# RNeasy® MinElute™ Cleanup Handbook

For RNA cleanup and concentration with small elution volumes

March 2003

QIAGEN

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# Contents

## Kit Contents

| **RNeasy® MinElute™ Cleanup Kit (50)**<br>**Catalog no.**<br>**Preps per kit** | <br>**74204**<br>**50** |
|---|---|
| RNeasy MinElute Spin Columns in collection tubes | 50 |
| Collection tubes (1.5 ml) | 50 |
| Collection tubes (2 ml) | 100 |
| Buffer RLT* | 45 ml |
| Buffer RPE† | 11 ml |
| RNase-free water | 10 ml |
| Handbook | 1 |

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

† *Buffer RPE is supplied as a concentrate. Before using for the first time add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution*

## Storage

The RNeasy MinElute Cleanup Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions.

## Product Use Limitations

The RNeasy MinElute Cleanup Kit is intended as a general-purpose device. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the RNeasy MinElute Cleanup Kit for any particular use, since the performance characteristics of this kit have not been validated for any specific organism. The RNeasy MinElute Cleanup Kit 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.

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

## Product Warranty and Satisfaction Guarantee

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

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

## Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of the RNeasy MinElute Cleanup Kit is monitored routinely on a lot-to-lot basis. All components are tested separately to ensure highest performance and reliability.

## 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 MinElute Cleanup 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.**

Buffer RLT contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach.

If liquid containing this buffer 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 MinElute Cleanup Kit.

**Buffer RLT**

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

**24-hour emergency information**

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

** 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 the container or label.*

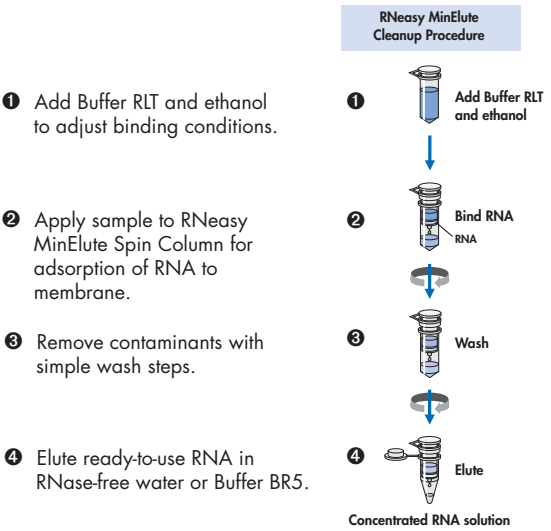
# Introduction

The RNeasy MinElute cleanup procedure represents a novel technology to purify and concentrate RNA (from 45 µg down to picogram amounts, less than 1 cell) from enzymatic reactions (e.g., in vitro transcription, DNase digestion, RNA labeling) or for desalting RNA samples (e.g., following phenol extraction and ethanol precipitation). The procedure uses minimal elution volumes, making it well-suited for concentration of RNA, for example, after RNeasy Mini or PAXgene™ Blood RNA purification protocols. QIAGEN provides a wide range of other kits for isolation of total RNA from different sample sources (see selection guide on page 8).

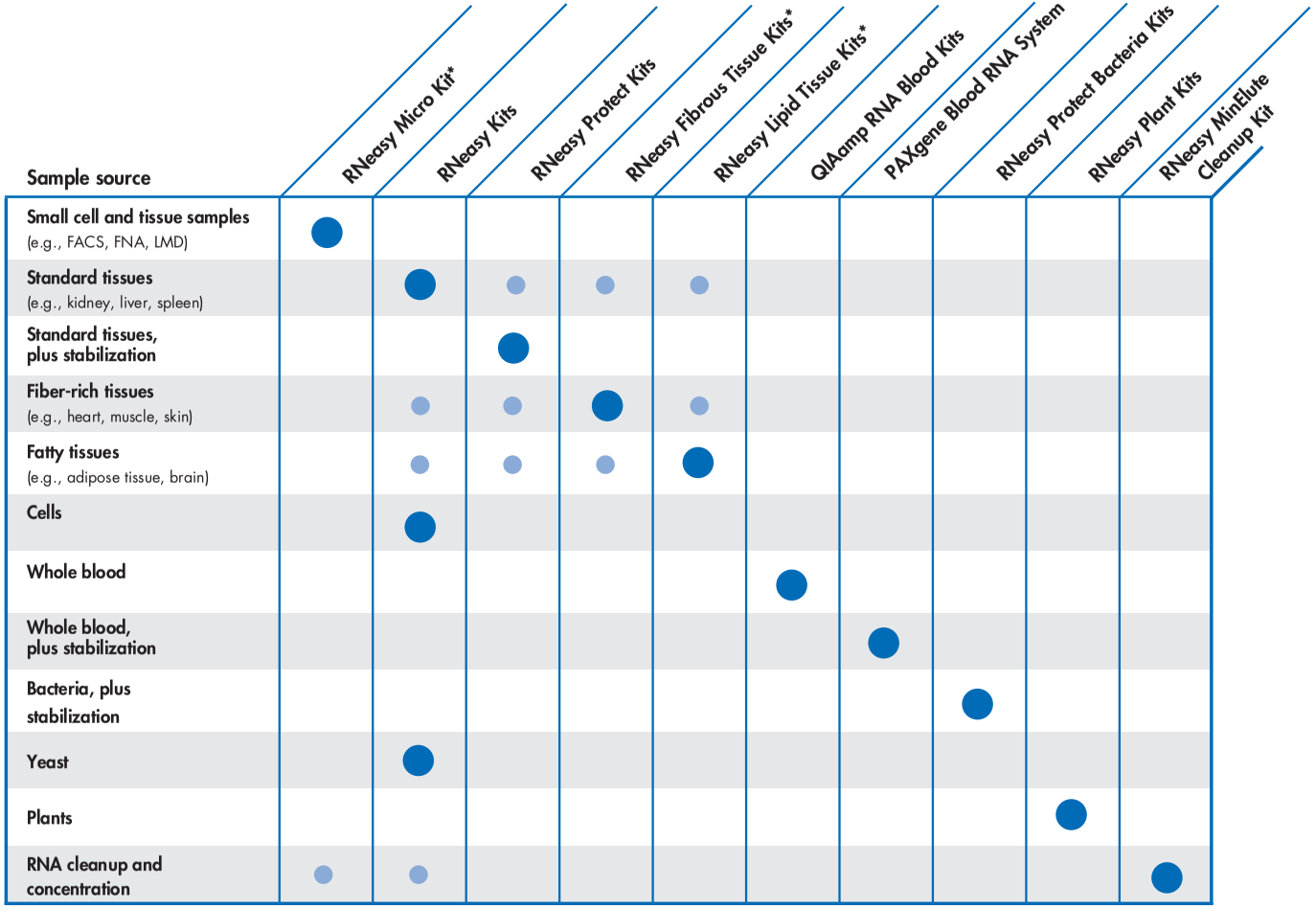
## The RNeasy MinElute principle and procedure

