps required before the BioRobot 9604 protocol can continue. Follow the instructions in each protocol message box before proceeding.

A protocol message box appears.

Protocol Message
Fill system liquid container with distilled water.
Empty waste container.
Empty vacuum trap.
Empty Tip Waste Bag.

22. **Click "Continue".**

The next protocol message box appears.

> **Protocol Message**
> Place buffer bottles in the external holder:
> make sure that buffer bottle RW1 is connected to the green
> and Buffer RPE to the red adapter.

23. **Click "Continue".**

The next protocol message box appears.

> **Protocol Message**
> Place the bottle containing RNase-free water into
> buffer slot B6.

Use only the RNase-free water supplied in the RNeasy 96 BioRobot 9604 Kit. Do not aliquot the RNase-free water: use it on the BioRobot 9604 in the bottle supplied.

24. **Click "Continue".**

The next protocol message box appears.

> **Protocol Message**
> Place a Square-Well Block
> into MP-Slot 5.

25. **Click "Continue".**

The next protocol message box appears.

> **Protocol Message**
> Fill a buffer trough with 20 ml of Buffer RLT, and place it into MP-Slot 2A.
> Fill a buffer trough with 20 ml of 70% Ethanol, and place it into MP-Slot 2C.

26. **Click "Continue".**

The next protocol message box appears.

> **Protocol Message**
> Place an elution microtube rack, assembled on top of the elution microtube adapter, into MP-Slot 4.

27. **Click "Continue".**

If the on-membrane DNase digestion is included, the next protocol message box appears.

> **Protocol Message**
> Place 4 x 2-ml microcentrifuge tubes, each containing
> 2 ml of Buffer RDD, RNase-free DNase, and water, into positions A1–A4 in the thermoblock.

28. Click "Continue".

The next protocol message box appears.

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the bevelled edges pointing to the right-hand side.

Or, when preparing less then 96 samples:

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the bevelled edges pointing to the right-hand side. Tape unused wells throughout the procedure with adhesive tape.

Do not use the AirPore Tape Sheets supplied in the RNeasy 96 BioRobot 9604 Kit. Use either adhesive tape or Tape Pads (cat. no. 19570) from QIAGEN.

29. Click "Continue".

The next protocol message box appears.

Protocol Message
Place sufficient tips into the tip racks. A run of 96 samples requires 3 x 96 tips. Make sure the tip racks are oriented and seated correctly on the holders.

30. Click "Continue".

The next protocol message box appears.

Protocol Message
Make sure that cassettes of the peristaltic pump are fitted.

31. Click "Continue".

The next protocol message box appears.

Protocol Message
Place cell-culture plate containing samples onto the MP-Slot in the 96-Well Shaker back/right position. Press "Continue" to start the protocol.

32. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol now starts.

A beeper sounds to let you know it is time for the first user interaction.

User interaction 1

33. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Seal RNeasy 96 plate with an AirPore tape. Transfer RNeasy 96 plate on top of a Square-Well Block into the centrifuge. Centrifuge for 10 min at 6000 rpm.

Use of AirPore tape reduces cross-contamination and allows evaporation of residual ethanol derived from Buffer RPE during centrifugation. To develop the heat required to evaporate the ethanol, centrifuge at room temperature. Residual ethanol will inhibit RT-PCR and must be removed by evaporation prior to elution.

Note: When using the Centrifuge 4K15C set the temperature to 40°C.

34. **Remove the assembly from the centrifuge bucket. Discard the waste in the Square-Well Block.**

35. **Click "Continue".**

    The next protocol message box appears.

> **Protocol Message**
> Place the 96-well centrifuge adapter onto the elution microtube rack, which is already on top of the elution microtube adapter in MP-Slot 4.
> Place the RNeasy 96 plate onto the elution microtube rack, remove the AirPore tape, and place the assembly, including the centrifuge adapter and the elution microtube adapter, into MP-Slot 1.

36. **Click "Continue".**

    The next protocol message box appears.

> **Protocol Message**
> Open bottle with RNase-free water.
>
> Press "Continue" to start elution.

37. **Click "Continue".**

    The RNeasy 96 BioRobot 9604 protocol continues.

    A beeper sounds to let you know it is time for the next user interaction.

User interaction 2

38. **Click the speaker icon to stop the beeper.**

    The next protocol message box appears.

> **Protocol Message**
> Seal the RNeasy 96 plate with AirPore tape. Lift the plate, centrifuge adapter, and elution microtube rack off the elution microtube adapter, and transfer the assembly into the centrifuge without the elution microtube adapter. Centrifuge for 2 min at 6000 rpm.
>
> Press "Continue".

39. **Click "Continue".**

    If only one elution cycle was selected, user interaction 3 is not required. Proceed directly to user interaction 4.

    If two elution cycles were selected, the next protocol message box appears.

> **Protocol Message**
> Remove the AirPore tape, and place the assembly back onto the elution microtube adapter in MP-Slot 1.
>
> Press "Continue"

40. **Click "Continue".**

    If two elution cycles were selected, the RNeasy 96 BioRobot 9604 protocol now continues.

    A beeper sounds to let you know it is time for the next user interaction.

User interaction 3 (only if two elution cycles were selected)

41. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Seal the RNeasy 96 plate with AirPore tape. Lift the plate, centrifuge adapter, and elution microtube rack off the elution microtube adapter, and transfer the assembly into the centrifuge without the elution microtube adapter. Centrifuge for 2 min at 6000 rpm. Press "Continue".

42. Click "Continue". Proceed directly to user interaction 4.

User interaction 4

43. The next protocol message box appears.

Protocol Message
Discard the RNeasy 96 plate, and close the elution microtubes with the caps provided in the kit. Store at –20°C. Press "Continue".

44. Click "Continue".

The Report File appears, in which comments can be inserted.

45. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know the main protocol has finished.

46. Click the speaker icon to stop the beeper.

Wash procedure

47. Click "Continue".

The next protocol message box appears.

Protocol Message
Connect the buffer bottle connectors to the adapters on the wash bottle filled with distilled water. Press "Continue" to start the wash procedure.

If you wish to perform another run immediately after the current run has finished, this wash step can be omitted; enter "no", and click "Continue".

48. Click "Continue".

After the wash procedure is finished, a protocol message box appears.

Protocol Message
Process done! Close all buffer bottles. Release cassettes of the peristaltic pump.

