January 2022

QIAwave RNA Mini Handbook

For purification of total RNA from animal cells, animal tissues, bacteria, and yeast, and for RNA cleanup

Sample to Insight

QIAGEN

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Kit Contents

QIAwave RNA Mini Kit **Catalog no.** **Number of preps**	**(250)** **74536** **250**
RNeasy® Mini Spin Columns (pink)	250
Waste Tubes (2 ml)*	250
Buffer RLT*†	220 ml
Buffer RW1†	220 ml
Buffer RPE/C‡ (concentrate)	5 ml
RNase-Free Water	30 ml

* Also available separately. See page 86 for Ordering Information.

† Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for Safety Information.

‡ Before using for the first time, mix with 60 ml ultrapure water and 260 ml ethanol (96–100%) as indicated on the label to obtain a working solution.

Storage

The QIAwave RNA Mini Kit should be stored dry at room temperature (15–25°C) and is stable for at least 12 months after delivery, if not otherwise stated on the label.

Intended Use

The QIAwave RNA Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.

Buffer RLT contains guanidine thiocyanate, and Buffer RW1 contains a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is split, 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

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAwave RNA Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAwave RNA Mini Kit can be used for purification of total RNA from animal cells, animal tissues, and yeast, and for cleanup of RNA from crude RNA preps and enzymatic reactions (e.g., DNase digestion, proteinase digestion, RNA ligation, and labeling reaction).

The QIAwave RNA Mini Kit can also be used to purify total RNA from bacteria. In this case, we strongly recommend using the kit in combination with RNAprotect® Bacteria Reagent (available separately), which provides in vivo stabilization of RNA in bacteria to ensure reliable gene expression analysis. Various protocols for stabilizing and purifying RNA from different bacteria species are included in the *RNAprotect Bacteria Reagent Handbook*. For Ordering Information, see page 86. It is also possible to use the RNeasy Mini Kit to purify cytoplasmic RNA from animal cells. The protocol can be downloaded at **www.qiagen.com/resources/RNeasyMini**.

The QIAwave RNA Mini Kit is designed to purify RNA from small amounts of starting material. They provide a fast and simple method for preparing up to 100 µg total RNA per sample. The purified RNA is ready for use in downstream applications such as:

- RNA-seq
- RT-PCR and real-time RT-PCR
- Differential display
- cDNA synthesis
- Northern, dot, and slot blot analyses
- Primer extension
- Poly A+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

The QIAwave RNA Mini Kit allow the parallel processing of multiple samples in less than 30 minutes. Time-consuming and tedious methods, such as CsCl step-gradient ultracentrifugation and alcohol precipitation, or methods involving the use of toxic substances, such as phenol and/or chloroform, are replaced by the QIAwave RNA procedure.

Principle and procedure

RNA purification using QIAwave RNA technology

The QIAwave RNA procedure represents a well-established technology for RNA purification. This technology combines the selective binding properties of a silica-based membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bases to bind to the RNeasy silica membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate–containing buffer, which immediately inactivates RNases to ensure purification of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNeasy Mini spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30–100 µl water.

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

In this handbook, different protocols are provided for different starting materials. The protocols differ primarily in the lysis and homogenization of the sample and in the adjustment of the conditions for binding RNA to the RNeasy membrane. Once the sample is bound to the membrane, the protocols are similar (see Figure 1, page 9).

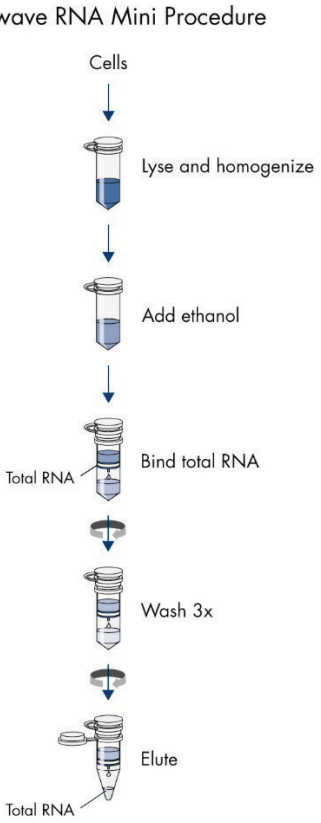

Figure 1. QIAwave RNA Mini procedure

RNA stabilization using RNAprotect technology

RNA stabilization is an absolute prerequisite for reliable gene expression analysis. Immediate stabilization of RNA in biological samples is necessary because, directly after harvesting the samples, changes in the gene expression pattern occur due to specific and nonspecific RNA degradation as well as to transcriptional induction. Such changes need to be avoided for all reliable quantitative gene expression analyses, such as microarray analyses, quantitative RT-PCR, such as TaqMan® and LightCycler® technology, and other nucleic acid-based technologies.