RNeasy MinElute technology combines the selective binding properties of a silica-gel–based membrane with the speed of microspin technology. Guanidine-isothiocyanate–containing lysis buffer and ethanol are added to the sample to create conditions that promote selective binding of RNA to the RNeasy MinElute membrane. The sample is then applied to the RNeasy MinElute Spin Column. RNA binds to the silica-gel membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water (see flowchart).

With the RNeasy MinElute procedure, 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.



## Selection guide for low-throughput total RNA isolation




● : *recommended kit;* ● : *compatible kit.* * *Stabilization of RNA in tissues is also available for these kits; please inquire.*

## Equipment and Reagents to Be Supplied by User

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

- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Ethanol (80% and 96–100%)*
- Disposable gloves
- Optional: 14.3 M β-mercaptoethanol (β-ME)† (commercially available solutions are usually 14.3 M)

**Additional equipment and reagent for RNA cleanup after isolation using the PAXgene Blood RNA Kit:**

- Buffer BR5 (included in the PAXgene Blood RNA Kit)
- Heating block or water bath

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

† *Generally β-ME is not necessary when cleaning up RNeasy or PAXgene RNA preps. Adding β-ME to Buffer RLT may be helpful when cleaning up other, crude preps of RNA or samples that contain large amounts of RNases. Add 10 µl of β-ME per 1 ml of Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. The solution is stable for 1 month after the addition of β-ME.*

# Protocol: RNA Cleanup

This protocol is designed to clean up RNA from enzymatic reactions, for desalting RNA samples, and for concentration of RNA isolated by various methods. For concentration of total cellular RNA purified using the PAXgene Blood RNA Kit, see the RNA cleanup protocol on page 13.

**Important points before starting**

- A maximum of 45 µg RNA in a maximum starting volume of 200 µl can be used. This amount corresponds to the binding capacity of the RNeasy MinElute Spin Columns. Do not overload the column. Overloading will significantly reduce yield and purity.
- If working with RNA for the first time, read Appendix A (page 18).
- Generally, DNase digestion is not required since the RNeasy MinElute silica-membrane technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan® or LightCycler® RT-PCR analysis with a low-abundance target). We recommend a DNase digest of the reaction mixture before starting the procedure (see protocol in Appendix C, page 24). The DNase is then removed during the cleanup procedure. (Note: On-column DNase digestion using the RNase-Free DNase Set requires an additional buffer and is not possible with the RNeasy MinElute Cleanup Kit. For RNA cleanup and concentration with integrated on-column DNase digestion, the RNeasy Micro Kit is recommended. See page 28 for ordering information.)
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information. Take appropriate safety measures and wear gloves when handling.
- All steps of the protocol, including centrifugation, should be performed at room temperature. During the procedure, work quickly.
- **Blue** (marked with a ▲) denotes amounts for starting volumes ≤100 µl; **red** (marked with a ●) denotes amounts for starting volumes of 100–200 µl.

**Things to do before starting**

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.
- Optional: Adding β-ME to Buffer RLT may be helpful when cleaning up crude preps of RNA (e.g., after salting-out methods) or samples that contain large amounts of RNases. Add 10 µl of β-ME per 1 ml of Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. The solution is stable for 1 month after the addition of β-ME.