RNeasy 96 BioRobot 9604 Protocol for Isolation of Cytoplasmic RNA from Animal Cells

Important notes before starting

- If preparing RNA for the first time, please read Appendix A in the *RNeasy 96 Handbook*. If using the RNeasy 96 BioRobot 9604 Kit for the first time, please read ''Important Notes before Using the RNeasy 96 BioRobot 9604 Kit'' (page 10).
- All centrifugation steps in the vacuum–spin protocol are performed in a Centrifuge 4-15C or Centrifuge 4K15C. Pelleting of nuclei can also be performed in a standard 96-well–microplate centrifuge. However, if nuclei from RNase-rich cells are pelleted at 4°C to avoid degradation of RNA, a refrigerated 96-well–microplate centrifuge or Centrifuge 4K15C, the refrigerated version of Centrifuge 4-15C, is required.
- If using flat-bottom cell-culture plates, use of a multichannel pipet is recommended for transfer of the cytoplasmic lysate (see page 11). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package. When using round-bottom cell-culture plates, transfer is carried out by the BioRobot 9604, and a multichannel pipet is not required.
- Only use freshly harvested cells since ice crystals form during freezing and thawing and destroy the nuclear membranes, releasing DNA and other nuclear molecules.
- Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to the right-hand side. For safety reasons, do not use plates that have been damaged in any way.
- In step 26 of all RNeasy 96 BioRobot 9604 protocols, place the elution microtube rack on top of the new elution microtube adapter before placing the assembly into MP-Slot 4.
- Buffer RLT may form a precipitate upon storage. If necessary, warm to 37°C to redissolve.
- When isolating RNA from cells containing high amounts of RNases, in some cases it may be necessary to add β-mercaptoethanol (β-ME) to Buffer RLT and RNase inhibitor and DTT to Buffer RLN to avoid degradation of RNA. β-ME supports the inactivation of RNases by GITC. Add 10 µl of 14.5 M β-ME per 1 ml of Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME. Add 1000 U/ml RNase inhibitor and 1 mM DTT to Buffer RLN just before use. In most cases, it is not necessary to add β-ME to Buffer RLT or an RNase inhibitor to Buffer RLN.
- 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.
- One bottle of RNase-free water in the RNeasy 96 BioRobot 9604 Kit contains sufficient buffer for two runs of 96 samples each.
- For most preparations, all steps of the RNeasy protocol should be performed at room temperature. In rare cases, when analyzing transcripts from RNase-rich cells or when analyzing exceptionally labile transcripts, it may be advantageous to perform cell lysis in Buffer RLN on ice.
- Using the cytoplasmic protocol, DNase digestion is generally not required: most of the DNA is removed by pelleting the nuclei during the procedure, and the RNeasy 96 silica-membrane technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. See "DNA contamination" in Appendix B of the *RNeasy 96 Handbook* for more information. See page 12 for details to prepare the DNase I incubation mix for the optional on-membrane DNase step included in the protocol.

RNeasy 96 BioRobot 9604 Protocol for Isolation of Cytoplasmic RNA from Animal Cells

I. Using vacuum technology

This protocol is a brief description of the steps performed by the BioRobot 9604 for preparation of cytoplasmic RNA using the RNeasy 96 BioRobot 9604 Kit and vacuum technology. Refer to the *BioRobot 9604 User Manual* and the context-sensitive help in the QIAsoft Operating System for further information.

1. **Prepare all reagents required for the protocol.**
   Check that Buffer RLT, Buffer RLN, and Buffer RPE have been prepared according to the instructions on page 27. If using the optional on-membrane DNase digestion, see page 12 for details to prepare the DNase I incubation mix.
2. **Make sure that the High-Speed Pipetting System is switched on.**
   Note: Always switch on the High-Speed Pipetting System before switching on the BioRobot 9604.
3. **Make sure that the BioRobot 9604 is switched on (switch is on the rear panel, lower right, looking from the front).**
   Note: The BioRobot 9604 should be left on at all times.
4. **Switch on the computer and monitor.**
5. **Launch the QIAsoft Operating System if necessary.**
6. **Select "RNeasy 96 Vac Cytoplasmic RNA" from the protocol field.**
7. **Click "RUN" on the toolbar.**
   The "Run Protocol: Slot Configuration" dialog box appears.
8. **Select the type of 96-well cell-culture plate used. Click "OK".**
   The "Run Protocol: No. of Samples" dialog box appears.
9. **Enter the number of samples to be processed (4 to 96) or the well positions of the first and last samples.**
   Note: Only multiples of 4 samples can be selected.
10. **Click "OK".**
11. **The first Enter Variable Value box appears.**

Enter Variable Value
Enter a text into this variable…
report file name report =

Enter a name as required with the suffix ".txt".

12. **Click "Continue".**
    The second Enter Variable Value box appears.

Enter Variable Value
Enter a text into this variable…
Operator name Operator =

Enter a name or identification code for the operator as required.

13. Click "Continue".

The third Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
Plate Identification barcode	PlateID =

Enter the identification number for the 96-well plate as required. The identification number can be entered manually or using the Labware Identification System hand scanner.

14. Click "Continue".

A protocol message box appears showing the identification number entered for the plate in the Enter Variable Value box.

Protocol Message
Plate Identification Number:

15. Click "Continue" to confirm the identification number and continue the protocol.

The fourth Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
DNase Treatment?	dnase = no

If the optional on-membrane DNase digestion is desired, enter "yes" for this variable. The default value is "no". Using the cytoplasmic protocol, DNase digestion is generally not required: most of the DNA is removed by pelleting the nuclei, and the RNeasy 96 silica-membrane technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. Using the cytoplasmic protocol with the optional DNase digestion results in undetectable levels of DNA, even by sensitive TaqMan analysis. See page 12 for details to prepare the DNase I incubation mix.

16. Click "Continue".

The fifth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Number of elution cycles ecycl = 2.00
(Value range: 1.00 … 2.00)

Enter a value of either 1.00 or 2.00 for the number of elution cycles desired. The default value is 2.00.

Note: We recommend initially testing the RNeasy 96 procedure both with one elution cycle and with two elution cycles in order to determine which gives optimal results for a particular sample type.

17. **Click "Continue".**

The sixth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 1 Evol1 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 70.00 µl.

Or, if only one elution cycle was selected:

Enter Variable Value
Enter a number for this variable…
Elution volume Evol = 100.00 µl
(Value range: 80.00 … 150.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 100.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

18. **Click "Continue."**

If two elution cycles were selected, the seventh Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 2 Evol2 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the second elution cycle. The default value is 70.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

19. **Click "Continue".**

20. **Wait for the BioRobot 9604 to initialize and calibrate itself.**

21. **A series of protocol message boxes appear detailing the preparation steps required before the BioRobot 9604 protocol can continue. Follow the instructions in each protocol message box before proceeding.**

A protocol message box appears.