To prevent RNA degradation, RNAprotect Reagents can be purchased additionally to the QIAwave RNA Mini Kit. After harvesting, tissues are immediately submerged in RNAprotect Tissue Reagent, which rapidly permeates the tissues to stabilize and protect cellular RNA in situ. The reagent preserves RNA for up to 1 day at 37°C, 7 days at 15–25°C, or 4 weeks at 2–8°C, allowing transportation, storage, and shipping of samples without ice or dry ice. Alternatively, the samples can be archived at –30 to –15°C or –90 to –65°C. During storage or transport in RNAprotect Tissue Reagent, even at elevated temperatures (e.g., room temperature or 37°C), the cellular RNA remains intact and undegraded. RNAprotect technology allows large numbers of samples to be easily processed and replaces inconvenient, dangerous, and equipment-intensive methods, such as snap-freezing of samples in liquid nitrogen, storage at –90 to –65°C, cutting and weighing on dry ice, or immediate processing of harvested samples.

Note: RNAprotect Tissue Reagent is not for stabilization of RNA in animal cells, whole blood, plasma, or serum.

Description of protocols

Purification of total RNA from animal cells using spin technology

Up to 1 x 10^7 cells, depending on the cell line, are disrupted in Buffer RLT and homogenized. An overview of disruption and homogenization methods is given on page 22. Ethanol is then added to the lysate, creating conditions that promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water. All bind, wash, and elution steps are performed by centrifugation in a microcentrifuge.

Purification of total RNA from animal cells using vacuum/spin technology

Up to 1 x 10^6 cells, depending on the cell line, are disrupted in Buffer RLT and homogenized. An overview of disruption and homogenization methods is given on page 22. Ethanol is then

added to the lysate, creating conditions that promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water. The bind and wash steps are performed on a QIAvac 24 Plus vacuum manifold, and the final elution step is performed by centrifugation in a microcentrifuge.

Stabilization of RNA in harvested animal tissues

This protocol describes how to stabilize RNA in harvested animal tissues using RNAprotect Tissue Reagent. Purification of total RNA from the stabilized tissues can be subsequently carried out according to "Protocol: Purification of Total RNA from Animal Tissues" (page 44).

Purification of total RNA from animal tissues

Fresh, frozen, or RNAprotect-stabilized tissue (up to 30 mg, depending on the tissue type) is disrupted in Buffer RLT and homogenized. An overview of disruption and homogenization methods is given on page 22. Ethanol is then added to the lysate, creating conditions that promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

Purification of total RNA from yeast

This protocol is for the purification of total RNA from up to 5 x 10^7 yeast cells. Two alternative methods of disrupting yeast cell walls are provided: enzymatic lysis or mechanical disruption. In general, both methods function equally well. For some applications, enzymatic lysis might be preferable since no additional laboratory equipment is required. Mechanical disruption, however, is well suited for time-course experiments where enzymatic digestion incubations are not practical.

The enzymatic lysis method uses zymolyase or lyticase digestion of the cell walls to convert cells to spheroplasts, which are then used in the RNeasy procedure. For samples of up to 5 x 10^7 yeast cells, spheroplasts are separated from the digestion mixture by centrifugation

before being lysed. For samples of up to 2 x 10^7 yeast cells, the digestion mixture is used directly in the QIAwave RNA procedure without prior separation of the spheroplasts. After addition of Buffer RLT and ethanol, samples are loaded onto the RNeasy Mini spin column. Total RNA binds to the RNeasy membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

Using the mechanical disruption method, yeast cells are lysed and homogenized by high-speed agitation in the TissueLyser or other bead mill in the presence of glass beads and Buffer RLT. Ethanol is added to the lysate, creating conditions that promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

RNA cleanup

This protocol can be used to purify RNA from enzymatic reactions (e.g., DNase digestion, RNA labeling) or to desalt RNA samples (up to 100 µg RNA). Buffer RLT and ethanol are added to the sample to create conditions that promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

Automated purification of RNA on QIAcube instruments

Purification of RNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using RNeasy Mini Kits for purification of high-quality RNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.