**Procedure**

1. **Adjust sample to a volume of ▲ 100 µl or ● 200 µl with RNase-free water. Add ▲ 350 µl or ● 700 µl Buffer RLT, and mix thoroughly.**

   If starting with an RNA pellet, be sure that the pellet is dissolved in the RNase-free water (supplied) before adding Buffer RLT.

   **Optional:** Add β-ME to Buffer RLT before use (see "Things to do before starting").

2. **Add ▲ 250 µl or ● 500 µl of 96–100% ethanol to the diluted RNA, and mix thoroughly by pipetting. Do not centrifuge. Continue immediately with step 3.**

3. **Apply 700 µl of the sample to an RNeasy MinElute Spin Column in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

   **For ● samples >700 µl, apply the remaining sample (up to 700 µl) and repeat the centrifugation. Discard the flow-through and collection tube.***

4. **Transfer the spin column into a new 2 ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the spin column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through.**

   Reuse the collection tube in step 5.

   **Note:** Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

5. **Add 500 µl of 80% ethanol to the RNeasy MinElute Spin Column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the silica-gel membrane. Discard the flow-through and collection tube.**

   **Note:** Following the centrifugation, remove the RNeasy MinElute Spin Column from the collection tube carefully so the column does not come into contact with the flow-through as this will result in carryover of ethanol.

** Flow-through contains Buffer RLT and is therefore not compatible with bleach. See page 6 for safety information*

6. **Transfer the RNeasy MinElute Spin Column into a new 2 ml collection tube (supplied). Open the cap of the spin column, and centrifuge in a microcentrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

   To avoid damage to the caps, place the columns into the centrifuge with at least one empty position between each column. Place the caps so that they point in the opposite direction to the rotation of the rotor (i.e., if the rotor rotates in a clockwise direction, orient the caps in a counter-clockwise direction).

   It is important to dry the silica-gel membrane since residual ethanol may interfere with downstream reactions. Centrifuging with the caps open ensures that no ethanol is carried over during elution.

7. **To elute, transfer the spin column to a new 1.5 ml collection tube (supplied). Pipet 14 µl RNase-free water directly onto the center of the silica-gel membrane. Close the tube gently, and centrifuge for 1 min at maximum speed to elute.**

   Smaller volumes of RNase-free water can be used to obtain a higher total RNA concentration, but this will reduce the overall yield. The yield will be approximately 20% less when using 10 µl RNase-free water for elution. Elution with less than 10 µl may not be enough to sufficiently hydrate the silica-gel membrane and is therefore not recommended.

   The dead volume of the RNeasy MinElute Spin Column is 2 µl; elution with 14 µl of RNase-free water results in an eluate with a volume of 12 µl.

   **Note:** When performing RT-PCR with RNA isolated using this procedure we recommend using the QIAGEN OneStep RT-PCR Kit. This kit contains a specially formulated blend of Omniscript™ Reverse Transcriptase, designed for RNA amounts greater than 50 ng, and Sensiscript® Reverse Transcriptase, for very small amounts of RNA (<50 ng). See page 30 for ordering information. For quantitative real-time RT-PCR we recommend QIAGEN QuantiTect™ RT-PCR Kits.

# Protocol: RNA Cleanup after Isolation Using the PAXgene Blood RNA Kit

This protocol is designed to clean up and concentrate total cellular RNA purified using the PAXgene Blood RNA Kit.

**Important points before starting**

- A maximum of 45 µg RNA in a maximum starting volume of 200 µl can be used. This amount corresponds to the binding capacity of the RNeasy MinElute Spin Columns.
- If desired, PAXgene Blood RNA eluates can be pooled before cleanup as long as the total amount of RNA is ≤45 µg and the total volume is ≤200 µl. In order to keep the pooled volume ≤200 µl, perform the second elution step in the PAXgene RNA isolation protocol by re-applying the first eluate to the PAXgene column (instead of using another 40 µl of Buffer BR5).
- The final denaturation step of the PAXgene Blood RNA Kit protocol is not necessary before cleanup. Denaturation of the RNA is included as a step at the end of this protocol.
- Buffer BR5 (included in the PAXgene Blood RNA Kit) is required for elution in this protocol.
- If working with RNA for the first time, read Appendix A (page 18).
- Generally, DNase digestion is not required since the RNeasy MinElute silica-membrane technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan or LightCycler RT-PCR analysis with a low-abundance target). We recommend performing the PAXgene Blood RNA protocol with the optional on-column DNase digestion, as described in the PAXgene protocol. Alternatively, the PAXgene eluate can be treated with DNase before starting the RNeasy MinElute cleanup procedure (see protocol in Appendix C, page 24). The DNase is then removed during the cleanup procedure. (Note: On-column DNase digestion using the RNase-Free DNase Set requires an additional buffer and is not possible with the RNeasy MinElute Cleanup Kit. For RNA cleanup and concentration with integrated on-column DNase digestion during RNA cleanup, the RNeasy Micro Kit is recommended. See page 28 for ordering information.)
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information. Take appropriate safety measures and wear gloves when handling.

- Unless otherwise indicated, all steps of the protocol, including centrifugation, should be performed at room temperature. During the procedure, work quickly.
- **Blue** (marked with a ▲) denotes amounts for starting volumes ≤100 µl; **red** (marked with a ●) denotes amounts for starting volumes of 100–200 µl.

