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RNeasy® 96 Universal Tissue 8000 Handbook

For high-throughput RNA purification from all types of animal tissue, automated on the BioRobot® Universal System

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy 96 Universal Tissue 8000 Kit	**(12)**
Catalog no.	**967852**
Number of preps	**12 x 96**
RNeasy 96 plates	12
Register Cards (96-well)	12
Collection Microtubes (racked)	12 x 96
Collection Microtube Caps	320 x 8
S-Blocks	14
Elution Microtubes CL	12 x 96
Caps for Strips	165 x 8
Buffer RW1*	6 x 325 ml
Buffer RPE†	6 x 100 ml
RNase-Free Water	96 x 1.9 ml
Top Elute Fluid	48 x 1.48 ml
QIAzol® Lysis Reagent*‡	5 x 200 ml
Quick-Start Protocol	1

* CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 4 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.

Additional QIAzol Lysis Reagent and other kit components are available separately. See ordering information (page 39).

Storage

The RNeasy 96 Universal Tissue 8000 Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions.

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.

Intended Use

The RNeasy 96 Universal Tissue 8000 Kit is intended for high-throughput RNA purification from all types of animal tissue, automated on the BioRobot Universal System or BioRobot 8000. The RNeasy 96 Universal Tissue 8000 Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

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

Safety Information

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

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

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 buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The RNeasy 96 Universal Tissue 8000 Kit is well-suited for simultaneous purification of RNA from 96 samples of all types of animal or human tissue. The RNeasy 96 Universal Tissue 8000 Kit facilitates efficient, high-throughput RNA sample preparation.

In less than 2 hours (including homogenization and RNA purification), highly pure RNA can be obtained from 96 tissue samples. The final 70 minutes of the procedure is fully automated on the BioRobot Universal System or BioRobot 8000. The RNeasy 96 Universal Tissue 8000 procedure replaces current time-consuming and tedious methods involving alcohol-

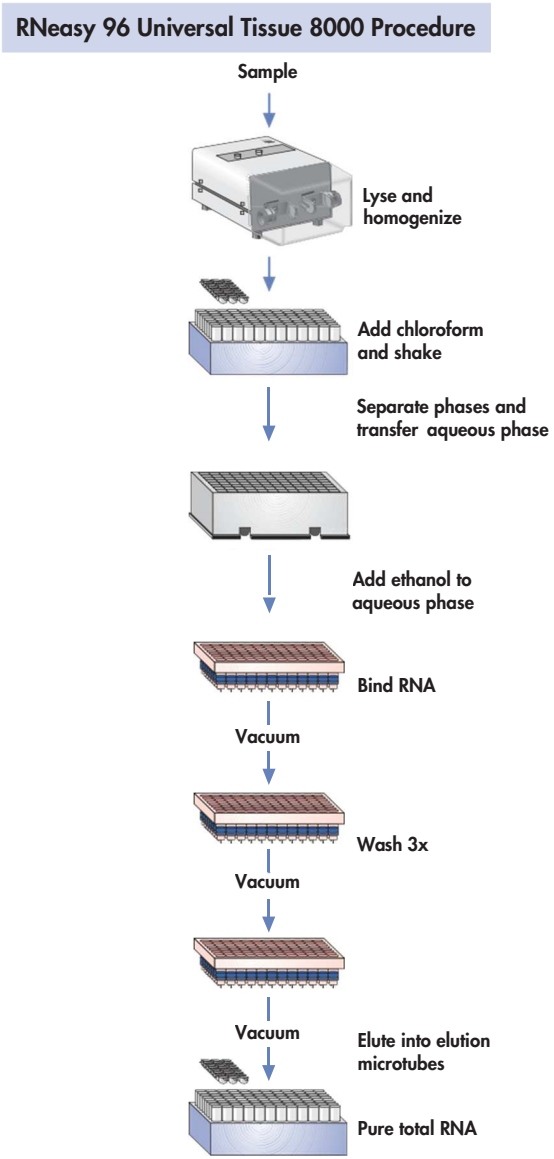
precipitation steps or large numbers of wash steps. The purified RNA is ready to use in any downstream application, including quantitative RT-PCR using QIAGEN QuantiNova® technology.

