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RNeasy® 96 Universal Tissue Handbook

For high-throughput RNA purification from all types of animal tissue

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy 96 Universal Tissue Kit **Catalog no.** **Number of preps**	**(4)** **74881** **4 x 96**
RNeasy 96 Plates	4
Register Cards (96-well)	4
Collection Microtubes (racked)	4 x 96
Collection Microtube Caps	100 x 98
Square-Well Blocks (2.2 ml)	6
Elution Microtubes CL	4 x 96
Caps for Strips	50 x 8
AirPore Tape Sheets	3 x 5
Buffer RW1*	2 x 220 ml
Buffer RPE†	4 x 55 ml
RNase-Free Water	2 x 50 ml
QIAzol Lysis Reagent*‡	2 x 200 ml
Quick-Start Protocol	1

* Not compatible with disinfecting reagents containing bleach. Contains a guanidine salt. See page 5 for safety information.

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

‡ Packaged separately

Storage

The RNeasy 96 Universal Tissue Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions, if not otherwise stated on the label. QIAzol Lysis Reagent can be stored at room temperature or at 2–8°C and is stable for at least 12 months under these conditions, if not otherwise stated on the label.

Intended Use

The RNeasy 96 Universal Tissue Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

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

Safety Information

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

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

QIAzol Lysis Reagent contains guanidine thiocyanate and Buffer RW1 contains between 2.5 and 10% guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If liquid containing these reagents 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.

Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of RNeasy 96 Universal Tissue Kits is monitored routinely on a lot-to-lot basis. All components are tested separately to ensure highest performance and reliability.

Introduction

The RNeasy 96 Universal Tissue Kit is well-suited for simultaneous purification of 96 or 192 RNA samples from all types of animal or human tissue. The RNeasy 96 Universal Tissue Kit facilitates efficient, high-throughput RNA sample preparation for research use (see "Intended Use", page 5).

In less than 2 hours (including homogenization and RNA purification), 96 high-purity RNA samples can be obtained. The RNeasy 96 Universal Tissue procedure replaces current time-consuming and tedious methods involving alcohol-precipitation steps or large numbers of washing steps. The purified RNA is ready to use in any downstream application including:

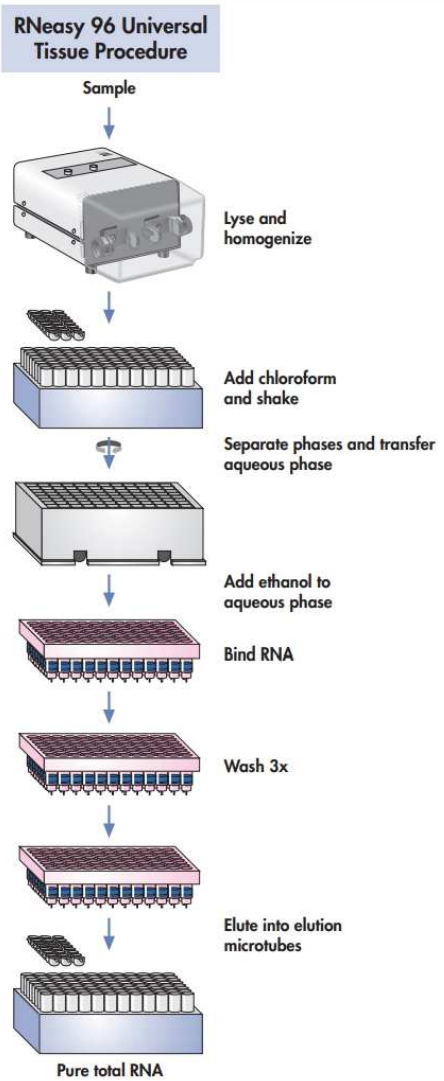
- RNA-seq
- RT-PCR
- Quantitative RT-PCR, including QIAGEN QuantiNova® technology
- Differential display
- DNA synthesis
- Northern, dot and slot blot analysis
- Primer extension
- Poly A+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

Principle and procedure

The RNeasy 96 Universal Tissue Kit represents an advanced technology for high-throughput RNA preparation. This technology integrates efficient phenol/guanidine-based lysis and silica-gel–membrane purification with the speed of vacuum and/or spin processing.

Tissue is first efficiently lysed using QIAzol Lysis Reagent and the TissueLyser II. This provides rapid and parallel disruption of cells and inactivation of RNases to ensure purification of intact RNA. After phase separation by centrifugation and recovery of the aqueous phase, ethanol is added to provide appropriate binding conditions. The sample is then applied to the wells of the RNeasy 96 Universal Tissue plate, where total RNA binds and contaminants are efficiently washed away. High-performance RNA is then eluted in a small volume of water, ready for use in any downstream application.

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



RNA purification with the RNeasy 96 Universal Tissue Kit. Protocol steps can be performed in a specially designed centrifuge system (page 13) with optional use of the QIAvac 96 vacuum manifold (page 14).

Description of protocols

The protocols in this handbook provide 2 different handling options, using a combination of vacuum and spin technology or spin technology alone. Both handling options provide high yields of high-performance RNA.

I. Vacuum/spin technology

Using vacuum/spin technology, all protocol steps from the binding step to the first Buffer RPE wash step are performed on the QIAvac 96 vacuum manifold (see page 14). Phase separation, the final Buffer RPE wash step, including membrane drying, and the elution steps are performed in the Centrifuge 4-16KS (see page 13). The Plate Rotor 2 x 96 holds 2 RNeasy 96 plates, allowing up to 192 RNA samples to be prepared in parallel. Residual traces of salt are removed by centrifugation in the final wash step. RNA purified using vacuum/spin technology can be used for any non-enzymatic or enzymatic downstream application, including quantitative RT-PCR analysis by QuantiNova technology.*

II. Spin technology

Using spin technology, all protocol steps are performed in the Centrifuge 4-16KS (see page 13). The Plate Rotor 2 x 96 holds two RNeasy 96 plates, allowing up to 192 RNA samples to be prepared in parallel. RNA purified using spin technology can be used for any non-enzymatic or enzymatic downstream application, including quantitative RT-PCR analysis by QuantiNova technology.†

* For more information about quantitative, real-time RT-PCR, request our application guide *Critical Factors for Successful Real-Time RT-PCR*.