**Things to do before starting**

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

**Procedure**

1. **Heat a heating block or water bath to 65°C for use in step 9.**
2. **Adjust sample to a volume of ▲ 100 µl or ● 200 µl with RNase-free water. Add ▲ 350 µl or ● 700 µl Buffer RLT, and mix thoroughly.**
3. **Add ▲ 250 µl or ● 500 µl of 96–100% ethanol to the diluted RNA, and mix thoroughly by pipetting. Do not centrifuge. Continue immediately with step 4.**
4. **Apply 700 µl of the sample to an RNeasy MinElute Spin Column in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

   **For ● samples >700 µl, apply the remaining sample (up to 700 µl) and repeat the centrifugation. Discard the flow-through and collection tube.***
5. **Transfer the spin column into a new 2 ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the spin column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through.**

   Reuse the collection tube in step 6.

   **Note:** Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").
6. **Add 500 µl of 80% ethanol to the RNeasy MinElute Spin Column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the silica-gel membrane. Discard the flow-through and collection tube.**

   **Note:** Following the centrifugation, remove the RNeasy MinElute Spin Column from the collection tube carefully so the column does not come into contact with the flow-through as this will result in carryover of ethanol.

** Flow-through contains Buffer RLT and is therefore not compatible with bleach. See page 6 for safety information.*

7. **Transfer the RNeasy MinElute Spin Column into a new 2 ml collection tube (supplied). Open the cap of the spin column, and centrifuge in a microcentrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

   To avoid damage to the caps, place the columns into the centrifuge with at least one empty position between each column. Place the caps so that they point in the opposite direction to the rotation of the rotor (i.e., if the rotor rotates in a clockwise direction, orient the caps in a counter-clockwise direction).

   It is important to dry the silica-gel membrane since residual ethanol may interfere with downstream reactions. Centrifuging with the caps open ensures that no ethanol is carried over during elution.

8. **To elute, transfer the spin column to a new 1.5 ml collection tube (supplied). Pipet 14 µl Buffer BR5 (from the PAXgene Blood RNA Kit) directly onto the center of the silica-gel membrane. Close the tube gently, and centrifuge for 1 min at maximum speed to elute.**

   Smaller volumes of Buffer BR5 can be used to obtain a higher total RNA concentration, but this will reduce the overall yield. The yield will be approximately 20% less when using 10 µl Buffer BR5 for elution. Elution with less than 10 µl may not be enough to sufficiently hydrate the silica-gel membrane and is therefore not recommended.

   The dead volume of the RNeasy MinElute Spin Column is 2 µl; elution with 14 µl of Buffer BR5 results in an eluate with a volume of 12 µl.

9. **Incubate the eluate for 5 min at 65°C in a heating block or water bath. Following incubation, chill immediately on ice.**

   Denaturation of the eluate is essential for maximum efficiency in downstream applications such as RT-PCR, other amplification reactions, or cDNA synthesis. It is not necessary to denature samples more than once, and samples remain denatured after freezing and thawing.

   **Note:** When performing RT-PCR with RNA isolated using this procedure we recommend using the QIAGEN OneStep RT-PCR Kit. This kit contains a specially formulated blend of Omniscript Reverse Transcriptase, designed for RNA amounts greater than 50 ng, and Sensiscript Reverse Transcriptase, for very small amounts of RNA (<50 ng). See page 30 for ordering information. For quantitative real-time RT-PCR we recommend QIAGEN QuantiTect RT-PCR Kits.

# 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 |
|---|---|
| **Low or no recovery** | |
| a) RNase-free water/Buffer BR5 incorrectly dispensed | For elution, pipet RNase-free water/Buffer BR5 to the center of the RNeasy MinElute membrane to ensure that the buffer completely covers the membrane. |
| b) Ethanol carryover | After the 80% ethanol wash step, be sure to dry the RNeasy MinElute silica-gel membrane by centrifugation at full speed for 5 min, as described in the protocol. Following centrifugation, remove the RNeasy MinElute Spin Column from the centrifuge tube carefully so the column does not contact the flow-through as this will result in carryover of the ethanol. |
| **Clogged column** | |
| a) Too much starting RNA | In subsequent preps, reduce amounts of starting RNA. A maximum of 45 µg RNA can be used, including any carrier RNA. This amount corresponds to the binding capacity of the RNeasy MinElute Spin Columns. |
| b) Centrifugation temperature too low | Centrifugation at low temperatures (e.g., under refrigeration) can cause precipitates to form that can clog the RNeasy MinElute Spin Column. All steps of the protocol, including centrifugation, should be performed at room temperature. |
| **Low $A_{260}/A_{280}$ value** | |
| Water used for dilution | Use 10 mM Tris·Cl,* pH 7.5, not RNase-free water to dilute the sample before measuring purity (see Appendix B, page 20) |

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

**RNA degraded**

| | |
|---|---|
| RNase contamination | Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling (see Appendix A page 18).<br><br>Do not put RNA samples into a vacuum dryer or microcentrifuge that has been used in DNA preparation where RNases may have been used.<br><br>Adding β-ME to Buffer RLT may be helpful when cleaning up other, crude preps of RNA (e.g., after salting-out methods) or samples that contain large amounts of RNases (see "Things to do before starting", page 10). |