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

- Ultrapure water
- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free pipette tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Microcentrifuge tubes for elution (1.5 ml or 2 ml)
- Optional: Waste Tubes (2 ml) (cat. no. 19211), in case customers need additional Waste Tubes
- 96–100% ethanol*
- Disposable gloves
- Equipment for sample disruption and homogenization (see page 22). Depending on the method chosen, one or more of the following are required:
  - Trypsin and PBS
  - QIAshredder homogenizer (see Ordering Information, page 86)
  - Blunt needle and syringe
  - Mortar and pestle
  - TissueLyser (see Ordering Information, page 86)
  - Rotor–stator homogenizer

For RNA purification from animal cells

- 70% ethanol*

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

For RNA purification from animal cells using vacuum technology

- QIAvac 24 Plus (cat. no. 19413); with the QIAvac Luer Adapter Set (cat. no. 19541); or other vacuum manifold with luer connectors and capable of dealing with vacuum pressures of –800 to –900 mbar
- QIAGEN Vacuum Pump (see page 86 for Ordering Information); or other vacuum pump capable of generating a vacuum pressure of –800 to –900 mbar and with a capacity of 18–20 liter/min.

  Note: Use of insufficient vacuum pressure may reduce RNA yield and purity. The RNeasy procedure requires higher vacuum pressures compared with other QIAGEN procedures. Most water pumps or house vacuums do not provide sufficient vacuum pressure.
- Optional: Vacuum Regulator (cat. no. 19530) to measure the pressure difference between the inside and outside of a vacuum system
- A vacuum pressure of –800 to –900 mbar should develop when RNeasy Mini spin columns are used on the vacuum manifold. Vacuum pressures exceeding –900 mbar should be avoided. The vacuum pressure is the pressure difference between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 mbar or 760 mm Hg) and can be regulated and measured using a pressure gauge or vacuum regulator. Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere.
- Optional: VacConnectors (cat. no. 19407)
- These disposable connectors fit between the RNeasy Mini spin columns and the luer extensions on the QIAvac 24 Plus. They prevent direct contact between the RNeasy Mini spin columns and luer connectors during RNA purification, avoiding any cross-contamination between samples. VacConnectors are discarded after single use.

For RNA purification from animal tissues

- 70% ethanol*
- Optional: Dithiothreitol (DTT)

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

For RNA purification from yeast using enzymatic lysis

- 70% ethanol*
- Buffer for enzymatic lysis
- In most cases, Buffer Y1 (containing sorbitol, EDTA, β-ME, and lyticase or zymolyase) can be used. See the protocol on page 52 for details on preparing Buffer Y1.

For RNA purification from yeast using mechanical disruption

- 70% ethanol*
- Glass beads, 0.45–0.55 mm diameter
- Concentrated nitric acid, deionized water, and baking oven
- TissueLyser or other bead-mill homogenizer

Suppliers of equipment for disruption and homogenization†

Bead-mill homogenizers and stainless steel and tungsten carbide beads can be purchased from:

- QIAGEN (TissueLyser system, see page 86 for Ordering Information)

Glass, stainless steel, and tungsten carbide beads can be purchased from:

- Retsch (**www.retsch.de**)

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

Important Notes

Working with QIAwave products

Preparation of functional buffers

Selected buffers are provided as concentrates in 15 ml bottles to shrink buffer bottles and reduce the amount of plastics used. Before using the kit for the first time, concentrates have to be reconstituted to receive the functional buffer. This is done with either water or water and ethanol. To reconstitute, the entire volume of the buffer concentrate should be transferred from the 15 ml bottle into a suitably sized glass bottle, either by using a pipette or by pouring. Subsequently, the appropriate volume of water or water and ethanol should be added as indicated on the 15 ml bottle. Afterwards, the glass bottle should be capped tightly and the reconstituted buffer mixed thoroughly by inverting.

In case of the QIAwave RNA Mini Kit, the above is true for Buffer RPE/C. For detailed instructions, see "Things to do before starting" on page 29 or watch our educational "how-to video" **www.qiagen.com/qiawavebuffer**.

Water quality used for preparation of functional buffers

We strongly recommend using highly pure water for reconstitution. Ultrapure water (also known as type 1 water) with a resistivity of 18.2 MΩ-cm at 25 °C, such as from a Milli-Q® system, works well. As fallback in case customer does not have access to type 1 water, QIAGEN offers nuclease-free water (5 liters, cat. no. 129117; 1000 ml, cat. no. 129115). Please note that these items need to be purchased separately. Usage of tap water should be avoided as this can have detrimental impact on the extraction of the target analyte.

Glassware

We suggest the use of glass bottles for the reconstitution of buffers. Glass bottles can be cleaned, sterilized, and reused more easily than plastic bottles, which will further reduce the plastic footprint of the kit.

Glassware should be treated before use to ensure that it is RNase free. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 h) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Labeling of functional buffers in glass bottles

Buffer concentrates are labelled with piggyback labels from which the upper label can be peeled off and transferred onto the glass bottles containing the functional buffer prepared before using the kit for the first time.