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

Equipment and Reagents to Be Supplied by User

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

For all protocols

- Multichannel pipet with tip. For the most efficient sample processing in the RNeasy 96 Universal Tissue protocol, we recommend the use of an electric multichannel pipet with a minimum capacity of 650 µl per pipet tip. Good options are the Matrix® Impact® or the Matrix Multi-8 Electrapette® cordless electronic multichannel pipets, both of which have a unique expandable tip-spacing system allowing the user to transfer liquid directly from racks of tubes to 96-well microplates. Matrix Impact or Multi-8 Electrapette pipets can be purchased from Matrix Technologies Corporation (**www.matrixtechcorp.com**).*
- Reagent reservoirs for multichannel pipets (Note: Make sure that reagent reservoirs used for chloroform are chloroform-resistant.)
- Disposable gloves
- QIAGEN TissueLyser system, comprising the TissueLyser II, the TissueLyser Adapter Set 2 x 96, Stainless Steel Beads, 5 mm, and the TissueLyser 5 mm Bead Dispenser, 96-Well (see ordering information, page 53). Alternatively, a rotor–stator homogenizer can be used.
- Centrifuge 4-16KS (see page 13)
- Plate Rotor 2 x 96 (see page 13)
- Chloroform (without added isoamyl alcohol)

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

- 96–100% ethanol *
- 70% ethanol in water †
- Dry ice
- **Optional**: Additional square-well blocks (cat. no. 19573). Two square-well blocks are supplied with the kit for use as waste-trays. If several RNeasy 96 plates are processed per day, it may be convenient to keep extra square-well blocks on hand.

For protocol using vacuum/spin technology

- QIAvac 96 vacuum manifold (see page 14)
- Vacuum source capable of generating a vacuum pressure of –800 to –900 mbar (see page 14). Vacuum pumps (18 liter/min) can be purchased from KNF Neuberger (**www.knf.com**).†

Optional reagents

- RNase-Free DNase Set (cat. no. 79254), containing RNase-free DNase I, Buffer RDD and RNase-free water. For optional on-plate DNase digestion, the RNeasy 96 Universal Tissue procedure requires 2 RNase-Free DNase Sets per 96-well plate.

TissueLyser system

The TissueLyser system provides high-throughput processing for simultaneous, rapid and effective disruption of up to 192 biological samples, including all types of animal tissue. Processing of up to 2 x 96 samples takes as little as 2–5 minutes.

Disruption and homogenization using the TissueLyser II gives yields comparable or better than with traditional rotor–stator homogenization methods. With rotor–stator homogenization, the samples must be processed individually, and the rotor–stator homogenizer must be cleaned

* Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.
† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

after each sample to prevent cross-contamination. In contrast, the TissueLyser II provides simultaneous disruption for high-throughput processing of a variety of animal tissues.

The TissueLyser system includes a number of different accessories for ease of use with different sample sizes and throughputs. In the RNeasy 96 Universal Tissue procedure, the TissueLyser Adapter Set 2 x 96 allows simultaneous processing of up to 192 samples in collection microtubes. Stainless steel beads with a diameter of 5 mm are optimal to use for animal tissues in combination with the RNeasy 96 Universal Tissue Kit. The TissueLyser 5 mm Bead Dispenser, 96-Well, is also available to conveniently deliver 96 beads in parallel into collection microtubes. See page 53 for ordering information.

Centrifuge 4-16KS

RNeasy 96 Universal Tissue protocols utilize a streamlined centrifugation procedure that allows preparation of RNA from up to 2 x 96 samples in parallel for direct use in any downstream application. For optimal handling, QIAGEN, in cooperation with the centrifuge manufacturer Sigma Laborzentrifugen GmbH, has developed a centrifugation system consisting of the Plate Rotor 2 x 96 and the refrigerated table-top Centrifuge 4-16KS (see ordering information, page 53). A temperature of 4°C is necessary during phase separation for optimal removal of genomic DNA. A wide range of other rotors can be used with Centrifuge 4-16KS in addition to the Plate Rotor 2 x 96.

Standard table-top centrifuges and 96-well–microplate rotors are not suitable for the RNeasy 96 Universal Tissue procedure. Usually 96-well–microplate buckets are not deep enough to carry the complete RNeasy assembly without interfering with how the buckets swing out. Furthermore, high g-forces (>5500 x *g*) are required for optimal performance of the RNeasy 96 Universal Tissue procedure.

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

Warning: Do not centrifuge the Plate Rotor 2 x 96 metal holders without the RNeasy 96 plates and square-well blocks, collection microtubes or elution microtubes. If unsupported, the holders will collapse under high g-force. Therefore, remove the holders during test runs. Standard 96-well microplates may be centrifuged in the holders if a g-force of 500 x *g* is not exceeded.

QIAvac 96 vacuum manifold

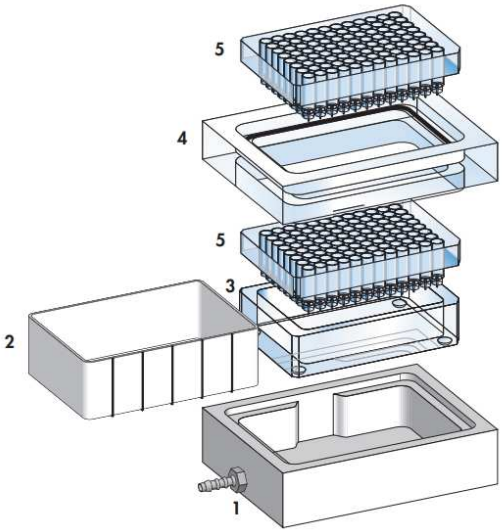


Figure 1. Components of the QIAvac 96 vacuum manifold.
1. QIAvac base, which holds a waste tray, a plate holder or a microtube rack
2. Waste tray
3. Plate holder (shown with 96-well plate) — not used in RNeasy 96 Universal Tissue protocols
4. QIAvac 96 top plate with aperture for 96-well plate
5. 96-well plate*

* Not included with QIAvac 96. Included in the RNeasy 96 Universal Tissue Kit

QIAvac 96 handling guidelines

QIAvac 96 facilitates the RNeasy 96 Universal Tissue procedure by providing a convenient, modular vacuum manifold (Figure 1) for use with the RNeasy 96 Universal Tissue Kit. The following recommendations should be followed when handling the QIAvac 96 vacuum manifold.