Principle and procedure

The RNeasy 96 Universal Tissue 8000 Kit represents an advanced technology for high-throughput RNA preparation. The kit integrates efficient phenol/guanidine-based lysis and silica-membrane purification with the speed of vacuum processing. Following homogenization and phase separation, the BioRobot workstation provides walkaway automation of the procedure, for total RNA purification from all types of animal or human tissue.

Tissue is first efficiently lysed using QIAzol Lysis Reagent and the TissueLyser II. This provides rapid and parallel disruption of tissue and inactivation of RNases to ensure purification of intact RNA. After phase separation by centrifugation, the BioRobot workstation removes the aqueous phase and adds ethanol to provide appropriate binding conditions. The workstation then applies the samples 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 8000 procedure (Figure 1), all RNA molecules longer than 200 nucleotides are purified. The procedure provides an enrichment for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. The size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently.


RNeasy 96 Universal Tissue 8000 Procedure
Sample
Lyse and homogenize
Add chloroform and shake
Separate phases and transfer aqueous phase
Add ethanol to aqueous phase
Bind RNA
Vacuum
Wash 3x
Vacuum
Vacuum
Elute into elution microtubes
Pure total RNA

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.

- BioRobot Universal System with Application Pack Gene Expression (see ordering information, page 39) or BioRobot 8000*
- Multichannel pipet with tips. For the most efficient sample processing, we recommend an electric multichannel pipet with a minimum capacity of 650 µl per pipet tip.
- Reagent reservoirs for multichannel pipets

  Note: Ensure that reagent reservoirs used for chloroform are chloroform-resistant.
- Disposable gloves
- QIAGEN TissueLyser II system, comprising the TissueLyser II (cat. no. 85300), the TissueLyser Adapter Set 2 x 96 (cat. no. 69984), Stainless Steel Beads, 5 mm (cat. no. 69989) and the TissueLyser 5 mm Bead Dispenser, 96-Well (cat. no. 69975). Alternatively, a rotor–stator homogenizer can be used.
- Centrifuge 4-16KS (see ordering information, page 39)
- Plate Rotor 2 x 96 (cat. no. 81031)
- Chloroform (without added isoamyl alcohol)
- 96–100% ethanol†
- 70% ethanol in water†
- Dry ice
- Disposable Filter-Tips, 1100 µl (cat. no. 9012598)

* The BioRobot 8000 is no longer available.

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

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 8000 procedure requires 2 RNase-Free DNase Sets per 96-well plate.
- Screw-cap tubes (2 ml) for use with the optional DNase treatment (Safe-Lock microtubes; Eppendorf*)

  Note: Use of other tubes may require modification of the QIAsoft protocol; for assistance, contact QIAGEN. Other tubes also may not fit in the reagent holder.

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

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 8000 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 8000 procedure is optimized for use with a maximum of 40 mg animal tissue (flash-frozen). With adipose tissue, up to 80 mg can be used. With liver, thymus, spleen intestine, only 20 mg should be used to avoid clogging the RNeasy 96 Universal Tissue plate. For tissues stabilized with RNAprotect® Tissue Reagent or Allprotect® Tissue Reagent,* half of these amounts should be used.

Table 1 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 2 gives examples of expected RNA yields from various tissues.

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

Table 1. RNeasy 96 Universal Tissue plate specifications

Parameter	Specification
Preps per plate	96
Amount of starting material	40 mg (up to 80 mg adipose tissue; 20 mg flash-frozen liver, thymus, spleen or intestine; half of these amounts for 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 expected. 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.

Table 2. Typical total RNA yields using the RNeasy 96 Universal Tissue 8000 Kit

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

* Amounts can vary due to 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.

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 (volume, 27 mm^3) of most animal tissues weighs 25–35 mg.

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 –90°C to –65°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 two 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 3 gives an overview of different disruption and homogenization methods. The methods are described in more detail below.

Table 3. Disruption and homogenization methods

Disruption method	Homogenization method	Comments
TissueLyser II system	TissueLyser II system	Simultaneously disrupts and homogenizes up to 192 samples in parallel. The TissueLyser II 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 TissueLyser II 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 provide optimal disruption of animal tissues in the RNeasy 96 Universal Tissue 8000 procedure. All other disruption parameters should be determined empirically for each application. The protocol in this handbook gives 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.