Waste Tubes

The newly introduced Waste Tube is made of recycled plastic recovered from post-consumer plastic waste and can differ in color from lot to lot due to slight differences in composition of the raw material. This however has no effect on its intended use to collect the flow-through from sample binding and membrane washing. After each of these steps, the flow-through is discarded and the Waste Tube is reused. The Waste Tube is only used for process waste and never comes into direct contact with the analyte of interest.

For detailed instructions watch our educational "how-to video"

Elution tubes

Elution tubes are not included in the kit. This allows the flexibility to use elution tubes of one´s own choice and purchase them in, for example, eco-friendlier big packs.

Recycling information

Please visit **www.qiagen.com/recycling-card** to learn more about how to recycle kit components.

Determining the amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. The maximum amount that can be used is determined by:

- The type of sample and its RNA content
- The volume of Buffer RLT required for efficient lysis
- The RNA binding capacity of the RNeasy spin column

When processing samples containing high amounts of RNA, less than the maximum amount of starting material shown in Table 1 should be used, so that the RNA binding capacity of the RNeasy spin column is not exceeded.

Table 1. RNeasy Mini Spin Column specifications

Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
RNA size distribution	RNA >200 nucleotides
Minimum elution volume	30 µl
Maximum amount of starting material	
Animal cells	1×10^7
Animal tissues	30 mg
Yeast	5×10^7

When processing samples containing average or low amounts of RNA, the maximum amount of starting material shown in Table 1 can be used. However, even though the RNA binding capacity of the RNeasy spin column is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy spin column membrane, resulting in lower RNA yield and purity.

More information on using the correct amount of starting material is given in each protocol. Table 2 (page 21) shows expected RNA yields from various sources.

Note: If the binding capacity of the RNeasy spin column is exceeded, RNA yields will not be consistent and may be reduced. If lysis of the starting material is incomplete, RNA yields will be lower than expected, even if the binding capacity of the RNeasy spin column is not exceeded.

Handling and storing starting material

RNA in animal and plant tissues is not protected after harvesting until the sample is treated with RNAprotect Tissue Reagent (animal tissues only), flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –90 to –65°C, or immediately immersed in RNAprotect Tissue Reagent.

The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RLT (lysis buffer), samples can be stored at –90 to –65°C for months.

Table 2. Typical yields of total RNA with RNeasy Mini Spin Columns

Source	Yield of total RNA* (µg)
Cell cultures (1 x 10^6 cells)	
NIH/3T3	10
HeLa	15
COS-7	35
LMH	12
Huh	15
Mouse/rat tissues (10 mg)	
Embryo (13 day)	25
Kidney	20–30
Liver	40–60
Spleen	30–40
Thymus	40–50
Lung	10–20
Yeast (1 x 10^7 cells)	
Saccharomyces cerevisiae	25

* Amounts can vary due to factors such as species, developmental stage, and growth conditions. Since the RNeasy procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA, and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Animal and yeast cells can be pelleted and then stored at –90 to –65°C until required for RNA purification. However, if performing RNA purification from yeast cells with enzymatic lysis, only freshly harvested samples can be used.

Disrupting and homogenizing starting material

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 cell walls and 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 RNA 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 spin column membrane and therefore significantly reduced RNA yields.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 3 (page 23) gives an overview of different disruption and homogenization methods and is followed by a detailed description of each method. This information can be used as a guide to choose the appropriate methods for your starting material.

Note: After storage in RNAprotect Tissue Reagent, tissues become slightly harder than fresh or thawed tissues. Disruption and homogenization of these tissues, however, is usually not a problem.

Table 3. Disruption and homogenization methods

Sample	Disruption method	Homogenization method	Comments
Animal cells	Addition of lysis buffer	Rotor–stator homogenizer or QIAshredder homogenizer or syringe and needle	If processing ≤1 x 10^5 cells, lysate can be homogenized by vortexing
Animal tissues	TissueLyser	TissueLyser	The TissueLyser gives results comparable to using a rotor–stator homogenizer
	Rotor–stator homogenizer	Rotor–stator homogenizer	Simultaneously disrupts and homogenizes
	Mortar and pestle	QIAshredder homogenizer or syringe and needle	Rotor–stator homogenizer usually gives higher yields than mortar and pestle
Yeast	Enzymatic digestion of cell wall followed by lysis of spheroplasts	Vortexing	
	TissueLyser with glass beads	TissueLyser with glass beads	TissueLyser simultaneously disrupts and homogenizes; cannot be replaced by vortexing