**DNA contamination in downstream experiments**

| | |
|---|---|
| No DNase treatment | DNase digest the sample before RNA cleanup (see protocol in Appendix C, page 24). Alternatively, for RNA cleanup and concentration with integrated on-column DNase digestion, the RNeasy Micro Kit is recommended. See page 28 for ordering information. For cleanup and concentration of PAXgene Blood RNA eluates, on-column digestion can be carried out during the PAXgene RNA isolation procedure, as described in the PAXgene protocol. |

**RNA does not perform well in downstream experiments**

| | |
|---|---|
| Ethanol carryover | After the 80% ethanol wash step, be sure to dry the RNeasy silica-gel membrane by centrifugation at full speed for 5 min, as described in the protocol. Following centrifugation, remove the RNeasy MinElute Spin Column from the centrifuge tube carefully so the column does not contact the flow-through as this will result in carryover of the ethanol. |

# Appendix A: General Remarks on Handling RNA

### Handling RNA

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

### General handling

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

### Disposable plasticware

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

### Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 19). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

### Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC (diethyl pyrocarbonate).* Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

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

**Electrophoresis tanks**

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

**Solutions**

Solutions (water and other solutions)* should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and $CO_2$. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris* to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carboxymethylation. Carboxymethylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

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

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

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

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

### Storage of RNA

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

### Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm ($A_{260}$) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may be difficult to determine amounts photometrically. Small amounts of RNA can be accurately quantified using an Agilent 2100 Bioanalyzer, quantitative RT-PCR, or fluorometric quantification (see "Fluorometric quantification of small amounts of RNA" on page 21).

### Spectrophotometric quantification of RNA

To ensure significance, $A_{260}$ readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml ($A_{260}$=1 ⇒ 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 22), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be done by washing cuvettes with 0.1 M NaOH, 1 mM EDTA* followed by washing with RNase-free water (see "Solutions", page 19). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

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

An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 10 µl

Dilution = 1 µl of RNA sample + 499 µl of 10 mM Tris·Cl,* pH 7.0 (1/500 dilution).

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

$A_{260}$ = 0.20

| | |
|---|---|
| Concentration of RNA sample | = 44 µg/ml x $A_{260}$ x dilution factor |
| | = 44 µg/ml x 0.20 x 500 |
| | = 4400 µg/ml |
| Total amount | = concentration x volume in milliliters |
| | = 4400 µg/ml x 0.01 ml |
| | = 44 µg of RNA |

**Fluorometric quantification of small amounts of RNA**

Small amounts of RNA may be difficult to measure photometrically. Fluorometric determination or quantitative RT-PCR are more sensitive and accurate methods for low amounts of RNA. Fluorometric measurements are carried out using an RNA-binding dye, such as RiboGreen® RNA quantitation reagent (Molecular Probes, Inc.),* Hoechst 33258,* or ethidium bromide.*

In the absence of a fluorometer, the following simplified method can be used.

**Procedure**

1. **Make a series of RNA reference solutions by diluting a stock solution containing a known amount of RNA (e.g., total RNA).**

   We recommend using a series of dilutions in RNase-free water with concentrations of 0, 2, 4, 6, 10, and 12 ng/µl (1 ng/µl = 1 µg/ml).
2. **Prepare a solution of 1.5 µg/ml ethidium bromide in 0.1 M ammonium acetate.***
3. **Mix 2 µl of each RNA reference with 8 µl of the ethidium bromide solution.**
4. **Prepare 3 different dilutions of the RNA solution to be measured. These dilutions should be chosen so that at least one lies within the range of the series of RNA references.**
5. **Mix 2 µl of each RNA dilution with 8 µl of the ethidium bromide solution.**

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

† *Ausubel, F.M. et al., eds. (1991)* Current Protocols in Molecular Biology. *New York: John Wiley & Sons, p. A.3D.3.*

6. **Pipet all mixtures (10 µl each) onto a UV transilluminator.* Keep the UV lamp turned off while pipetting.**

   To avoid contaminating the UV transilluminator surface with ethidium bromide, cover it first with disposable, transparent, plastic wrap.

7. **Turn on the UV lamp, and photograph the samples, taking care to adjust the timing and aperture settings so that the differences in signal intensity of the different RNA standards are clearly distinguishable.**

8. **Compare the signal intensities of the RNA dilutions with the series of RNA reference solutions. Determine which RNA reference(s) is nearest in signal intensity to the RNA dilution(s).**

   The original concentration of this RNA reference then corresponds to the RNA concentration in the RNA dilution. Multiply by the dilution factor to find the RNA concentration in the original, undiluted sample.

   For example, if a tenfold dilution of the RNA gives the same signal intensity as the 6 ng/µl RNA reference, then the original, undiluted RNA sample has a concentration of 10 x 6 ng/µl = 60 ng/µl. In this case, a fivefold dilution of the RNA should give the same signal intensity as the 12 ng/µl RNA reference (5 x 12 ng/µl = 60 ng/µl).