Protocol Message
Fill system liquid container with distilled water.
Empty waste container.
Empty vacuum trap.
Empty Tip Waste Bag.

22. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Place buffer bottles in the external holder:
> make sure that buffer bottle RLT is connected to the blue, RW1 to the green, 100% Ethanol to the yellow, and Buffer RPE to the red adapter.

23. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Place the bottle containing RNase-free water into
> buffer slot B6.

Use only the RNase-free water supplied in the RNeasy 96 BioRobot 9604 Kit. Do not aliquot the RNase-free water: use it on the BioRobot 9604 in the bottle supplied.

24. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Place a Square-Well Block each into MP-Slot 5 and into the
> 96-Well Shaker front/right position.

25. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Fill a buffer trough with 20 ml of Buffer RLN, and place it into MP-Slot 2A.

26. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Place an elution microtube rack, assembled on top of the elution microtube adapter, into MP-Slot 4.

27. Click "Continue".

If the on-membrane DNase digestion is included, the next protocol message box appears.

> **Protocol Message**
> Place 4 x 2-ml microcentrifuge tubes, each containing
> 2 ml of Buffer RDD, RNase-free DNase, and water, into positions A1–A4 in the thermoblock.

28. Click "Continue".

The next protocol message box appears.

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the bevelled edges pointing to the right-hand side.

Or, when preparing less then 96 samples:

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the bevelled edges pointing to the right-hand side. Tape unused wells throughout the procedure with adhesive tape.

Do not use the AirPore Tape Sheets supplied in the RNeasy 96 BioRobot 9604 Kit. Use either adhesive tape or Tape Pads (cat. no. 19570) from QIAGEN.

29. Click "Continue".

The next protocol message box appears.

Protocol Message
Place sufficient tips into the tip racks. A run of 96 samples requires 3 x 96 tips. Make sure the tip racks are oriented and seated correctly on the holders.

30. Click "Continue".

The next protocol message box appears.

Protocol Message
Make sure that cassettes of the peristaltic pump are fitted.

31. Click "Continue".

The next protocol message box appears.

Protocol Message
Place cell-culture plate containing samples onto the MP-Slot in the 96-Well Shaker back/right position. Press "Continue" to start the protocol.

32. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol now starts.

A beeper sounds to let you know it is time for the first user interaction.

User interaction 1

33. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Incubate cell-culture plate for 5 minutes at room temperature or on ice. Press "Continue".

For most preparations, cell lysis can be performed at room temperature. In rare cases, when analyzing transcripts from RNase-rich cells or when analyzing exceptionally labile transcripts, it may be advantageous to perform cell lysis on ice.

34. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know when 5 minutes has elapsed.

35. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Centrifuge cell-culture plate for 5 minutes at 500 x g. Place cell-culture plate back on 96-Well Shaker back/right position.

Note: For most preparations, centrifugation can be performed at room temperature. In some cases, when analyzing transcripts from RNase-rich cells or when analyzing labile transcripts, it may be advantageous to perform the centrifugation at 4°C.

36. Click "Continue".

If a round-bottom cell-culture plate is used, the RNeasy 96 BioRobot 9604 protocol now continues. A beeper sounds to let you know it is time for user interaction 2.

If a flat-bottom cell-culture plate is used, the next protocol message box appears.

Protocol Message
Remove the supernatant manually, and transfer into the the Square-Well Block with the pre-dispensed Buffer RLT. Place Square-Well Block back into 96-Well Shaker front/right position.

To transfer the supernatants, hold the cell-culture plate at an angle from horizontal, and position the pipet tip at the edge of the well bottom to minimize the risk of carrying over pelleted cell nuclei.

37. Click "Continue".

If a flat-bottom cell-culture plate is used, the RNeasy 96 BioRobot 9604 protocol now continues.

A beeper sounds to let you know it is time for the next user interaction.

User interaction 2

38. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Tap the RNeasy 96 plate firmly on a stack of paper towels. Repeat several times until no further liquid is released. Place the elution microtube rack, assembled on top of the elution microtube adapter, into the vacuum manifold base.

Residual Buffer RPE from the collars and nozzles of each well will be absorbed by the paper towels. Use a stack of paper towels approximately 4 cm high.

39. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place the RNeasy 96 plate into the vacuum manifold top plate with the bevelled edges pointing to the right-hand side. Open bottle with RNase-free water.

40. **Click "Continue".**

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know it is time for the next user interaction.

User interaction 3

41. **Click the speaker icon to stop the beeper.**

The next protocol message box appears.

Protocol Message
Discard the RNeasy 96 plate, and close the elution microtubes with the caps provided in the kit. Store at –20°C. Press "Continue".

42. **Click "Continue".**

The Report File appears, in which comments can be inserted.

43. **Click "Continue".**

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know the main protocol has finished.

44. **Click the speaker icon to stop the beeper.**

Wash procedure

45. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Connect the buffer bottle connectors to the adapters on the wash bottle filled with distilled water. Press "Continue" to start the wash procedure.

If you wish to perform another run immediately after the current run has finished, this wash step can be omitted; enter "no", and click "Continue".

46. **Click "Continue".**

After the wash procedure is finished, a protocol message box appears.

Protocol Message
Process done! Close all buffer bottles. Release cassettes of the peristaltic pump.

RNeasy 96 BioRobot 9604 Protocol for Isolation of Cytoplasmic RNA from Animal Cells

II. Using vacuum/spin technology

This protocol is a brief description of the steps performed by the BioRobot 9604 for preparation of cytoplasmic RNA using the RNeasy 96 BioRobot 9604 Kit and vacuum/spin technology. The protocol involves centrifugation steps at the end of the procedure and must be used in connection with the QIAGEN Centrifuge 4-15C or Centrifuge 4K15C. Refer to the BioRobot 9604 User manual and the context-sensitive help in QIAsoft 3.0 for further information.