Preparation of reagents and worktable

Buffer RW1

One bottle of Buffer RW1 (325 ml) contains sufficient buffer for 2 runs of 96 samples. Buffer RW1 left over after a run should be stored at room temperature (15–25°C) for the next run.

Buffer RPE

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

RNase-free water

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

Top Elute Fluid

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

RNase-free DNase I

The RNeasy 96 Universal Tissue 8000 procedure provides the option of performing DNase digestion during RNA purification. Generally, DNase digestion is not required, since the procedure efficiently removes most of the DNA without the use of DNase. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small

amounts of DNA (e.g., QuantiNova RT-PCR analysis with a low-abundance target). For further details, see Appendix D, page 37.

Plasticware

One RNeasy 96 plate, one rack of collection microtubes and one S-Block are required for a single run of 96 samples. When placing these items of plasticware on the BioRobot worktable, make sure that position A1 is located at the upper-left corner. Discard the plasticware after use.

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

Table 4. Loading buffers and reagents

Item	Position	Volume*
Ethanol (70%)	Reagent carousel	106 ml
Buffer RW1	Reagent carousel	162.5 ml
Buffer RPE†	Reagent carousel	250 ml (reconstituted)
Ethanol (96–100%)	Reagent carousel	145 ml
Distilled water	Reagent carousel	700 ml
RNase-free water	High-speed shaker system carrying reagent holder for 8 tubes	8 x 1.9 ml
Top Elute Fluid	High-speed shaker system carrying reagent holder for 8 tubes	4 x 1.48 ml
Optional: RNase-Free DNase I‡	Cooling and heating system carrying reagent holder for 8 tubes	4 x 1.99 ml

* Volumes for one run of 96 samples.

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

‡ See Appendix D, page 37, for details on preparing RNase-free DNase I.

Table 5. Loading plasticware (BioRobot Universal System)

Item	Position	Holder/adapter
RNeasy 96 plate	QIAplate Holder silver 27	Silver multiwall-plate holder
Collection microtubes	High-speed shaker system	–
S-Block	High-speed shaker system	–
Elution Microtubes CL	MP Slot 21	Blue elution microtube adapter
Channeling block	QIAplate Holder black 16	Black multiwall-plate holder
Rack of disposable filter-tips (1100 µl)	Tip-Rack Slot 2 Tip-Rack Slot 3 Tip-Rack Slot 4 Tip-Rack Slot 5 Tip-Rack Slot 10 Tip-Rack Slot 20 Tip-Rack Slot 25	Red tip-tray holders

Table 6. Loading plasticware (BioRobot 8000)

Item	Position	Holder/adapter
RNeasy 96 plate	QIAplate Slot 6	Silver multiwall-plate holder
Collection microtubes	High-speed shaker system	–
S-Block	High-speed shaker system	–
Elution Microtubes CL	MP Slot 21	Blue elution microtube adapter
Channeling block	QIAplate Slot 16	Black multiwall-plate holder
Rack of disposable filter-tips (1100 µl)	Tip-Rack Slot 3 Tip-Rack Slot 4 Tip-Rack Slot 5 Tip-Rack Slot 10	Red tip-tray holders

Protocol: Purification of Total RNA from Animal Tissues

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:

- 40 mg flash-frozen tissue
- 80 mg flash-frozen adipose tissue
- 20 mg flash-frozen liver, thymus, spleen or intestine
- Half of these amounts for RNAprotect- and Allprotect-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.

Typical RNA yields from various sources are given in Table 2 (page 11).

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

Things to do before starting

- If using the RNeasy 96 Universal Tissue 8000 Kit for the first time, read "Important Notes" (page 10).
- If preparing RNA for the first time, read Appendix A (page 27).

- 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 –90°C to –65°C. Tissue can be stored for several months at –90°C to –65°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 –90°C to –65°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 reagent are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 11.
- 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 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 15 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 4 for safety information.
- Use of a multichannel pipet is recommended. 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 nondisposable plasticware (see page 28). Make sure that reagent reservoirs used for chloroform are chloroform- resistant.
- All centrifugation steps in the protocol are performed in a Centrifuge 4-16KS.