Disruption and homogenization using the TissueLyser system

In bead milling, cells and 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
- Disintegration time

For animal tissues, the optimal beads are 3–7 mm diameter stainless steel beads, and for yeast cells, the optimal beads are 0.5 mm diameter glass beads. It is essential that glass beads are prewashed in concentrated nitric acid. All other disruption parameters must be determined empirically for each application. The protocol for RNA purification from yeast (page 52) describes how to perform mechanical disruption of yeast cells with glass beads. For guidelines on disruption and homogenization of animal tissues using the TissueLyser system, refer to the *TissueLyser Handbook*. 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 s depending on the toughness and size of the sample. Rotor–stator homogenizers can also be used to homogenize cell lysates. 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 the side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes. Probes with diameters of 5 and 7 mm are suitable for volumes up to 300 µl and can be used for homogenization in microcentrifuge tubes. Probes with a diameter of 10 mm or longer require larger tubes. In addition, round-bottomed tubes allow more efficient homogenization than conical-bottomed tubes.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the animal or plant tissue immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into a liquid-nitrogen cooled, appropriately sized tube and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer and continue as quickly as possible with the homogenization according to one of the 2 methods below.

Note: Grinding the sample using a mortar and pestle will disrupt the sample but will not homogenize it. Homogenization must be performed afterwards.

Homogenization using QIAshredder homogenizers

Using QIAshredder homogenizers is a fast and efficient way to homogenize cell and tissue lysates without cross-contamination of samples. Up to 700 µl of lysate is loaded onto a QIAshredder spin column placed in a 2 ml collection tube and spun for 2 min at maximum speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column. QIAshredder spin columns can be purchased separately for use with the QIAwave RNA Mini Kit. See page 86 for Ordering Information.

Homogenization using a syringe and needle

Cell and tissue lysates can be homogenized using a syringe and needle. Lysate is passed through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may be required to facilitate handling and minimize loss.

Eliminating genomic DNA contamination

Generally, DNase digestion is not required with the QIAwave RNA Mini Kit since RNeasy silica-membrane technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundance target). In these cases, residual DNA can be removed by optional on-column DNase digestion using the RNase-Free DNase Set (see Appendix D, page 77). The DNase is efficiently removed in subsequent wash steps. Alternatively, residual DNA can be removed by a DNase digestion after RNA purification (see Appendix E, page 80). The DNase digestion can then be cleaned up, if desired, using "Protocol: RNA Cleanup" (page 61).

If the purified RNA will be used in real-time, two-step RT-PCR, we recommend using the QuantiTect® Reverse Transcription Kit. The kit provides a fast and convenient procedure, enabling cDNA synthesis and genomic DNA removal in only 20 min. For Ordering Information, see page 86.

Protocol: Purification of Total RNA from Animal Cells Using Spin Technology

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. The minimum amount is generally 100 cells, while the maximum amount depends on:

- The RNA content of the cell type
- The RNA binding capacity of the RNeasy spin column (100 µg RNA)
- The volume of Buffer RLT required for efficient lysis (the maximum volume of Buffer RLT that can be used limits the maximum amount of starting material to 1 x 10^7 cells)

RNA content can vary greatly between cell types. The following examples illustrate how to determine the maximum amount of starting material:

- COS cells have high RNA content (approx. 35 µg RNA per 10^6 cells). Do not use more than 3 x 10^6 cells; otherwise, the RNA binding capacity of the RNeasy spin column will be exceeded.
- HeLa cells have average RNA content (approx. 15 µg RNA per 10^6 cells). Do not use more than 7 x 10^6 cells; otherwise, the RNA binding capacity of the RNeasy spin column will be exceeded.
- NIH/3T3 cells have low RNA content (approx. 10 µg RNA per 10^6 cells). The maximum amount of starting material (1 x 10^7 cells) can be used.

If processing a cell type not listed in Table 2 (page 21) and if there is no information about its RNA content, we recommend starting with no more than 3–4 x 10^6 cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and purity.

Counting cells is the most accurate way to quantitate the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is given in Table 4.

Table 4. Growth area and number of HeLa cells in various culture vessels

Cell-culture vessel	Growth area (cm^2)*	Number of cells†
Multiwell plates		
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x 10^6
Dishes		
35 mm	8	1 x 10^6
60 mm	21	2.5 x 10^6
100 mm	56	7 x 10^6
145–150 mm	145	2 x 10^7
Flasks		
40–50 ml	25	3 x 10^6
250–300 ml	75	1 x 10^7
650–750 ml	162–175	2 x 10^7

* Per well, if multiwell plates are used; varies slightly depending on the supplier.