1. **Prepare all reagents required for the protocol.**
   Check that Buffer RLT, Buffer RLN, and Buffer RPE have been prepared according to the instructions on page 27. If using the optional on-membrane DNase digestion, see page 12 for details to prepare the DNase I incubation mix.
2. **Make sure that the High-Speed Pipetting System is switched on.**
   Note: Always switch on the High-Speed Pipetting System before switching on the BioRobot 9604.
3. **Make sure that the BioRobot 9604 is switched on (switch is on the rear panel, lower right, looking from the front).**
   Note: The BioRobot 9604 should be left on at all times.
4. **Switch on the computer and monitor.**
5. **Launch QIAsoft 3.0 if necessary.**
6. **Select "RNeasy 96 VS Cytoplasmic RNA" from the protocol field.**
7. **Click "RUN" on the toolbar.**
   The "Run Protocol: Slot Configuration" dialog box appears.
8. **Select the type of 96-well cell-culture plate used. Click "OK".**
   The "Run Protocol: No. of Samples" dialog box appears.
9. **Enter the number of samples to be processed (4 to 96) or the well positions of the first and last samples.**
   Note: Only multiples of 4 samples can be selected.
10. **Click "OK".**
11. **The first Enter Variable Value box appears.**

Enter Variable Value
Enter a text into this variable…
report file name report =

Enter a name as required with the suffix ".txt".

12. **Click "Continue".**
    The second Enter Variable Value box appears.

Enter Variable Value
Enter a text into this variable…
Operator name Operator =

Enter a name or identification code for the operator as required.

13. Click "Continue".

The third Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
Plate Identification barcode	PlateID =

Enter the identification number for the 96-well plate as required. The identification number can be entered manually or using the Labware Identification System hand scanner.

14. Click "Continue".

A protocol message box appears showing the identification number entered for the plate in the Enter Variable Value box.

Protocol Message
Plate Identification Number:

15. Click "Continue" to confirm the identification number and continue the protocol.

The fourth Enter Variable Value box appears.

Enter Variable Value	
Enter a text into this variable…	
DNase Treatment?	dnase = no

If the optional on-membrane DNase digestion is desired, enter "yes" for this variable. The default value is "no". Using the cytoplasmic protocol, DNase digestion is generally not required: most of the DNA is removed by pelleting the nuclei, and the RNeasy 96 silica-membrane technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. Using the cytoplasmic protocol with the optional DNase digestion results in undetectable levels of DNA, even by sensitive TaqMan analysis. See page 12 for details to prepare the DNase I incubation mix.

16. Click "Continue".

The fifth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Number of elution cycles ecycl = 2.00
(Value range: 1.00 … 2.00)

Enter a value of either 1.00 or 2.00 for the number of elution cycles desired. The default value is 2.00.

Note: We recommend initially testing the RNeasy 96 procedure both with one elution cycle and with two elution cycles in order to determine which gives optimal results for a particular sample type.

17. Click "Continue".

The sixth Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 1 Evol1 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 70.00 µl.

Or, if only one elution cycle was selected:

Enter Variable Value
Enter a number for this variable…
Elution volume Evol = 100.00 µl
(Value range: 80.00 … 150.00 µl)

Enter the volume in microliters of RNase-free water to be used for the first elution cycle. The default value is 100.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

18. Click "Continue."

If two elution cycles were selected, the seventh Enter Variable Value box appears.

Enter Variable Value
Enter a number for this variable…
Elution volume 2 Evol2 = 70.00 µl
(Value range: 55.00 … 75.00 µl)

Enter the volume in microliters of RNase-free water to be used for the second elution cycle. The default value is 70.00 µl.

The elution volume will be slightly less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

19. Click "Continue".

20. Wait for the BioRobot 9604 to initialize and calibrate itself.

21. A series of protocol message boxes appear detailing the preparation steps required before the BioRobot 9604 protocol can continue. Follow the instructions in each protocol message box before proceeding.

A protocol message box appears.

Protocol Message
Fill system liquid container with distilled water.
Empty waste container.
Empty vacuum trap.
Empty Tip Waste Bag.

22. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place buffer bottles in the external holder: make sure that buffer bottle RLT is connected to the blue, RW1 to the green, 100% Ethanol to the yellow, and Buffer RPE to the red adapter.

23. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place the bottle containing RNase-free water into buffer slot B6.

Use only the RNase-free water supplied in the RNeasy 96 BioRobot 9604 Kit. Do not aliquot the RNase-free water: use it on the BioRobot 9604 in the bottle supplied.

24. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place a Square-Well Block each into MP-Slot 5 and into the 96-Well Shaker front/right position.

25. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Fill a buffer trough with 20 ml of Buffer RLN, and place it into MP-Slot 2A.

26. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place an elution microtube rack, assembled on top of the elution microtube adapter, into MP-Slot 4.

27. **Click "Continue".**

If the on-membrane DNase digestion is included, the next protocol message box appears.

Protocol Message
Place 4 x 2-ml microcentrifuge tubes, each containing 2 ml of Buffer RDD, RNase-free DNase, and water, into positions A1–A4 in the thermoblock.

28. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the bevelled edges pointing to the right-hand side.

Or, when preparing less then 96 samples:

Protocol Message
Place the RNeasy 96 plate into the top plate of the vacuum manifold with the bevelled edges pointing to the right-hand side. Tape unused wells throughout the procedure with adhesive tape.

Do not use the AirPore Tape Sheets supplied in the RNeasy 96 BioRobot 9604 Kit. Use either adhesive tape or Tape Pads (cat. no. 19570) from QIAGEN.

29. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place sufficient tips into the tip racks. A run of 96 samples requires 3 x 96 tips. Make sure the tip racks are oriented and seated correctly on the holders.

30. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Make sure that cassettes of the peristaltic pump are fitted.

31. **Click "Continue".**

The next protocol message box appears.

Protocol Message
Place cell-culture plate containing samples onto the MP-Slot in the 96-Well Shaker back/right position. Press "Continue" to start the protocol.

32. **Click "Continue".**

The RNeasy 96 BioRobot 9604 protocol now starts.

A beeper sounds to let you know it is time for the first user interaction.

User interaction 1

33. **Click the speaker icon to stop the beeper.**

The next protocol message box appears.

Protocol Message
Incubate cell-culture plate for 5 minutes at room temperature or on ice. Press "Continue".

For most preparations, cell lysis can be performed at room temperature. In rare cases, when analyzing transcripts from RNase-rich cells or when analyzing exceptionally labile transcripts, it may be advantageous to perform cell lysis on ice.

34. **Click "Continue".**

    The RNeasy 96 BioRobot 9604 protocol continues.

    A beeper sounds to let you know when 5 minutes has elapsed.

35. **Click the speaker icon to stop the beeper.**

    The next protocol message box appears.

Protocol Message
Centrifuge cell-culture plate for 5 minutes at 500 x g. Place cell-culture plate back on 96-Well Shaker back/right position.