### Purity of RNA

The ratio of the readings at 260 nm and 280 nm ($A_{260}/A_{280}$) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. However, the $A_{260}/A_{280}$ ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting $A_{260}/A_{280}$ ratio can vary greatly. Lower pH results in a lower $A_{260}/A_{280}$ ratio and reduced sensitivity to protein contamination.† For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an $A_{260}/A_{280}$ ratio of 1.9–2.1‡ in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration ($A_{260}$ reading of 1 = 40 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Quantification of RNA", page 20).

** UV radiation is dangerous, especially to the eyes. Make sure that the UV source is appropriately shielded. Wear a face shield that blocks UV radiation while the transilluminator is switched on.*

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

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

### DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. To prevent any interference by DNA in RT-PCR applications, such as TaqMan and LightCycler RT-PCR analyses, we recommend working with intron-spanning primers so that genomic DNA will not be amplified. Alternatively, DNA contamination can be detected on agarose gels* following RT-PCR by performing control experiments in which no reverse transcriptase is added before the PCR step.

Before performing the RNeasy MinElute cleanup protocol, the solution containing the RNA can be treated with DNase following the protocol in Appendix C (page 24). The RNA can then be purified with the RNeasy MinElute cleanup protocol. The DNase is efficiently removed during the cleanup procedure.

Alternatively, the RNeasy Micro Kit is recommended for RNA cleanup and concentration with integrated on-column DNase digestion. See page 28 for ordering information.

### Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining or using an Agilent 2100 Bioanalyzer. The respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S RNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation before cleanup.

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

# Appendix C: DNase Digestion of RNA before RNA Cleanup

This protocol gives reaction conditions for DNase digestion using the QIAGEN RNase-Free DNase Set (see page 30 for ordering information; see the RNase-Free DNase Set product insert for product description and more information). Buffer RDD in the set is optimized for on-column DNase digestion.* The buffer is also well-suited for efficient DNase digestion in solution, as described below.

**Important points before starting**

- **Do not vortex reconstituted DNase I**. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.
- For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

**Things to do before starting**

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. **Do not vortex**.

**Procedure**

**C1. Mix the following in a microcentrifuge tube:**

| | |
|---|---|
| **≤87.5 µl** | **RNA solution (contaminated with genomic DNA)** |
| **10 µl** | **Buffer RDD** |
| **2.5 µl** | **DNase I stock solution** |

**Make the volume up to 100 µl with RNase-free water.**

The reaction volumes can be doubled if necessary (to 200 µl final volume).

**C2. Incubate on the benchtop (20–25°C) for 10 min.**

**C3. Clean up the RNA following an RNeasy MinElute cleanup protocol.**

** On-column DNase digestion using the RNase-Free DNase Set requires an additional buffer and is not possible with the RNeasy MinElute Cleanup Kit. For RNA cleanup and concentration with on-column DNase digestion, the RNeasy Micro Kit is recommended. See page 28 for ordering information.*

# Appendix D: RNA Cleanup after Lysis and Homogenization with QIAzol Lysis Reagent

QIAzol Lysis Reagent is a monophasic solution of phenol and guanidine thiocyanate, which can be used for lysis and partial purification of total RNA (see page 28 for ordering information). Cleanup using the RNeasy MinElute Cleanup Kit is recommended to remove any contaminating phenol.*

### Important points before starting

- A maximum of 45 µg RNA can be used. This amount corresponds to the binding capacity of the RNeasy MinElute Spin Columns. If the expected RNA yield is >45 µg, use an appropriate proportion of the QIAzol lysate per RNeasy MinElute Spin Column.
- This protocol is designed for QIAzol preps with a maximum starting volume of 1 ml QIAzol Lysis Reagent. This corresponds to approximately 600 µl final volume (aqueous phase) for RNA cleanup.
- If working with RNA for the first time, read Appendix A (page 18).
- Generally, DNase digestion is not required since the combination of QIAzol and RNeasy MinElute technologies efficiently removes nearly all of the DNA without DNase treatment. For further DNA removal, the RNeasy Micro Kit is recommended for RNA cleanup and concentration with integrated on-column DNase digestion. See page 28 for ordering information. (Note: On-column DNase digestion using the RNase-Free DNase Set requires an additional buffer and is not possible with the RNeasy MinElute Cleanup Kit.)
- QIAzol Lysis Reagent contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the *QIAzol Handbook* for safety information. Take appropriate safety measures and wear gloves when handling.
- Unless otherwise indicated, all steps of the protocol, including centrifugation, should be performed at room temperature. During the procedure, work quickly.

### Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

** This protocol also works well with some other reagents containing phenol and guanidine thiocyanate. Please contact QIAGEN Technical Services for more details (see inside front cover for contact information).*

**Procedure**

**D1. Carry out homogenization of the sample in QIAzol Lysis Reagent, followed by phase separation, as described in the *QIAzol Handbook* (steps 1–7 of the QIAzol protocol for lysis and homogenization).**

**D2. Transfer the upper aqueous phase to a new collection tube. Add 1 volume of 70% ethanol, and mix thoroughly by vortexing. Do not centrifuge. Continue immediately with step D3.**

**D3. Apply up to 700 µl of the sample to an RNeasy MinElute Spin Column in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

**If the sample is >700 µl, apply another aliquot of the sample (up to 700 µl) and repeat the centrifugation. Discard the flow-through.***

**D4. Transfer the spin column into a new 2 ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the spin column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through.**

Reuse the collection tube in step D5.