† Cell numbers are given for HeLa cells (approx. length = 15 µm), assuming confluent growth. Cell numbers will vary for different kinds of animal cells, which vary in length from 10 to 30 µm.

Important points before starting

- If using the QIAwave RNA Min Kit for the first time, read "Important Notes" (page 17).
- If working with RNA for the first time, read Appendix A (page 68).
- Cell pellets can be stored at –90 to –65°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2. Homogenized cell

lysates from step 3 can be stored at –90 to –65°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4. Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.

- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β-ME to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.
- Preparation of Buffer RPE/C: Transfer the entire volume of Buffer RPE/C from the 15 ml bottle into a glass bottle larger than 325 ml, either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (5 liters, cat. no. 129117; 1000 ml, cat. no. 129115) and 260 ml ethanol (96–100%) to obtain a final volume of 325 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- Preassemble RNeasy Mini spin columns with Waste Tubes.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 77).

Procedure

1. Harvest cells according to step 1a or 1b.

   1a. Cells grown in suspension (do not use more than 1 x 10^7 cells):
   Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and proceed to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.

   1b. Cells grown in a monolayer (do not use more than 1 x 10^7 cells):
   Cells can be either lysed directly in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

   To lyse cells directly:

   Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.

   To trypsinize and collect cells:

   Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min. Completely aspirate the supernatant, and proceed to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.

2. Disrupt the cells by adding Buffer RLT.

   For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of Buffer RLT (see Table 5). Vortex or pipet to mix, and proceed to step 3.

   Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

Table 5. Volumes of Buffer RLT for lysing pelleted cells

Number of pelleted cells	Volume of Buffer RLT (µl)
<5 x 10^6	350
5 x 10^6 – 1 x 10^7	600

   For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RLT (see Table 6) to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

Table 6. Volumes of Buffer RLT for direct cell lysis

Dish diameter (cm)	Volume of Buffer RLT (µl)*
<6	350
6–10	600

* Regardless of the cell number, use the buffer volumes indicated to completely cover the surface of the dish.

3. Homogenize the lysate according to step 3a, 3b, or 3c.

   See “Disrupting and homogenizing starting material”, page 22, for more details on homogenization. If processing ≤1 x 10^5 cells, homogenize by vortexing for 1 min. After homogenization, proceed to step 4.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy spin column. Homogenization with a rotor–stator or QIAshredder homogenizer generally results in higher RNA yields than with a syringe and needle.

3a. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Proceed to step 4.

3b. Homogenize the lysate for 30 s using a rotor–stator homogenizer. Proceed to step 4.

3c. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.

4. Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting.
   Important: Do not centrifuge.

   Note: The volume of lysate may be less than 350 or 600 µl due to loss during homogenization.

   Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

5. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a RNeasy spin column placed in a 2 ml Waste Tube (both supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and reuse the Waste Tube in step 6.*
   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.*

   Optional: If performing optional on-column DNase digestion (see "Eliminating genomic DNA contamination", page 26), follow Appendix D (page 77), steps 1–4, after performing this step.

6. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step in step 7.*
   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

* The flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

Skip this step if performing optional on-column DNase digestion (page 77).

7. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step 8.
   Note: Buffer RPE/C is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see "Things to do before starting").
8. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.
   The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
   Note: After centrifugation, carefully remove the RNeasy spin column from the Waste Tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
9. **Optional**: Discard the flow-through and reuse the Waste Tube. Close the lid gently, and centrifuge at full speed for 1 min.
   Perform this step to eliminate any possible carryover of Buffer RPE/C, or if residual flow-through remains on the outside of the RNeasy spin column after step 8.
10. Place the RNeasy spin column in a new 1.5 ml collection tube (not supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.
11. If the expected RNA yield is >30 µg, repeat step 10 using another 30–50 µl RNase-free water, or using the eluate from step 10 (if high RNA concentration is required). Reuse the collection tube from step 10.
    If using the eluate from step 10, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Protocol: Purification of Total RNA from Animal Cells Using Vacuum/Spin Technology

Determining the correct amount of starting material

- See "Determining the correct amount of starting material", page 27.

Important points before starting

- If using the RNeasy Kit for the first time, read "Important Notes" (page 17). If working with RNA for the first time, read Appendix A (page 68).
- Cell pellets can be stored at –90 to –65°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2. Homogenized cell lysates from step 3 can be stored at –90 to –65°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- Do not use more than 10^6 cells per prep. The cell numbers in each prep should be similar (no more than a two-fold difference between the highest and lowest) to allow uniform flow rates on the vacuum manifold.