    Note: For most preparations, centrifugation can be performed at room temperature. In some cases, when analyzing transcripts from RNase-rich cells or when analyzing labile transcripts, it may be advantageous to perform the centrifugation at 4°C.

36. **Click "Continue".**

    If a round-bottom cell-culture plate is used, the RNeasy 96 BioRobot 9604 protocol now continues. A beeper sounds to let you know it is time for user interaction 2.

    If a flat-bottom cell-culture plate is used, the next protocol message box appears.

Protocol Message
Remove the supernatant manually, and transfer into the Square-Well Block with the pre-dispensed Buffer RLT. Place Square-Well Block back into 96-Well Shaker front/right position.

    To transfer the supernatants, hold the cell-culture plate at an angle from horizontal, and position the pipet tip at the edge of the well bottom to minimize the risk of carrying over pelleted cell nuclei.

37. **Click "Continue".**

    If a flat-bottom cell-culture plate is used, the RNeasy 96 BioRobot 9604 protocol now continues.

    A beeper sounds to let you know it is time for the next user interaction.

User interaction 2

38. **Click the speaker icon to stop the beeper.**

    The next protocol message box appears.

Protocol Message
Seal RNeasy 96 plate with an AirPore tape. Transfer RNeasy 96 plate on top of a Square-Well Block into the centrifuge. Centrifuge for 10 min at 6000 rpm.

    Use of AirPore tape reduces cross-contamination and allows evaporation of residual ethanol derived from Buffer RPE during centrifugation. To develop the heat required to evaporate the ethanol, centrifuge at room temperature. Residual ethanol will inhibit RT-PCR and must be removed by evaporation prior to elution.

    Note: When using the Centrifuge 4K15C set the temperature to 40°C.

39. **Remove the assembly from the centrifuge bucket. Discard the waste in the Square-Well Block.**

40. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Place the 96-well centrifuge adapter onto the elution microtube rack, which is already on top of the elution microtube adapter in MP-Slot 4.
> Place the RNeasy 96 plate onto the elution microtube rack, remove the AirPore tape, and place the assembly, including the centrifuge adapter and the elution microtube adapter, into MP-Slot 1.

41. Click "Continue".

The next protocol message box appears.

> **Protocol Message**
> Open bottle with RNase-free water.
>
> Press "Continue" to start elution.

42. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know it is time for the next user interaction.

User interaction 3

43. Click the speaker icon to stop the beeper.

The next protocol message box appears.

> **Protocol Message**
> Seal the RNeasy 96 plate with AirPore tape. Lift the plate, centrifuge adapter, and elution microtube rack off the elution microtube adapter, and transfer the assembly into the centrifuge without the elution microtube adapter. Centrifuge for 2 min at 6000 rpm.
>
> Press "Continue".

44. Click "Continue".

If only one elution cycle was selected, user interaction 4 is not required. Proceed directly to user interaction 5.

If two elution cycles were selected, the next protocol message box appears.

> **Protocol Message**
> Remove the AirPore tape, and place the assembly back onto the elution microtube adapter in MP-Slot 1.
>
> Press "Continue"

45. Click "Continue".

If two elution cycles were selected, the RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know it is time for the next user interaction.

User interaction 4 (only if two elution cycles were selected)

46. Click the speaker icon to stop the beeper.

The next protocol message box appears.

Protocol Message
Seal the RNeasy 96 plate with AirPore tape. Lift the plate, centrifuge adapter, and elution microtube rack off the elution microtube adapter, and transfer the assembly into the centrifuge without the elution microtube adapter. Centrifuge for 2 min at 6000 rpm. Press "Continue".

47. Click "Continue". Proceed directly to user interaction 5.

User interaction 5

48. The next protocol message box appears.

Protocol Message
Discard the RNeasy 96 plate, and close the elution microtubes with the caps provided in the kit. Store at –20°C. Press "Continue".

49. Click "Continue".

The Report File appears, in which comments can be inserted.

50. Click "Continue".

The RNeasy 96 BioRobot 9604 protocol continues.

A beeper sounds to let you know the main protocol has finished.

51. Click the speaker icon to stop the beeper.

Wash procedure

52. Click "Continue".

The next protocol message box appears.

Protocol Message
Connect the buffer bottle connectors to the adapters on the wash bottle filled with distilled water. Press "Continue" to start the wash procedure.

If you wish to perform another run immediately after the current run has finished, this wash step can be omitted; enter "no", and click "Continue".

53. Click "Continue".

After the wash procedure is finished, a protocol message box appears.

Protocol Message
Process done! Close all buffer bottles. Release cassettes of the peristaltic pump.

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 protocols in this handbook or molecular biology applications (see inside front cover for contact information).

	Comments and suggestions
Clogged plate wells	
Too much starting material	Reduce amount of starting material. It is essential to use the correct amount of starting material (see "Sample size" in the *RNeasy 96 Handbook*).
Little or no RNA eluted	
a) Too much starting material	Overloading significantly reduces yield. Reduce the amount of starting material (see "Sample size" in the *RNeasy 96 Handbook*).
b) Buffer temperatures too low	All buffers must be at room temperature throughout the procedure.
c) Residual liquid in cell-culture plate after removal of medium	Make sure the correct type of 96-well cell-culture plate (flat-bottom or round-bottom) is entered in the "Run Protocol: Slot Configuration" dialog box. Use of plates from some suppliers may result in incomplete removal of cell-culture medium. See appendix, page 46, for recommended suppliers and ordering information.
d) RNase contamination	Make sure that the DNase used in the optional on-membrane DNase digestion is supplied RNase-free. See appendix, page 46, for recommended suppliers and ordering information. For elution, use only the elution microtube racks included in the RNeasy 96 BioRobot 9604 Kit. Other collection devices, such as deep-well plates, are not compatible and may not be RNase-free.
Low A_{260}/A_{280} value	Use 10 mM Tris·Cl, not RNase-free water, to dilute the sample before measuring purity (see Appendix B in the *RNeasy 96 Handbook*).