- QIAvac 96 operates with a house vacuum or a vacuum pump. If the house vacuum is weak or inconsistent, we recommend using a vacuum pump with a capacity of 18 liter/min. Use of insufficient vacuum pressure may reduce RNA yield and purity.
- A vacuum pressure of –800 to –900 mbar should develop when an RNeasy 96 plate sealed with tape is used on the QIAvac 96. Vacuum pressures exceeding –900 mbar should be avoided. The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 millibar or 760 mm Hg) and can be regulated and measured using a pressure gauge or vacuum regulator (see ordering information, page 53). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere. Table 1 provides pressure conversions to other units.
- Between loading steps, the vacuum must be switched off and the manifold ventilated to maintain uniform conditions for each sample. This can be done with a vacuum regulator (see ordering information, page 53) inserted between the vacuum source and the QIAvac 96 vacuum manifold.
- Wear safety glasses when working near a manifold under pressure.
- For safety reasons, do not use 96-well plates that have been damaged in any way.
- Always place the QIAvac 96 vacuum manifold on a secure bench top or work area. If dropped, the manifold may crack.

- Always store the QIAvac 96 vacuum manifold clean and dry. To clean, simply rinse all components with water, and dry with paper towels. Do not air dry, as the screws may rust and need to be replaced. Do not use abrasives. After rinsing and drying, wipe manifold components with paper towels wetted with 70% ethanol, and dry with fresh paper towels.
- The QIAvac 96 vacuum manifold and components are not resistant to ethanol, methanol or other organic solvents when exposed for long periods. If solvents are spilled on the unit, rinse thoroughly with distilled water after the RNeasy preparation. Ensure that no residual buffers remain in the vacuum manifold.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 96 vacuum manifold. The spring lock on the top plate and the self-sealing gasket provide an airtight seal when vacuum is applied to the assembled unit. To maximize gasket life, rinse the gasket free of salts and buffers after each use, and dry with paper towels before storage.

Table 1. Pressure Conversions

To convert from millibars (mbar) to:	Multiply by:
Millimeters of mercury (mm Hg)	075
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr	0.75
Atmospheres (atmos)	0.000987
Pounds per square inch (psi)	0.0145

Important Notes

Amount of tissue

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

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

The RNeasy 96 Universal Tissue procedure is optimized for use with a maximum of 50 mg animal tissue (flash-frozen). With adipose tissue, up to 100 mg can be used. With liver, thymus, spleen or tissues stabilized with RNAprotect® Tissue Reagent,* only 25 mg should be used to avoid clogging the RNeasy 96 Universal Tissue plate.

Table 2 gives specifications for the RNeasy 96 Universal Tissue plate. Each well of the plate has a maximum binding capacity of 100 µg of RNA, but actual yields depend on the sample type used. Table 3 gives examples of expected RNA yields from various tissues.

* See the *RNAprotect Tissue Handbook* for more information about RNAprotect Tissue Reagent.

Table 2. RNeasy 96 Universal Tissue Plate specifications

Preps per plate	96
Amount of starting material	50 mg (up to 100 mg adipose tissue; 25 mg flash-frozen liver, thymus or spleen tissue; 25 mg RNAprotect-stabilized tissue)
Binding capacity per well	100 µg RNA*
Maximum loading volume per well	1 ml
RNA size distribution	All RNA >200 nucleotides

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

Note: If the binding capacity of the RNeasy 96 Universal Tissue plate is exceeded, yields of total RNA will not be consistent and less than 100 µg total RNA may be recovered. If lysis of the starting material is incomplete, yields of total RNA will be lower than expected, even if the binding capacity of the RNeasy 96 Universal Tissue plate is not exceeded.

Weighing tissue is the most accurate way to quantify the amount of starting material. However, the following may be used as a guide. A 3 mm cube (approximately this size: ■ ; volume, 27 mm^3) of most animal tissues weighs 25–35 mg.

Table 3. Typical total RNA yields using the RNeasy 96 Universal Tissue Kit

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

* Amounts can vary due to species, age, gender, physiological state, etc. Since the RNeasy procedure enriches for RNA >200 bases long, the total RNA yield does not include 5.8S rRNA, tRNA and other low-molecular weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storage of starting material

RNA is not protected until the sample material is treated with RNAprotect Tissue Reagent, flash-frozen or disrupted and homogenized in the presence of RNase-inhibiting or denaturing agents. It is therefore important that samples are immediately frozen in liquid nitrogen* and stored at –70°C or immediately immersed in RNAprotect Tissue Reagent. Frozen tissue should not be allowed to thaw during handling or weighing. The relevant procedures should be carried out as quickly as possible.

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

Disruption and homogenization of starting materials

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA purification procedures. Disruption and homogenization are 2 distinct steps.

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

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

Some disruption methods simultaneously homogenize the sample while others require an additional homogenization step. Table 4 gives an overview of different disruption and homogenization methods. The different disruption and homogenization methods listed in Table 4 are described in more detail below.

Table 4. Disruption and Homogenization Methods

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

Disruption and homogenization using the QIAGEN TissueLyser system

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. Disruption efficiency is influenced by:

- Size and composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed and configuration of the TissueLyser II
- Disintegration time

Stainless steel beads with a diameter of 5 mm are optimal to use for animal tissues in combination with the RNeasy 96 Universal Tissue Kit. All other disruption parameters should be determined empirically for each application. The protocols in this handbook give guidelines for disruption and homogenization of tissues using the TissueLyser II and stainless-steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

Disruption and homogenization using rotor–stator homogenizers

Rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize, in the presence of lysis buffer, single samples of animal tissues in 15–90 seconds depending on the toughness and size of the sample. The rotor turns at a very high speed causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. Foaming of the sample should be kept to a minimum by using properly sized vessels, keeping the tip of the homogenizer submerged and holding the immersed tip to one side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes.