**Note:** Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

**D5. Add 500 µl of 80% ethanol to the RNeasy MinElute Spin Column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the silica-gel membrane. Discard the flow-through and collection tube.**

**Note:** Following the centrifugation, remove the RNeasy MinElute Spin Column from the collection tube carefully so the column does not come into contact with the flow-through as this will result in carryover of ethanol.

**D6. Transfer the RNeasy MinElute Spin Column into a new 2 ml collection tube (supplied). Open the cap of the spin column, and centrifuge in a microcentrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

To avoid damage to the caps, place the columns into the centrifuge with at least one empty position between each column. Place the caps so that they point in the opposite direction to the rotation of the rotor (i.e., if the rotor rotates in a clockwise direction, orient the caps in a counter-clockwise direction).

It is important to dry the silica-gel membrane since residual ethanol may interfere with downstream reactions. Centrifuging with the caps open ensures that no ethanol is carried over during elution.

** Flow-through contains QIAzol Lysis Reagent and is therefore not compatible with bleach. See the* QIAzol Handbook *for safety information.*

**D7. To elute, transfer the spin column to a new 1.5 ml collection tube (supplied). Pipet 14 µl RNase-free water directly onto the center of the silica-gel membrane. Close the tube gently, and centrifuge for 1 min at maximum speed to elute.**

Smaller volumes of RNase-free water can be used to obtain a higher total RNA concentration, but this will reduce the overall yield. The yield will be approximately 20% less when using 10 µl RNase-free water for elution. Elution with less than 10 µl may not be enough to sufficiently hydrate the silica-gel membrane and is therefore not recommended.

The dead volume of the RNeasy MinElute Spin Column is 2 µl; elution with 14 µl of RNase-free water results in an eluate with a volume of 12 µl.

**Trademarks and disclaimers**

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| **RNeasy MinElute Cleanup Kit — for RNA cleanup with small elution volumes** | | |
| RNeasy MinElute Cleanup Kit (50) | 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers | 74204 |
| **Related products** | | |
| **RNeasy Micro Kit — for isolation of total RNA from small amounts of tissue or small numbers of cells** | | |
| RNeasy Micro Kit (50) | 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free DNase I, Carrier RNA RNase-free Reagents and Buffers | Inquire |
| **RNeasy Fibrous Tissue Kits — for isolation of total RNA from fiber-rich tissues** | | |
| RNeasy Fibrous Tissue Mini Kit (50) | 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers | 74704 |
| RNeasy Fibrous Tissue Midi Kit (10) | 10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers | 75742 |
| **RNeasy Lipid Tissue Kits — for isolation of total RNA from fatty tissues** | | |
| RNeasy Lipid Tissue Mini Kit (50) | 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers | 74804 |
| RNeasy Lipid Tissue Midi Kit (10) | 10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers | 75842 |
| **QIAzol Lysis Reagent — for efficient lysis of fatty tissues before RNA isolation** | | |
| QIAzol Lysis Reagent (200 ml) | 200 ml QIAzol Lysis Reagent | 79306 |

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| **RNeasy Protect Kits — for stabilization and isolation of RNA from tissues** | | |
| RNeasy Protect Mini Kit (50)* | RNA*later*™ RNA Stabilization Reagent† (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers | 74124 |
| **RNeasy Protect Bacteria Kits — for stabilization and isolation of RNA from bacteria** | | |
| RNeasy Protect Bacteria Mini Kit (50)* | RNeasy Mini Kit (50) and RNAprotect™ Bacteria Reagent† (2 x 100 ml) | 74524 |
| **RNA*later* TissueProtect Tubes — for stabilization of RNA in animal tissues and storage of stabilized tissues** | | |
| RNA*later* TissueProtect Tubes (50 x 1.5 ml)* | For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA*later* RNA Stabilization Reagent each | 76154 |
| **RNeasy Kits — for isolation of RNA from a wide variety of samples** | | |
| RNeasy Mini Kit (50)* | 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers | 74104 |
| **PAXgene Blood RNA System — for integrated collection of blood samples with stabilization and purification of their RNA** | | |
| PAXgene Blood RNA Kit (50) | 50 PAXgene RNA Spin Columns, Processing Tubes, RNase-free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes | 762134 |
| PAXgene Blood RNA Tubes (100) | 100 blood collection tubes. To be used in conjunction with the PAXgene Blood RNA Kit (50) | 762115‡<br>762125§ |
| **RNeasy 96 Kit — for high-throughput manual isolation of RNA from animal cells and tissues** | | |
| RNeasy 96 Kit (4)*¶ | For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Elution Microtubes CL, Caps, RNase-free Reagents and Buffers | 74181 |

** Larger kit sizes and/or formats available; please inquire. † Also available separately; please inquire.*
*‡ US and Canada § Other countries*
*¶ Requires use of either QIAvac 96 or the QIAGEN 96-Well-Plate Centrifugation System.*