	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 and "Handling and storage of starting material" in the *RNeasy 96 Handbook* and the "Important notes before starting" for each protocol.
b) RNase contamination	Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNase during the procedure or later handling. See Appendix A in the *RNeasy 96 Handbook* for general remarks on handling RNA.
c) Cytoplasmic protocol: cell lysis not performed on ice	For most preparations, cell lysis can be performed at room temperature. In rare cases, when analyzing transcripts from RNase-rich cells or when analyzing exceptionally labile transcripts, it may be advantageous to perform cell lysis in Buffer RLN on ice.
DNA contamination in downstream experiments	
a) Optimal procedure not used	The cytoplasmic RNA protocol is recommended for applications where the absence of DNA contamination is critical since the intact nuclei are removed at the start of the procedure. Follow the protocol for isolation of cytoplasmic RNA (see pages 8–9 and cytoplasmic protocol).
b) No DNase treatment	Follow the optional on-membrane DNase digest step 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 the RNeasy or RNeasy 96 RNA cleanup protocol (see the *RNeasy Handbook* or the *RNeasy 96 Handbook* for details).
c) Improper setup of DNase solutions	Make sure that the tubes of DNase I incubation mix contain a full 2.0 ml of DNase I mix. Use 2 ml screw-top tubes from Sarstedt. Use of other tubes may require adjusting the QIAsoft programming on the BioRobot 9604. Other tubes also may not fit in the BioRobot 9604 thermoblock. See appendix, page 46, for ordering information.

	Comments and suggestions
RNA does not perform well in downstream experiments	
a) Salt carryover during elution	Ensure that Buffer RPE is at 20 to 30°C. If using vacuum technology, be sure to strike the bottom side of the RNeasy 96 plate repeatedly on a stack of paper towels until no further liquid is released (see protocols). Follow the protocol using vacuum/spin technology (see page 9).
b) Ethanol carryover	After the Buffer RPE wash, be sure to dry the plate-well membranes by centrifuging the plate at 6000 rpm (~5600 x *g*) for 10 min at room temperature (vacuum/spin technology). If using vacuum technology, be sure to strike the bottom side of the RNeasy 96 plate repeatedly on a stack of paper towels until no further liquid is released (see protocols).

Appendix: Equipment and Reagent Suppliers*

Round-bottom cell-culture plates can be purchased from:

- Greiner (cat. no. 650180)
  Note: Use of other round-bottom cell-culture plates may require adjusting the QIAsoft programming on the BioRobot 9604.

Flat-bottom cell-culture plates can be purchased from:

- Costar (cat. no. 3599)
  Note: Use of other flat-bottom cell-culture plates may require adjusting the QIAsoft programming on the BioRobot 9604.

DNase I, RNase-free, can be purchased from:

- QIAGEN (as part of the RNase-Free DNase Set)
- Worthington Biochemical Corp.
- Amersham Biosciences
- Roche Molecular Biochemicals
- Stratagene

2 ml screw-cap tubes for use with the optional DNase treatment, can be purchased from:

- Sarstedt (cat. no. 72.6940.06)
  Note: Use of other tubes may require adjusting the QIAsoft programming on the BioRobot 9604. Other tubes also may not fit in the BioRobot 9604 thermoblock.

RNase inhibitors can be purchased from:

- Promega
- Applied Biosystems
- Pharmacia
- Stratagene

Matrix Impact or Multi-8 Electrapette can be purchased from:

- Matrix Technologies Corporation (**www.matrixtechcorp.com**)

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

Ordering Information

Product	Contents	Cat. No.
RNeasy 96 BioRobot 9604 Kit		
RNeasy 96 BioRobot 9604 Kit (12)	For 12 x 96 total RNA preps: 12 RNeasy 96 Plates, Elution Microtubes (1.2 ml), Caps, RNase-free Reagents and Buffers	967142
BioRobot 9604*		
BioRobot 9604	System includes: robotic workstation with 4 dilutor drives; microprocessor-controlled vacuum pump; vacuum manifold; High-speed Pipetting System; Tip-Change System; QIAsoft 3.0 Operating System, Basic Edition; computer, cables; installation and training; 1-year warranty on parts and labor	900300
96-Well Shaker System†	Microprocessor-controlled shaker system for cell lysis, resuspension, and mixing	900560
Labware Identification System‡	Hand-held bar-code reader for labware identification	900542
Accessories		
Disposable Filter-Tips, 1100 µl (960)	Conducting disposable filter-tips, pack of 960	990252
Disposable Filter-Tips, 1100 µl (10 x 960)	Conducting disposable filter-tips, 10 packs of 960 each	990255
Disposable Troughs, 20 ml (10)	Troughs holding up to 20 ml of liquid for use with multiple-probe systems, pack of 10	Inquire
Centrifuge 4-15C§	Universal laboratory centrifuge with brushless motor	Inquire
Centrifuge 4K15C§	Universal refrigerated laboratory centrifuge with brushless motor	Inquire
Plate Rotor 2 x 96¶	Rotor for 2 QIAGEN 96 plates, for use with QIAGEN Centrifuges	81031
Centrifuge Adapter (96-well-plate)	Adapter for ensuring correct orientation during centrifugation of 96-well plates in the 96-Well-Plate Centrifugation System	Inquire
Elution Microtube Adapter	Adapter for using Elution Microtube Racks on the QIAvac 96 and the BioRobot vacuum manifold	Inquire
Buffer RLT	220 ml RNeasy Lysis Buffer for 6 RNeasy 96 plates	79216
Square-Well Blocks (24)	96-well blocks with 2.2 ml wells, 24 blocks per case	19573
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Elution Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	Inquire
Elution Microtubes (loose)	Nonsterile polypropylene tubes (1.2 ml), 960 in strips of 8, loose in bag	Inquire
Caps for Elution Microtubes	Nonsterile polypropylene caps for elution microtubes (1.2 ml), 960 in strips of 8	Inquire

* *QIAGEN Robotic Systems are not available in all countries; please inquire.*

† *Standard component of the BioRobot 9604, configuration C*

‡ *Optional component of the BioRobot 9604, configuration C*

§ *Centrifuges 4-15C and 4K15C are not available in all countries; please inquire.*

¶ *The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-15C and 4K15C from QIAGEN, and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.*