Square-well blocks

Square-well blocks are supplied with the 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 53.

Fresh square-well blocks must be used to take up the aqueous phase. Do not reuse cleaned square-well blocks to collect the aqueous phase.

Square-well blocks may be cleaned and reused as waste trays. To reuse the square-well blocks as waste trays, rinse them thoroughly with tap water,* and incubate for 2 hours or overnight in 0.1 N 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 QIAzol Lysis Reagent and Buffer RW1 on the square-well blocks.

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

Protocol: Purification of Total RNA from Animal Tissues using Vacuum/Spin Technology

Determining the correct amount of starting material

It is essential to begin with the correct amount of tissue to obtain optimal RNA yields and purity with RNeasy 96 Universal Tissue plates. Generally, this protocol can be used with a maximum of

- 50 mg flash-frozen tissue
- 100 mg flash-frozen adipose tissue
- 25 mg flash-frozen liver, thymus or spleen tissue
- 25 mg RNAprotect-stabilized tissue

Using fresh tissue is not recommended since RNA in unstabilized fresh tissue is not protected until the sample is homogenized in QIAzol Lysis Reagent. RNA is therefore likely to degrade during the time that it takes to excise 96 tissue samples. Average RNA yields from various sources are given in Table 3 (page 16).

Do not overload the plates. Overloading will significantly reduce yields and quality and may cause clogging of the RNeasy 96 Universal Tissue plate.

Important points before starting.

- If using the RNeasy 96 Universal Tissue Kit for the first time, read “Important Notes” (page 17).
- If preparing RNA for the first time, read Appendix A (page 41).

- Flash-frozen or RNAprotect-stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen,* and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates can also be stored at –70°C for several months. To process frozen homogenized lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the QIAzol Lysis Reagent are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of RNA. Continue with step 9.
- Generally, DNase digestion is not required since integrated QIAzol and RNeasy 96 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA remaining can be removed using the optional on-plate DNase digestion or by a DNase digestion after RNA purification (please contact QIAGEN Technical Services for a protocol). For on-plate DNase digestion, prepare the DNase I stock solution as described on page 12 before beginning the procedure.
- QIAzol Lysis Reagent and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information. Take appropriate safety measures and wear gloves when handling.
- Use of a multichannel pipet is recommended (see page 5). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for square-well blocks (see page 22). Make sure that reagent reservoirs used for chloroform are chloroform-resistant.
- A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is required (see page 14). The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

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

- All centrifugation steps in the protocol are performed in a Centrifuge 4-16KS (see page 13).
- The preliminary centrifugation step (step 9) and the centrifugation step to separate the aqueous from the organic phase (step 12) should be done at 4°C. All other steps of the RNeasy 96 Universal Tissue protocol should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- Check that all buffers are at room temperature (15–25°C). If using the optional on-plate DNase digestion, see Appendix D, page 50, for details to prepare the DNase I incubation mix.

Procedure

1. Preparation of QIAvac 96 vacuum manifold: Place the waste tray inside the QIAvac base. Place the top plate squarely over the base. Place the RNeasy 96 plate in the QIAvac top plate, making sure that the plate is seated tightly. Attach the QIAvac 96 manifold to the vacuum source. Keep vacuum switched off.

   Note: Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to the right-hand side.

2. Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube), and transfer the collection microtube rack to a box with dry ice.

   Note: We recommend using the TissueLyser II for parallel disruption and homogenization of up to 192 samples in parallel. Alternatively a rotor–stator homogenizer can be used for tissue disruption and homogenization. This protocol describes RNA purification from flash-frozen tissue using the TissueLyser II. For RNAprotect-stabilized tissue, cooling the collection microtube rack on dry ice is not necessary.

3. Remove the tissue sample from RNAprotect Tissue Reagent or from cold storage.

   Do not allow the tissue to thaw before it is placed in QIAzol Lysis Reagent.

4. Determine the amount of tissue. Do not use more than 50 mg flash-frozen tissue, 25 mg liver, thymus, spleen or RNAprotect-stabilized tissue, or 100 mg adipose tissue. Transfer it immediately to a cooled collection microtube. Repeat this until all required pieces of tissues are placed in the collection microtubes.

   Weighing tissue is the most accurate way to determine the amount. See page 19 for guidelines to determine the amount of starting material.

   RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen or disrupted and homogenized in protocol steps 6 and 7. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

5. Remove the collection microtube rack from the dry ice, and immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube.
6. Close the collection microtube rack using the supplied strips of collection microtube caps, and homogenize on the TissueLyser II for 5 min at 25 Hz.
7. Rotate the TissueLyser II rack to allow even homogenization, and homogenize for another 5 min at 25 Hz.

   Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

8. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.
9. Load the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 1 min at 4°C to collect residual liquid from the caps of the tubes.
10. Add 150 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps, and shake it vigorously while inverting the rack for 15 s. Thorough mixing is important for subsequent phase separation.
11. Place the collection microtube rack on the benchtop at room temperature for 2–3 min.

12. Centrifuge at 6,000 x *g* for 15 min at 4°C. After centrifuging, heat the centrifuge to room temperature if the same centrifuge is to be used for the following centrifugation steps of the protocol. After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 400 µl.
13. Transfer all of the upper, aqueous phases to a new square-well block. Then add 1 volume (usually 400 µl) of 70% ethanol to each transferred aqueous phase, and mix by pipetting up and down. Do not centrifuge. Continue with step 14. If some of the aqueous phase is lost during this step, adjust the volume of ethanol accordingly.
14. Pipet the samples (approximately 800 µl) from step 13 into the wells of the RNeasy 96 plate, and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum, and ventilate the QIAvac 96 manifold. Make sure that the QIAvac 96 vacuum manifold is assembled correctly before loading. The flow-through is collected in the waste tray. The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample. Take care not to wet the rims of the wells to avoid cross-contamination in subsequent steps

    Note: Tape unused wells with adhesive tape. Do not use the AirPore Tape Sheets supplied with the RNeasy 96 Universal Tissue Kit. Use either adhesive tape or tape pads (cat. no. 19570) from QIAGEN.