* 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 2 centrifugation steps (steps 11 and 14) should be performed at 4°C. All other steps of the RNeasy 96 Universal Tissue 8000 procedure 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 37, for details to prepare the DNase I incubation mix.

Procedure

1. Make sure that the BioRobot workstation is switched on.
2. Switch on the computer and monitor.
3. Launch the QIAsoft Operating System.

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

   If using the BioRobot 8000, the QIAsoft 4.2 Operating System is required. Start the software from the Microsoft Windows "Start" menu, where it is located under Programs/QIAsoft 4.2/QIAsoft 4.2.
4. 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.

5. 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.

6. Determine the amount of tissue. Do not use more than 40 mg flash-frozen tissue, 20 mg liver, thymus, spleen or intestine or 80 mg adipose tissue. Use half of these amounts when working with RNAprotect stabilized tissues. 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 10 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 8 and 9. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

7. Remove the collection microtube rack from the dry ice, and immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube.

8. 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.

9. 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.

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

11. Load the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 x *g* for 1 min at 4°C to collect residual liquid from the caps of the tubes.

12. 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.

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

14. Centrifuge at 6000 x *g* for 15 min at 4°C.

    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 400 µl.

15. Select "RNeasy 96 Universal Tissue" from the protocol selection box and click "Run" to start the protocol.

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

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

    - Number of samples: Select 96 samples.
    - DNase treatment: Enter "yes" to perform DNase digestion on the RNeasy 96 plate (see page 37).
    - Elution volume: Choose the elution volume. For most applications, we recommend the default elution volume.
    - Automatic clog detection (BioRobot Universal System only): Enter "yes" to perform clog detection. The BioRobot workstation will then check for clogged membranes on the RNeasy 96 plate. Any wells with clogged membranes will not be processed further in the RNA purification procedure. Note that this option requires the use of more disposable tips and increases run time.

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

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

   Use the elution microtube caps (caps for strips) provided to seal the microtubes for storage. Store RNA at –90°C to –65°C.

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

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

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

   If using the BioRobot 8000, refer to the *BioRobot 8000 User Manual* for details about maintenance procedures.

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

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Phases do not separate completely	
a) No chloroform added or chloroform not pure	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
b) Homogenate not sufficiently mixed before centrifugation	After addition of chloroform (step 12), the homogenate must be vigorously shaken. If the phases are not well-separated, shake the rack vigorously while inverting it for at least 15 seconds, and repeat the incubation and centrifugation (steps 13 and 14, pages 21 and 22).
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 13) 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 (page 10).

* 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 10).
b)	Buffer temperatures too low	All buffers must be at room temperature (15–25°C) throughout the procedure.
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 for 5 minutes 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 31).
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 sure not to introduce any RNases during the procedure or later handling. See Appendix A (page 27) for general remarks on handling RNA.
DNA contamination in downstream experiments		
a)	Phase separation performed at too high a temperature	The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
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.

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

		Comments and suggestions
c)	No DNase treatment	Follow the optional on-plate DNase digestion using the RNase-Free DNase Set (Appendix D, page 37) 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*).
RNA 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		
	Salt carryover during elution	Ensure that Buffer RPE is at room temperature.
Low well-to-well reproducibility		
a)	Incomplete homogenization	Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample.
b)	Variability between samples	RNA yields from tissue samples can vary more than, for example, cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability.

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 28), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g.,

* 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.

0.5% SDS),* rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant) and allow to dry.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier. Glassware 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

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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.

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 31), 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 28). 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**)

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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 30).

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. QuantiNova Primer

* 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.

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 DNA cannot be avoided, we recommend using the QuantiNova Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see ordering information, page 39).

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: Formaldehyde Agarose Gel Electrophoresis

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

FA gel preparation

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

1.2 g agarose*

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

Add RNase-free water to 100 ml

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

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

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

RNA sample preparation for FA gel electrophoresis

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

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

Gel running conditions

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

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

* 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.

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.

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

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

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

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

Important points before starting

- Generally, DNase digestion is not required since 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.