Product	Contents	Cat. No.
Related products		
RNeasy 96 Kit — for high-throughput RNA isolation from cells		
RNeasy 96 Kit* (4)	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Elution Microtubes (1.2 ml), Caps, RNase-free Reagents and Buffers	74181
RNeasy 96 Kit* (12)	For 12 x 96 total RNA preps: 12 RNeasy 96 Plates, Elution Microtubes (1.2 ml), Caps, RNase-free Reagents and Buffers	74182
RNeasy 96 BioRobot 8000 Kit — for high-throughput, walk-away RNA isolation on the BioRobot 8000		
RNeasy 96 BioRobot 8000 Kit (12)	For 12 x 96 total and cytoplasmic RNA preps on the BioRobot 8000: 12 RNeasy 96 Plates, Elution Microtubes (1.2 ml), Caps, Square-Well Blocks, RNase-free Reagents and Buffers	Inquire
BioRobot 8000† — for high-throughput, walk-away nucleic acid purification		
BioRobot 8000	System includes: robotic workstation comprised of 8 dilutor units and selected system components; variable spacing system; QIAsoft 4.1 Operating System; computer; installation and training; 1 year warranty on parts and labor	900500
QIAamp 96 Virus BioRobot Kit — for automated high-throughput purification of viral RNA and DNA from cell-free body fluids		
QIAamp 96 Virus BioRobot Kit (12)	For 12 x 96 nucleic acid preps: 12 QIAamp 96 Plates, RNase-free Buffers, QIAGEN Protease, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Collection Microtubes (1.2 ml), Carrier RNA, Caps	965642
DNeasy® 96 Tissue Kit — for high-throughput DNA isolation from animal tissues and cells		
DNeasy 96 Tissue Kit (4)*	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Proteinase K, Buffers, Square-Well Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 ml), Caps, 96-well Plate Registers	69581
QIAamp® 96 DNA Blood Kit† — for high-throughput DNA isolation from blood and body fluids		
QIAamp 96 DNA Blood Kit (4)	For 4 x 96 DNA preps: 4 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, Collection Vessels	51161

** Larger kit sizes available; please inquire. Requires use of the QIAGEN 96-Well-Plate Centrifugation System.*

† QIAGEN Robotic Systems are not available in all countries; please inquire.

Product	Contents	Cat. No.
RNeasy Kits — for total RNA isolation from animal cells or tissues, yeast, or bacteria		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Midi Kit (10)*	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75162
RNeasy Plant Kit — for total RNA isolation from plants and fungi		
RNeasy Plant Mini Kit (20)*	20 RNeasy Mini Spin Columns, 20 QIAshredder™ Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74903
QIAamp RNA Blood Mini Kit — for total cellular RNA isolation from whole human blood		
QIAamp RNA Blood Mini Kit (50)	For 50 RNA preps: 50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304
RNase-Free DNase Set — for DNase digestion during RNA purification		
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer, and RNase-free water for 50 RNA minipreps	79254
Omniscript™ RT Kit — for reverse transcription using ≥50 ng RNA		
Omniscript RT Kit (10)*	For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,† RNase-free water	205110
Sensiscript™ RT Kit — for reverse transcription using <50 ng RNA		
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,† RNase-free water	205211
QIAGEN OneStep RT-PCR Kit — for easy and sensitive one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit (25)*	For 25 reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,‡ dNTP Mix,§ 5x Q-Solution, RNase-free water	210210
***Taq* DNA Polymerase — for standard and specialized PCR applications**		
Taq DNA Polymerase (250 U)	250 units *Taq* DNA Polymerase, 10x PCR Buffer,¶ 5x Q-Solution, 25 mM $MgCl_2$	201203
HotStarTaq™ DNA Polymerase — for highly specific hot-start PCR		
HotStarTaq DNA Polymerase (250 U)	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,¶ 5x Q-Solution, 25 mM $MgCl_2$	203203

* *Larger kit sizes available; please inquire.*

† *Contains 5 mM of each dNTP*

‡ *Contains 12.5 mM $MgCl_2$*

§ *Contains 10 mM of each dNTP*

¶ *Contains 15 mM $MgCl_2$*

Product	Contents	Cat. No.
QuantiTect™ SYBR® Green PCR and RT-PCR Kits — for quantitative, real-time PCR and RT-PCR using SYBR Green		
QuantiTect SYBR Green PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix;* 2 x 2.0 ml RNase-free water	204143
QuantiTect SYBR Green RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green RT-PCR Master Mix;* 1 x 100 µl QuantiTect RT Mix; 2 x 2.0 ml RNase-free water	204243
QuantiTect Probe PCR and RT-PCR Kits — for quantitative, real-time PCR and RT-PCR using sequence-specific probes		
Quanti Tect Probe PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix;† 2 x 2.0 ml RNase-free water	204343
QuantiTect Probe RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe RT-PCR Master Mix;† 1 x 100 µl QuantiTect RT Mix; 2 x 2.0 ml RNase-free water	204443
QIAGEN Operon Oligonucleotide Synthesis Service — high-quality oligos, modified oligos, and longmers		
Oligonucleotide Synthesis Service	Custom-made oligonucleotides and a wide range of modified oligos, including Molecular Beacons, dual-labeled probes, FRET probes, and many more	Inquire

* *Contains 5 mM MgCl2*

† *Contains 8 mM MgCl2*

Trademarks

Patented or patent-pending technology and/or registered or registration-pending trademark of QIAGEN: QIAGEN®, QIAamp®, QIAshredder™, QIAsoft™, QIAvac, AirPore™, BioRobot®, DNeasy®, HotStarTaq™, Omniscript™, Operon®, QuantiTect™, RNeasy®, Sensiscript™.

Electrapette and Impact are registered trademarks of MATRIX Technologies Corporation, USA. Nonidet is a registered trademark of Shell Chemicals.

SYBR is a registered trademark of Molecular Probes, Inc.

TaqMan is a registered trademark of Roche Molecular Systems, Inc.

The PCR process is covered by US Patents 4,683,195 and 4,683,202 and foreign equivalents issued to Hoffmann-La Roche AG.

QIAGEN sample preparation products may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

Purchase of QIAGEN products for PCR is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

LC Red 640 hybridization probes are licensed from Hoffman La Roche Ltd.

Molecular Beacons are licensed under patents owned by The Public Health Research Institute of the City of New York, Inc. and come with licensed rights for use only in the purchaser's research and development activities.

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

QIAGEN Companies

Please see the inside front cover for contact information for your local QIAGEN office.