    Optional DNase digest: Generally, DNase digestion is not required since integrated QIAzol and RNeasy 96 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. See Appendix D, page 50, for more information and for details to prepare the DNase I incubation mix.
15. Add 800 µl of Buffer RW1 to each well of the RNeasy 96 plate. Switch on the vacuum source, and apply vacuum until transfer is complete (1–5 min). Switch off the vacuum, and ventilate the QIAvac 96 manifold. Collect the wash fraction in the same waste tray used in step 14.

16. Lift the top plate carrying the RNeasy 96 plate off the base, and empty the waste tray.* Reassemble the QIAvac 96 vacuum manifold.
17. Add 800 µl of Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum, and ventilate the QIAvac 96 manifold.

    Note: Ensure that ethanol is added to Buffer RPE (see "Things to do before starting", page 32).
18. Place the RNeasy 96 plate on top of a square-well block.
19. Add another 800 µl of Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the square-well block and RNeasy 96 plate into the holder, and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature to dry the plate membranes.

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.
20. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing 0.85 ml elution microtubes.

* Flow-through contains QIAzol Lysis Reagent or Buffer RW1 and is therefore not compatible with bleach. See page 10 for safety information.

21. To elute the RNA, add 45–70 µl RNase-free water to each well, and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.

    Note: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

    Although eluting with the minimum amount of RNase-free water results in increased RNA concentrations, total yields might be reduced, especially for tissues with low amounts of RNA, such as adipose tissue or skin.

22. Remove the AirPore Tape Sheet. Repeat the elution step (step 21) once with a second volume of 45–70 µl RNase-free water.

    Note: Repeating the elution step is required for complete recovery of RNA. Use elution microtube caps (caps for strips) provided to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Protocol: Purification of Total RNA from Animal Tissues using Spin Technology

Determining the correct amount of starting material

It is essential to begin with the correct amount of tissue to obtain optimal RNA yield and purity with RNeasy 96 Universal Tissue plates. Generally, this protocol can be used with a maximum of

- 50 mg flash-frozen tissue
- 100 mg flash-frozen adipose tissue
- 25 mg flash-frozen liver, thymus or spleen tissue
- 25 mg RNAprotect Tissue-stabilized tissue

Using fresh tissue is not recommended since RNA in unstabilized fresh tissue is not protected until the sample is homogenized in QIAzol Lysis Reagent. RNA is therefore likely to degrade during the time that it takes to excise 96 tissue samples.

Average RNA yields from various sources are given in Table 3 (page 19).

Do not overload the plates. Overloading will significantly reduce yield and quality and may cause clogging of the RNeasy 96 Universal Tissue plate.

Important points before starting

- If using the RNeasy 96 Universal Tissue Kit for the first time, read "Important Notes" (page 17).
- If preparing RNA for the first time, read Appendix A (page 41).

- Flash-frozen or RNAprotect-stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen,* and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates can also be stored at –70°C for several months. To process frozen homogenized lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 8.
- Generally, DNase digestion is not required since integrated QIAzol and RNeasy 96 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA remaining can be removed using the optional on-plate DNase digestion or by a DNase digestion after RNA purification (please contact QIAGEN Technical Services for a protocol). For on-plate DNase digestion, prepare the DNase I stock solution as described on page 40 before beginning the procedure.
- QIAzol Lysis Reagent and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information. Take appropriate safety measures and wear gloves when handling.
- Use of a multichannel pipet is recommended (see page 11). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for square-well blocks (see page 22). Make sure that reagent reservoirs used for chloroform are chloroform-resistant.
- All centrifugation steps in the protocol are performed in a Centrifuge 4-16KS (see page 13).

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

- The preliminary centrifugation step (step 8) and the centrifugation step to separate the aqueous from the organic phase (step 11) should be done at 4°C. All other steps of the RNeasy 96 Universal Tissue protocol should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- Check that all buffers are at room temperature (15–25°C). If using the optional on-plate DNase digestion, see Appendix D, page 50, for details to prepare the DNase I incubation mix.

Procedure

1. Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube), and transfer the collection microtube rack to a box with dry ice.

   Note: We recommend using the TissueLyser II for parallel disruption and homogenization of up 192 samples in parallel. Alternatively a rotor–stator homogenizer can be used for tissue disruption and homogenization. This protocol describes RNA purification from flash-frozen tissue using the TissueLyser II. For RNAprotect-stabilized tissue, cooling the collection microtube rack on dry ice is not necessary.

2. Remove the tissue sample from RNAprotect Tissue Reagent or from cold storage.

   Do not allow the tissue to thaw before it is placed in QIAzol Lysis Reagent.

3. Determine the amount of tissue. Do not use more than 50 mg flash-frozen tissue, 25 mg liver, thymus, spleen or RNAprotect-stabilized tissue, or 100 mg adipose tissue. Transfer it immediately to a cooled collection microtube. Repeat this until all required pieces of tissues are placed in the collection microtubes.

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

   RNA in unstabilized tissues is not protected after harvesting until the sample is flash-frozen or disrupted and homogenized in protocol steps 5 and 6. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible

4. Remove the collection microtube rack from the dry ice, and immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube.
5. Close the collection microtube rack using the supplied strips of collection microtube caps and homogenize on the TissueLyser II for 5 min at 25 Hz.
6. Rotate the TissueLyser II rack to allow even homogenization, and homogenize for another 5 min at 25 Hz.

   Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized after 2 x 5 min. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

7. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.
8. Load the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 1 min at 4°C to collect residual liquid from the caps of the tubes.
9. Add 150 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps, and shake it vigorously while inverting the rack for 15 s.

   Thorough mixing is important for subsequent phase separation.