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 8000 procedure requires 2 RNase-Free DNase Sets per 96-well plate. Dissolve 2 vials of solid DNase I (2 x 1500 Kunitz units) in 2 x 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vials. Inject RNase-free water into the vials using an RNase-free needle and syringe. Mix gently by inverting the vials. Do not vortex.
- Unused DNase I stock solution can be stored at –90°C to –30°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

1. 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.
2. Aliquot into four 2 ml Safe-Lock tubes with 1.99 ml DNase I incubation mix per tube, and keep on ice until use.

   Note: Use of other tubes may require modification of the QIAsoft protocol; for assistance, contact QIAGEN. Other tubes also may not fit in the reagent holder.

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 Universal Tissue 8000 Kit	For 12 x 96 total RNA preps on the BioRobot 8000 or BioRobot Universal System: 12 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	967852
TissueLyser II		
TissueLyser II	Bead mill; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96*	Various
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Accessories		
Centrifuge 4-16KS	Universal laboratory centrifuge with brushless motor	Various
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD and RNase-free water for 50 RNA minipreps	79254
QIAzol Lysis Reagent (200ml)	200 ml QIAzol Lysis Reagent	79306

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x96.

Product	Contents	Cat. no.
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
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585
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; 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
Disposable Filter-Tips, 1100 µl (960)	Conducting disposable filter-tips; pack of 960	9012598

Product	Contents	Cat. no.
Related products for RNA purification		
RNeasy 96 BioRobot 8000 Kit – for fully automated high-throughput RNA minipreps from cells		
RNeasy 96 BioRobot 8000 Kit (12)	For 12 x 96 total RNA preps on the BioRobot 8000 or BioRobot Universal System: 12 RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, RNase-Free Reagents and Buffers	967152
RNeasy 96 Universal Tissue Kit – for high-throughput RNA purification from any type of animal tissue		
RNeasy 96 Universal Tissue Kit (4)*†	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	74881
RNeasy 96 Kit – for high-throughput RNA minipreps from cells		
RNeasy 96 Kit (4)‡§	For 4 x 96 total and cytoplasmic RNA preps: 4 RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, RNase-Free Reagents and Buffers	74181
Related products for one-step RT-PCR and real-time one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit – for fast and successful one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit (25)*	For 25 x 50 µl reactions: QIAGEN OneStep RT-PCR Enzyme Mix (1 x 50 µl), 5x QIAGEN OneStep RT-PCR Buffer (1 x 250 µl), dNTP Mix (1 x 50 µl, 10 mM each), 5x Q-Solution (1 x 400 µl), RNase-Free Water (1 x 1.9 ml)	210210

Product	Contents	Cat. no.
QuantiNova SYBR Green RT-PCR Kit – for quantitative, real-time, one-step RT-PCR using SYBR Green I		
QuantiNova SYBR Green RT-PCR Kit (100) (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
QuantiNova Probe RT-PCR Kit – for quantitative, real-time, one-step RT-PCR using sequence-specific probes		
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
QuantiNova Multiplex RT-PCR Kits – for quantitative, multiplex, real-time, one-step RT-PCR using sequence-specific probes		
QuantiNova Multiplex RT-PCR Kit (100)*	For 100 x 20 µl reactions: 0.5 ml 4x QuantiNova Multiplex RT-PCR Master Mix, 20 µl QuantiNova Multiplex RT-Mix, 20 µl QuantiNova IC RNA, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml RNase-Free Water	208552
QuantiNova Multiplex RT-PCR Kit (2500)*	For 2500 x 20 µl reactions: 10 x 1.3 ml 4x QuantiNova Multiplex RT-PCR Master Mix, 5 x 100 µl QuantiNova Multiplex RT-Mix, 3 x 100 µl QuantiNova IC RNA, 3 x 500 µl QuantiNova Yellow Template Dilution Buffer, 3 x 1 ml QN ROX Reference Dye, 20 x 1.9 ml RNase-Free Water	208556

* Larger kit sizes available; please inquire.
† Requires use of the Plate Rotor 2 x 96 and Centrifuge 4-16S (TissueLyser II recommended for disruption and homogenization; QIAvac 96 optional).
‡ Larger kit sizes available; please inquire.
§ Requires use of QIAvac 96 or the QIAGEN 96-Well-Plate Centrifugation system.

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
September 2020	Updated branding of RNA protection products.