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| **RNeasy 96 BioRobot® 8000 Kit — for fully automated, high-throughput isolation of RNA from animal cells and tissues** | | |
| RNeasy 96 BioRobot 8000 Kit (12) | For 12 x 96 fully automated total RNA preps on the BioRobot Gene Expression: 12 RNeasy 96 Plates, Elution Microtubes CL, Caps, Square-Well Blocks, RNase-Free Reagents and Buffers | 967152 |
| **RNase-Free DNase Set — for DNase digestion during RNA purification*** | | |
| RNase-Free DNase Set (50) | 1500 units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps | 79254 |
| **Omniscript Reverse Transcriptase — for standard 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 (containing 5 mM each dNTP), RNase-free water | 205110 |
| **Sensiscript Reverse Transcriptase — for reverse transcription using small amounts of RNA (<50 ng)** | | |
| Sensiscript RT Kit (50)† | For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water | 205211 |
| **QIAGEN OneStep RT-PCR Kit — for fast and efficient 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 (containing 12.5 mM $MgCl_2$), dNTP Mix (containing 10 mM each dNTP), 5x Q-Solution, RNase-free water | 210210 |

** On-column DNase digestion using the RNase-Free DNase Set requires an additional buffer and is not possible with the RNeasy MinElute Cleanup Kit. For RNA cleanup and concentration with integrated on-column DNase digestion, the RNeasy Micro Kit is recommended. See page 28 for ordering information.*

*† Larger kit sizes available; please inquire.*

# QIAGEN Companies

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

# QIAGEN Distributors

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

**Austria**
VWR International GmbH
Tel: (01) 576 00 0
Fax: (01) 576 00 350
E-mail: merck-wien@merckeurolab.at
Web site: www.vwr.com

**Belgium/Luxemburg**
Westburg b.v.
Tel: 0800-1-9815
Fax: (31) 33-4951222
E-mail: info@westburg.nl
Web site: www.westburg.nl

**Brazil**
Uniscience do Brasil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

**China**
Gene Company Limited
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

**Croatia**
INEL Medicinska Tehnika d.o.o.
Tel: (01) 2984-898
Fax: (01) 6520-966
E-mail:
inel-medicinska-tehnika@zg.tel.hr

**Cyprus**
Scientronics Ltd
Tel: 02-357 22 765416
Fax: 02-357 22 764614
E-mail: a.sarpetsas@biotronics.com.cy

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

**Denmark**
VWR International A/S
Tel: 43 86 87 88
Fax: 43 86 87 90
E-mail: info@dk.vwr.com
Web site: www.vwr.com

**Egypt**
Clinilab
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

**Finland**
VWR International Oy
Tel: (09) 804 551
Fax: (09) 8045 5200
E-mail: info@fi.vwr.com
Web site: www.vwr.com

**Greece**
BioAnalytica S.A.
Tel: (210)-640 03 18
Fax: (210)-646 27 48
E-mail: bioanalyt@hol.gr

**Hungary**
Kasztel-Med Co. Ltd.
Tel: (01) 385 3887
Fax: (01) 381 0695
E-mail: info@kasztel.hu
Web site: www.kasztel.hu

**India**
Genetix
Tel: (011)-542 1714
or (011)-515 9346
Fax: (011)-546 7637
E-mail: genetix@nda.vsnl.net.in

**Israel**
Westburg (Israel) Ltd.
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.
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.
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.
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: qvalaner@infosel.net.mx

**The Netherlands**
Westburg b.v.
Tel: (033)-4950094
Fax: (033)-4951222
E-mail: info@westburg.nl
Web site: www.westburg.nl

**New Zealand**
Biolab Ltd
Tel: (09) 980 6700
or 0800 933 966
Fax: (09) 980 6788
E-mail:
biosciences@nzl.biolabgroup.com
Web site: www.biolabgroup.com/nzl

**Norway**
VWR International AS
Tel: 22 90 00 00
Fax: 22 90 00 40
E-mail: info@no.vwr.com
Web site: www.vwr.com

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

**Portugal**
IZASA PORTUGAL, LDA
Tel: (21) 424 7312
Fax: (21) 417 2674

**Singapore**
Research Biolabs Pte Ltd
Tel: 2731066
Fax: 2734914
E-mail: biolabs@singnet.com.sg

**Slovak Republic**
BIO-CONSULT Slovakia spol. s.r.o.
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@post.sk
Web site: www.bio-consult.cz

**Slovenija**
MEDILINE d.o.o.
Tel: (01) 830-80-40
Fax: (01) 830-80-70
or (01) 830-80-63
E-mail: mediline@siol.net

**South Africa**
Southern Cross Biotechnology
(Pty) Ltd
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za
Web site: www.scb.co.za

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

**Sweden**
VWR International AB
Tel: (08) 621 34 00
Fax: (08) 760 45 20
E-mail: info@se.vwr.com
Web site: www.vwr.com

**Taiwan**
TAIGEN Bioscience Corporation
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

**Thailand**
Theera Trading Co. Ltd.
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

**Turkey**
Medek Medikal Ürünler
ve Saglik Hizmetleri A. S.
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: makialp@med-ek.com

**All other countries**
QIAGEN GmbH, Germany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