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

11. Centrifuge at 6,000 x *g* for 15 min at 4°C. After centrifuging, heat the centrifuge to room temperature if the same centrifuge is to be used for the following centrifugation steps of the protocol.
    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 400 µl.
12. Transfer all of the upper, aqueous phases to a new square-well block. Then add 1 volume (usually 400 µl) of 70% ethanol to each transferred aqueous phase, and mix by pipetting up and down. Do not centrifuge. Continue with step 13.
    If some of the aqueous phase is lost during this step, adjust the volume of ethanol accordingly.
13. Place an RNeasy 96 plate on top of a square-well block.
14. Pipet the samples (approximately 800 µl) from step 12 into the wells of the RNeasy 96 plate. Take care not to wet the rims of the wells to avoid cross-contamination in subsequent steps.
15. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the square-well block and RNeasy 96 plate into the holder, and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature. Centrifugation with sealed plates prevents cross-contamination.
    Optional DNase digestion: Generally, DNase digestion is not required since integrated QIAzol and RNeasy 96 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. See Appendix D, page 50, for more information and for details to prepare the DNase I incubation mix.

16. Empty the square-well block,* and remove the AirPore Tape Sheet. Add 800 µl of Buffer RW1 to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.
17. Empty the square-well block, and remove the AirPore Tape Sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.
Note: Ensure that ethanol is added to Buffer RPE (see "Things to do before starting", page 51).
18. Empty the square-well block, and remove the AirPore Tape Sheet. Add another 800 µl Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with a new AirPore Tape Sheet. Centrifuge at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature. It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.
19. Remove AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing 0.85 ml elution microtubes.
20. To elute the RNA, add 45–70 µl of RNase-free water to each well, and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approximately 5600 x *g*) for 4 min at room temperature.
Note: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate. Although eluting with the minimum amount of RNase-free water results in increased RNA concentrations, total yields might be reduced, especially for tissues with low amounts of RNA, such as adipose tissue or skin.

* Flow-through contains QIAzol Lysis Reagent or Buffer RW1 and is therefore not compatible with bleach. See page 10 for safety information.

21. Remove the AirPore Tape Sheet. Repeat the elution step (step 20) once with a second volume of 45–70 µl RNase-free water.

    Note: Repeating the elution step is required for complete recovery of RNA. Use elution microtube caps (caps for strips) provided to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

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 back cover for contact information).

		Comments and suggestions
Phases do not separate completely		
a)	No chloroform added or chloroform not pure	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
b)	Homogenate not sufficiently mixed before centrifugation	After addition of chloroform (vacuum/spin step 10 on page 26; spin step 9 on page 33), the homogenate must be vigorously shaken. If the phases are not well separated, shake the rack vigorously while inverting it for at least 15 s, and repeat the incubation and centrifugation (vacuum/spin steps 11 and 12 on pages 26 and 27, respectively; spin steps 10 and 11 on pages 33 and 34, respectively).
c)	Organic solvents in samples used for purification	Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers or alkaline reagents.* These can interfere with the phase separation.
Clogged plate wells		
a)	Inefficient disruption and/or homogenization	See "Disruption and homogenization of starting materials" (page 20) for a detailed description of disruption and homogenization methods.
b)	Too much starting material	Reduce amount of starting material. It is essential not to exceed the maximum amount of starting material (see page 17).
c)	Centrifugation temperature too low	Except for phase separation, all centrifugation steps should be performed at room temperature (15–25°C). Some centrifuges may cool to below 20°C even when set at 20°C. This can cause precipitates to form that can clog the RNeasy 96 plate. If this happens, set the centrifugation temperature to 25°C, and warm the ethanol-containing lysate to 37°C before transferring to the RNeasy 96 plate.

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

		Comments and suggestions
Little or no RNA eluted		
a)	Too much starting material	Overloading significantly reduces yield. Reduce the amount of starting material (see page 17).
b)	Buffer temperatures too low	All buffers must be at room temperature (15–25°C) throughout the procedure
c)	throughout the procedure membrane	Repeat elution, but incubate the RNeasy 96 plate on the benchtop at room temperature for 10 min with RNase-free water before centrifuging
Low A_{260}/A_{280} value		
a)	Not enough QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time
b)	Sample not incubated for 5 min after homogenization	Place the sample at room temperature (15–25°C) for 5 min after homogenization, as indicated in the protocol.
c)	Water used to dilute RNA for A_{260}/A_{260} measurement	Use 10 mM Tris·Cl,* pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see "Purity of RNA", page 45).
RNA degraded		
a)	Inappropriate handling of starting material	Ensure that tissues have been properly handled and that the protocol has been performed without interruptions, especially the initial steps involving tissue lysis and homogenization. Some tissues (e.g., pancreas or intestine) contain high amounts of RNases. Care must be taken to excise these tissues from animals as fast as possible and to stabilize them either by freezing in liquid nitrogen* or by immersing them in RNAprotect Tissue Reagent† immediately after excision.
b)	RNase contamination	Check for RNase contamination of buffers. Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 41) for general remarks on handling RNA.

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

† See the *RNAprotect Tissue Handbook* for more information about RNAprotect Tissue Reagent.

		Comments and suggestions
DNA contamination in downstream experiments		
a)	Phase separation performed at too high a temperature	The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
b)	Interphase contamination of aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the eluate. Make sure to transfer the aqueous phase without interphase contamination.
c)	No DNase treatment	Follow the optional on-plate DNase digestion using the RNase-Free DNase Set (Appendix D, page 50) at the point indicated in the protocol. Alternatively, after the RNeasy procedure, DNase digest the eluate containing the RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment or repurified using an RNeasy RNA cleanup protocol (see the *RNeasy 96 Handbook* or the *RNeasy MinElute® Cleanup Handbook*).
NA concentration too low		
	Elution volume too high	Elute with less RNase-free water. Although eluting with less RNase-free water results in increased RNA concentrations, total yields might be reduced, especially for tissues with low amounts of RNA, such as adipose tissue or skin.
RNA does not perform well in downstream experiments		
a)	Salt carryover during elution	Ensure that Buffer RPE is at room temperature Ethanol carryover (15–25°C).
b)	Ethanol carryover	During the second Buffer RPE wash, be sure to dry the plate well membranes by centrifuging the plate at 6000 rpm (approximately 5600 x *g*) for 10 min at room temperature.
c)	Vacuum/spin protocol: Vacuum pressure too low	A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary to achieve efficient RNA binding to the membrane and washing.
Low well-to-well reproducibility		
a)	Elution volume too low	Use elution volumes of 2 x 50 or 2 x 70 µl to improve well-to-well reproducibility.
b)	Vacuum pressure too low	A vacuum source capable of generating a vacuum pressure of –800 to –900 mbar is necessary to achieve efficient RNA binding to the membrane and washing.