- Between loading steps, switch off the vacuum and ventilate the manifold to maintain uniform conditions for each sample. This can be done with a vacuum regulator inserted between the vacuum source and the vacuum manifold.
- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- Always leave the lids of the RNeasy spin columns open while applying vacuum.
- The flow-through from each vacuum step is collected in the QIAvac 24 Plus, which can hold the waste from 24 samples. At the end of the procedure, discard the liquid waste and clean the vacuum manifold as described in the *QIAvac 24 Plus Handbook*. If using other vacuum manifolds, follow the supplier's instructions.

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β-ME to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.
- Preparation of Buffer RPE/C: Transfer the entire volume of Buffer RPE/C from the 15 ml bottle into a glass bottle larger than 325 ml , either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (5 liters, cat. no. 129117; 1000 ml, cat. no. 129115) and 260 ml ethanol (96–100%) to obtain a final volume of 325 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 77).
- Set up the vacuum manifold according to the supplier's instructions. If using the QIAvac Plus 24, refer to the *QIAvac 24 Plus Handbook*. Insert each RNeasy spin column into a luer connector.

Procedure

1. Harvest cells according to step 1a or 1b.

   1a. Cells grown in suspension (do not use more than 1 x 10^6 cells):
   Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and proceed to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.

   1b. Cells grown in a monolayer (do not use more than 1 x 10^6 cells):
   Cells can be either lysed directly in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

   To lyse cells directly:

   Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.

   To trypsinize and collect cells:

   Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min. Completely aspirate the supernatant, and proceed to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.

2. Disrupt the cells by adding Buffer RLT.

   For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 350 µl Buffer RLT. Vortex or pipet to mix, and proceed to step 3.

   Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

   For direct lysis of cells grown in a monolayer, add 350 µl Buffer RLT to the cell-culture dish (if 350 µl is not enough to cover the dish, use 600 µl Buffer RLT instead; be sure then to use 600 µl of 70% ethanol in step 4). Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

3. Homogenize the lysate according to step 3a, 3b, or 3c.

   See "Disrupting and homogenizing starting material", page 22, for more details on homogenization. If processing ≤1 x 10^5 cells, homogenize by vortexing for 1 min. After homogenization, proceed to step 4.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy spin column. Homogenization with a rotor–stator or QIAshredder homogenizer generally results in higher RNA yields than with a syringe and needle.

   3a. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Proceed to step 4.

   3b. Homogenize the lysate for 30 s using a rotor–stator homogenizer. Proceed to step 4.

   3c. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.

4. Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting.

   Important: Do not centrifuge.

   Note: The volume of lysate may be less than 350 or 600 µl due to loss during homogenization.

Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

5. Transfer 700 µl of each sample from step 4, including any precipitate that may have formed, to each RNeasy spin column on the vacuum manifold.
6. Switch on the vacuum. Apply vacuum until transfer is complete. Switch off the vacuum and ventilate the vacuum manifold.

   Make sure that the vacuum manifold is assembled correctly before loading. The flow-through is collected in the QIAvac 24 Plus.* If a spin column clogs, switch off the vacuum, ventilate, and try again. If it still clogs, continue with "Protocol: Purification of Total RNA from Animal Cells Using Spin Technology", page 27.

   Note: Be sure to switch off the vacuum and ventilate the manifold between pipetting steps to maintain uniform conditions for each sample.
7. If necessary, repeat steps 5 and 6 with the remaining volume (approx. 500 µl) of each sample.

   The flow-through is collected in the QIAvac 24 Plus.*
8. Add 700 µl Buffer RW1 to each RNeasy spin column.
9. Switch on the vacuum. Apply vacuum until transfer is complete. Switch off the vacuum and ventilate the vacuum manifold.

   The flow-through is collected in the QIAvac 24 Plus.*
10. Add 500 µl Buffer RPE/C to each RNeasy spin column.

    Note: Buffer RPE/C is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see "Things to do before starting").
11. Switch on the vacuum. Apply vacuum until transfer is complete. Switch off the vacuum and ventilate the vacuum manifold.

    The flow-through is collected in the QIAvac 24 Plus
12. Add 500 µl Buffer RPE/C to each RNeasy spin column.

* The flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

13. Switch on the vacuum. Apply vacuum until transfer is complete. Switch off the vacuum and ventilate the vacuum manifold.

    The flow-through is collected in the QIAvac 24 Plus.

14. Remove the RNeasy spin columns from the vacuum manifold, and place each in a 2 ml Waste Tube (supplied). Close the lids gently, and centrifuge at full speed for 1 min.