QIAGEN Distributors

Argentina
Tecnolab S.A.
Charlone 144 - C1427BXD
Capital Federal
Tel: (011) 4555 0010
Fax: (011) 4553 3331
E-mail: info@tecnolab.com.ar
Web site: www.tecnolab.com.ar

Austria/Slovenia
Merck Eurolab GmbH
Zimbagasse 5
1147 Wien
Austria
Tel: (01) 576 00 0
Fax: (01) 576 00 350
E-mail: merck-wien@merckeurolab.at
Web site: www.merckeurolab.at

Belgium/Luxemburg
Westburg b.v.
P.O. Box 214
3830 AE Leusden
The Netherlands
Tel: 0800-1-9815
Fax: (31) 33-4951222
E-mail: info@westburg.nl
Web site: www.westburg.nl

Brazil
Uniscience do Brasil
Av. Cândido Portinari, 933/937
05114-001 São Paulo - SP
Brazil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

China
Gene Company Limited
Unit A, 8/F., Shell Industrial Building
12 Lee Chung Street
Chai Wan, Hong Kong, P.R.C.
Tel: (852)2896-6283
Fax: (852)2515-9371
E-mail:
Hong Kong: info@genehk.com
Beijing: gene@public2.bta.net.cn
Shanghai: gene@public.sta.net.cn
Chengdu: gene@public.cd.sc.cn
Guangzhou:
gzyitao@public.guangzhou.gd.cn

Cyprus
Scientronics Ltd
34, Zenonos Sozou Str.
1075 Lefkosia
Tel: 02-765 416
Fax: 02-764 614
E-mail: sarpetsa@spidernet.com.cy

Czech Republic
BIO-CONSULT spol. s.r.o.
Božejovická 145
142 01 Praha-Libuš
Tel/Fax: (420) 2 417 29 792
E-mail: bio-cons@login.cz
Web site: www.bio-consult.cz

Denmark
Merck Eurolab A/S
Roskildevej 16
2620 Albertslund
Tel: 43 86 87 88
Fax: 43 86 88 89
E-mail: info@merckeurolab.dk
Web site: www.merckeurolab.dk

Egypt
Clinilab
P.O. Box 12 El-Manial
4, 160 St., El-Etehad Square
Riham Tower, El-Maadi
Cairo
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

Finland
Merck Eurolab Oy
Niittyrinne 7
02270 Espoo
Tel: (09)-804 551
Fax: (09)-804 55200
E-mail: info@merckeurolab.fi
Web site: www.merckeurolab.fi

Greece
BioAnalytica S.A.
11, Laskareos Str.
11471 Athens
Tel: (01)-640 03 18
Fax: (01)-646 27 48
E-mail: bioanalyt@hol.gr

India
Genetix
C-88, Kirti Nagar
Lower Ground Floor
New Delhi-110 015
Tel: (011)-542 1714
or (011)-515 9346
Fax: (011)-546 7637
E-mail: genetix@nda.vsnl.net.in

Israel
Westburg (Israel) Ltd.
1, Habursekai St. Kiriat Ha'asakim
Beer Sheva 84899
Tel: 08-6650813/4
or 1-800 20 22 20 (toll free)
Fax: 08-6650934
E-mail: info@westburg.co.il
Web site: www.westburg.co.il

Korea
LRS Laboratories, Inc.
SongBuk P.O. Box 61
Seoul, 136-600
Tel: (02) 924-86 97
Fax: (02) 924-86 96
E-mail: webmaster@lrslab.co.kr
Web site: www.lrslab.co.kr

Malaysia
RESEARCH BIOLABS SDN. BHD.
11-A, Jalan BK 5A/2
Bandar Kinrara
47100 Puchong, Selangor Darul Ehsan
Tel: (603)-8070 3101
Fax: (603)-8070 5101
E-mail: biolabs@tm.net.my
Web site: www.researchbiolabs.com

Mexico
Quimica Valaner S.A. de C.V.
Jalapa 77, Col Roma
Mexico D.F. 06700
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: qvalaner@infosel.net.mx

The Netherlands
Westburg b.v.
P.O. Box 214
3830 AE Leusden
Tel: (033)-4950094
Fax: (033)-4951222
E-mail: info@westburg.nl
Web site: www.westburg.nl

New Zealand
Biolab Scientific Ltd.
244 Bush Road
Albany, Auckland
Tel: (09)9806700
or 0800933966
Fax: (09)9806788
E-mail: info@biolab.co.nz
Web site: www.biolab.co.nz

Norway
Merck Eurolab AS
Postboks 45, Kalbakken
0901 Oslo
Kakkelovnskroken 1
Tel: 22 90 00 00
Fax: 22 90 00 40
E-mail: info@merckeurolab.no
Web site: www.merckeurolab.no

Poland
Syngen Biotech Sp.z.o.o.
ul.Legnicka 62 A
54-204 Wroclaw
Tel: (071) 351 41 06
or 0601 70 60 07
Fax: (071) 351 04 88
E-mail: info@syngen.com.pl
Web site: www.syngen.com.pl

Portugal
IZASA PORTUGAL, LDA
Rua do Proletariado, 1 - Quinta do Paizinho
2795-648 Carnaxide
Tel: (21) 424 7312
Fax: (21) 417 2674

Singapore
Research Biolabs Pte Ltd
211 Henderson Road #14-01
Henderson Industrial Estate
Singapore 159552
Tel: 2731066
Fax: 2734914
E-mail: biolabs@singnet.com.sg

Slovak Republic
BIO-CONSULT Slovakia spol. s.r.o.
Ružová dolina 6
SK-821 08 Bratislava 2
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@post.sk
Web site: www.bio-consult.cz

South Africa
Southern Cross Biotechnology (Pty) Ltd
P.O. Box 23681
Claremont 7735
Cape Town
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za
Web site: www.scb.co.za

Spain
IZASA, S.A.
Aragón, 90
08015 Barcelona
Tel: (93) 902.20.30.90
Fax: (93) 902.22.33.66
E-mail: suministros@izasa.es

Sweden
Merck Eurolab AB
Fagerstagatan 18A
16394 Spånga
Tel: (08) 621 34 00
Fax: (08) 760 45 20
E-mail: info@merckeurolab.se
Web site: www.merckeurolab.se

Taiwan
TAIGEN Bioscience Corporation
3F. No. 306, Section 4
Chen-Der Road
111 Taipei
Taiwan, R.O.C.
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

Thailand
Theera Trading Co. Ltd.
64 Charan Sanit Wong Road
(Charan 13) Bangkokyai
Bangkok 10600
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

QIAGEN Importers

Central & South America
(except Argentina and Brazil)
Labtrade Inc.
6157 NW 167th Street F-26
Miami, FL 33015
USA
Tel: (305) 828-3818
Fax: (305) 828-3819
E-mail: labtrade@icanect.net
Web site: www.labtrade.com

Saudi Arabia
Abdulla Fouad Co. Ltd.
Medical Supplies Division
Prince Mohammed Street
P.O. Box 257, Dammam 31411
Kingdom of Saudia Arabia
Tel: (03) 8324400
Fax: (03) 8346174
E-mail:
sadiq.omar@abdulla-fouad.com

Turkey
Medek Medikal Ürünler
ve Saglik Hizmetleri A.S.
Bagdat Cad. 449 D.9 Suadiye
81070 Istanbul
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: akialp@turk.net

All other countries

QIAGEN GmbH, Germany