		Comments and suggestions
c)	Incomplete homogenization	Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample.
d)	Variability between samples	RNA yields from tissue samples can vary more than, for example, cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability.

Appendix A: General Remarks on Handling RNA

Handling RNA

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

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 42), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant) and allow to dry.

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

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier. Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with diethyl pyrocarbonate (DEPC)*, as described in "Solutions" below.

Solutions

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

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

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

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

Storage of RNA

Purified RNA may be stored at –70°C to –15°C in RNase-free 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 quantified using quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

Using the QIAxpert UV/VIS Spectrophotometer for microvolume analysis

To determine the concentration of your RNA sample purified with RNeasy QIAGEN kit, use the corresponding RNeasy App on the QIAxpert. For more information, see the QIAxpert product page (**www.qiagen.com/qiaxpert-system**).

Using a standard spectrophotometer

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 → 4 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 45), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 42). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample	= 100 µl
Dilution	= 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

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

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

Purity of RNA

The assessment of RNA purity will be performed routinely, when using the QIAxpert with the corresponding RNeasy App. See the QIAxpert user manual for more information (**www.qiagen.com/qiaxpert-system/user manual**)

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

For standard photometric measurements, 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, such as protein, that absorb in the UV spectrum. 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 when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution. For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 44).

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. RNeasy Kits will, however, remove the vast majority of cellular DNA. gDNA Eliminator Solution helps to further reduce genomic DNA contamination; however, trace amounts of genomic DNA may still remain, depending on the amount and nature of the sample. For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic

* 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 cannot be avoided, we recommend using the QuantiTect Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see ordering information, page 53).

Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Plus Universal Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel system or Agilent® 2100 Bioanalyzer. Ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification. As a useful measure of RNA integrity, the QIAxcel® Advanced system and the Agilent 2100 Bioanalyzer provide an RNA integrity score (RIS) and an RNA integrity number (RIN), respectively. Ideally, the value should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

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

Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

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

FA gel preparation

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

1.2 g	agarose*
10 ml	10x FA gel buffer (see composition below)

add RNase-free water to 100 ml

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

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

RNA sample preparation for FA gel electrophoresis

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

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

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

Gel running conditions

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

Composition of FA gel buffers

10x FA gel buffer

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

pH to 7.0 with NaOH*

1x FA gel running buffer

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

5x RNA loading buffer

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

RNase-free water to 10 ml
Stability: approximately 3 months at 4°C

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

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

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

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

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

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

Lysis and homogenization of the sample and binding of RNA to the silica-gel membrane are performed according to the standard protocols. After washing with a reduced volume of Buffer RW1, the RNA is treated with DNase I while bound to the silica-gel membrane. The DNase is removed by a second wash with Buffer RW1. Washing with Buffer RPE and elution are then performed according to the standard protocols.

Important points before starting

- Generally, DNase digestion is not required since integrated QIAzol and RNeasy 96 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., QuantiNova RT-PCR analysis with a low-abundance target). DNA can also be removed by a DNase digestion following RNA purification.
- Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.
- If desired, these DNase digestion steps can be carried out on the QIAvac 96 vacuum manifold instead of using centrifugation. Please contact QIAGEN Technical Services for more information.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. The RNeasy 96 Universal Tissue procedure requires 2 RNase-Free DNase Sets per 96-well plate. Dissolve 2 vials of solid DNase I (2 x 1500 Kunitz units) in 2 x 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. Do not vortex.
- Unused DNase I stock solution can be stored at –20°C for up to 9 months. Thawed stock solution can be stored at 2–8°C for up to 6 weeks. Do not refreeze the DNase I stock solution after thawing.

Procedure

Carry out steps 1–14 in the vacuum/spin protocol or 1–15 of the spin protocol. Instead of continuing with the next Buffer RW1 wash step, follow steps D1–D4 below. If the vacuum/spin protocol is used, centrifugation in steps D1–D4 can be replaced by processing on the QIAvac 96.

1. Pipet 400 µl Buffer RW1 into each well of the RNeasy 96 plate, and centrifuge at room temperature (15–25°C) for 4 min at 6000 rpm (approximately 5600 x *g*) to wash. Discard the flow-through.*

   Reuse the square-well block in step D3.

2. Add 670 µl DNase I stock solution (see above) to 7.3 ml Buffer RDD. Mix by gently inverting the tube. Buffer RDD is supplied with the RNase-Free DNase Set.

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

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for safety information.

3. Pipet the DNase I incubation mix (80 µl per well) directly onto the RNeasy silica-gel membrane in each well of the RNeasy 96 plate, and place on the benchtop at room temperature for 15 min.

   Note: Make sure to pipet the DNase I incubation mix directly onto the RNeasy silica-gel membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the RNeasy 96 plate wells.

4. Pipet 400 µl Buffer RW1 into each well of the RNeasy 96 plate, and centrifuge for 4 min at 6000 rpm (approximately 5600 x *g*). Discard the flow-through.* Continue with step 16 of the vacuum/spin protocol (page 28) or step 17 of the spin protocol (page 35).

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

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 Universal Tissue Kit (4)*	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes (racked), Elution Microtubes CL (0.85 ml), Caps, Square-Well Blocks, AirPore Tape Sheets, RNase-Free Reagents and Buffers	74881
Accessories		
TissueLyser II	Universal laboratory mixer-mill disruptor	Various
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates, for use with collection microtubes (racked, 1.2 ml) on the TissueLyser II	69984
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with 1.2 ml collection microtubes and 2.0 ml microcentrifuge tubes on the TissueLyser II	69989
TissueLyser 5 mm Bead Dispenser, 96-well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Centrifuge 4-16KS*	Universal refrigerated laboratory centrifuge with brushless motor	81610
Plate Rotor 2 x 96†	Rotor for 2 QIAGEN 96 plates for use with QIAGEN Centrifuges	81031

Product	Contents	Cat. no.
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder, Rack of Collection Microtubes (1.2 ml)	19504
Vacuum Regulator	For use with QIAvac manifolds	19530
RNase-Free DNase Set (50)	1500 units RNase-Free DNase I, RNase-Free Buffer RDD and RNase-Free Water for 50 RNA minipreps	79254
QIAzol Lysis Reagent (200 ml)	200 ml QIAzol Lysis Reagent	79306
RNAprotect Tissue Reagent (50 ml)	50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Reagent (250 ml)	250 ml RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106
RNAprotect Tissue Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAprotect Tissue Reagent each	76154
RNAprotect Tissue Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNAprotect Tissue Reagent each	76163
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

Product	Contents	Cat. no.
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
Collection Microtubes (racked, 10 x 96)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks, 960 in strips of 8	19566
Related products for RNA purification from tissues		
RNeasy Protect Kits — for RNA stabilization and RNeasy purification of total RNA from animal tissues		
RNeasy Protect Mini Kit (50)‡	RNAprotect Tissue Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	74124
RNeasy Protect Mini Kit (250)	RNAprotect Tissue Reagent (250 ml), 250 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74126
RNeasy Lipid Tissue Kits — for purification 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

Product	Contents	Cat. no.
RNeasy Fibrous Tissue Kits — for purification 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
Related products for RNA purification		
RNeasy 96 Kits — for 96-well purification of total RNA from cells using gDNA Eliminator plates		
RNeasy 96 Kit (12)[§]	For 12 x 96 total RNA preps: 12 RNeasy 96 Plates, Elution Microtubes CL, Caps, RNase-Free Reagents and Buffers	74182
RNeasy Plus Mini Kits — for purification of up to 100 µg total RNA from cells/tissues using gDNA Eliminator columns		
RNeasy Plus Mini Kit (50)	For 50 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-Free Water and Buffers	74134
RNeasy Plus Mini Kit (250)	For 250 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-Free Water and Buffers	74136
RNeasy Plus Micro Kit (50)	For 50 micropreps: RNeasy MinElute Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Water and Buffers	74034

Product	Contents	Cat. no.
RNeasy Kits — for purification of total RNA 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 (50)‡	50 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-Free Reagents and Buffers	75144
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-Free Reagents and Buffers	75162
RNeasy MinElute Cleanup Kit — for RNA cleanup and concentration 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
Products for downstream applications		
QuantiNova SYBR Green RT-PCR Kit — for one-step qRT-PCR using SYBR Green I for gene expression analysis		
QuantiNova SYBR Green RT-PCR Kit (100)	For 100 x 20 µl reactions: 1 ml QuantiNova SYBR Green RT-PCR Master Mix, 20 µl QuantiNova SYBR Green RT Mix, 20 µl Internal Control RNA, 500 µl Yellow Template Dilution Buffer, 250 µl ROX Reference Dye, 1.9 µl RNase-Free Water	208152

Product	Contents	Cat. no.
QuantiNova Probe RT-PCR Kit (100)	For 100 x 20 µl reactions: 1 ml QuantiNova Probe RT-PCR Master Mix, 20 µl QuantiNova Probe RT Mix, 20 µl Internal Control RNA, 500 µl Yellow Template Dilution Buffer, 250 µl ROX Reference Dye, 1.9 µl RNase-Free Water	208352
QIAGEN OneStep Ahead RT-PCR Kit— for faster one-step RT-PCR with high sensitivity, specificity and fidelity		
OneStep Ahead RT-PCR Kit (50)	6 vials for 50 reactions: 1 x 500 µl OneStep Ahead RT-PCR Master Mix, 1 x 50 µl OneStep Ahead RT Mix, 1 x 200 µl Template Tracer, 1 x 50 µl Master Mix Tracer, 1 x 1.9 ml water, 1 x 400 µl Q-Solution	220211
Omniscript® RT Kit — for standard reverse transcription with any amount of RNA from 50 ng to 2 µg per reaction		
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	205111
Sensiscript® RT Kit — for reverse transcription using small amounts of RNA (i.e., less than 50 ng RNA including carrier RNA)		
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

Product	Contents	Cat. no.
HotStarTaq® DNA Polymerase — for robust amplification in all applications		
HotStarTaq DNA Polymerase (250 U)‡	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203203
AllTaq Master Mix Kit (500)	For 500 x 20 µl PCR amplifications: 2 x 1.25 ml AllTaq Master Mix (4x), 1 x 200 µl Template Tracer (25x), 2 x 50 µl Master Mix Tracer (125x), 5 x 1.9 ml RNase-Free Water	203144
***Taq* DNA Polymerase — for standard and specialized PCR applications**		
Taq DNA Polymerase (250 U)‡	250 units *Taq* DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	201203
Taq PCR Core Kit (250 U)‡	250 units *Taq* DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix (containing 10 mM each dNTP)	201223
Taq PCR Master Mix Kit (250 U)‡	3 x 1.7 ml *Taq* PCR Master Mix, containing 250 units *Taq* DNA Polymerase total and providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP; 3 x 1.7 ml distilled water	201443

* Requires use of the Plate Rotor 2 x 96 and Centrifuge 4-16KS (QIAvac 96 optional).

† 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 4-15, 4-15C and 4-16KS from QIAGEN and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15 and 6K15 from Sigma Laborzentrifugen GmbH.

‡ Larger kit sizes available; please inquire.

§ Requires use of QIAvac 96 or the QIAGEN 96-Well-Plate Centrifugation system.

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

** For more information about quantitative, real-time RT-PCR, request our application guide *Critical Factors for Successful Real-Time RT-PCR*

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

Document Revision History

Date	Changes
February 2021	Updated branding of RNA protection products. Corrected centrifugation speed to 6000 rpm in protocol steps. Removed discontinued kit (cat. no. 74882).

Notes

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