15. Place each RNeasy spin column in a new 1.5 ml collection tube (not supplied). Add 30–50 µl RNase-free water directly to each spin column membrane. Close the lids gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

16. If the expected RNA yield is >30 µg, repeat step 15 using another 30–50 µl RNase-free water, or using the eluate from step 15 (if high RNA concentration is required). Reuse the collection tubes from step 15.

    If using the eluate from step 15, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Protocol: Stabilization of RNA in Harvested Animal Tissues

This protocol describes how to stabilize and store human and animal tissues in RNAprotect Tissue Reagent, For RNA purification from stabilized tissues, see “Protocol: Purification of Total RNA from Animal Tissues”, page 44.

Important notes about RNAprotect Tissue Reagent

RNA in harvested animal tissue is not protected until the tissue is completely submerged in a sufficient volume of RNAprotect Tissue Reagent. After harvesting, the tissue should be **immediately** placed in a**t least 10 volumes of the reagent (or approx. 10 µl reagent per 1 mg tissue)**. Larger volumes can be used if necessary or desired. Smaller volumes may lead to RNA degradation during storage. Storage containers should be wide enough so that the reagent covers the entire tissue. Storage containers or tubes with large diameters may require more reagent to completely cover the tissue. The procedures for tissue harvesting and RNA stabilization should be carried out as quickly as possible.

Tissue size is critical for successful RNA stabilization with RNAprotect Tissue Reagent. Immediately upon contact, the reagent diffuses into the surface layer and outer portions of solid tissues. To ensure rapid and reliable stabilization of RNA even in the inner parts of solid tissues, the sample must be cut into slices **less than 0.5 cm thick**. The slices can be any convenient size, provided one dimension of the sample is <0.5 cm. If the slices are thicker than 0.5 cm, the reagent will diffuse too slowly into the interior of the sample and RNA degradation will occur. Small organs such as rat kidney and spleen or most mouse organs (except liver) do not require slicing: the entire organ can be placed in RNAprotect Tissue Reagent.

The following guide may help you to determine the amount of RNAprotect Tissue Reagent required for RNA stabilization:

- A cube of rat kidney with a 5 mm edge length ([5 mm]^3 = 125 mm^3 = 125 µl) weighs 150–175 mg and requires at least 1.5–1.75 ml of the reagent.
- A 3 mm cube ([3 mm]^3 = 27 mm^3 = 27 µl) of most animal tissues weighs 30–35 mg and requires at least 300–350 µl of the reagent.

Although weighing tissues is generally more accurate, RNA in unstabilized tissues will degrade during weighing. In some cases, however, it may be more convenient to quickly estimate the weight of tissue pieces. Average weights of various entire adult mouse organs and the corresponding amounts of RNAprotect Tissue Reagent to use are given in Table 7.

Table 7. Tissue weights and amounts of RNAprotect Tissue Reagent

Mouse organ	Weight (mg)	Amount of RNAprotect Tissue Reagent (ml)
Kidney	180–250	≤2.5
Spleen	100–160	≤1.6
Lung	190–210	≤2.1
Heart	100–170	≤1.7
Liver	1000–1800	≤18

Important points before starting

- If using the QIAwave RNA Mini Kit for the first time, read "Important Notes" (page 17).
- RNAprotect Tissue Reagent may form a precipitate during storage below room temperature. Before using the reagent, redissolve the precipitate by heating to 37°C with agitation.
- Only fresh, unfrozen tissues can be stabilized using RNAprotect Tissue Reagent. Previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

Procedure

1. Before excising the tissue sample, estimate the volume (or weight) of the sample to be stabilized in RNAprotect Tissue Reagent.
2. Determine the appropriate volume of RNAprotect Tissue Reagent for preserving the tissue. At least 10 volumes of the reagent (or approx. 10 µl reagent per 1 mg of tissue) is required. Pipet the correct amount of reagent into an appropriate collection vessel.

   Note: Be sure to completely submerge the tissue in RNAprotect Tissue Reagent. For details, see "Important notes about RNAprotect Tissue Reagent", page 40.
3. Excise the tissue sample from the animal and, if necessary, cut it into slices less than 0.5 cm thick. Perform this step as quickly as possible and proceed immediately to step 4.

   Note: For effective RNA stabilization, the tissue sample must be less than 0.5 cm thick. For details, see "Important notes about RNAprotect Tissue Reagent", page 40.
4. Completely submerge the tissue piece(s) in the collection vessel containing RNAprotect Tissue Reagent from step 2.

   Note: The tissue sample must be immediately submerged in RNAprotect Tissue Reagent to protect the RNA.
5. Store the tissue submerged in RNAprotect Tissue Reagent for up to 4 weeks at 2–8°C, up to 7 days at 15–25°C, or up to 1 day at 37°C.

   For archival storage at –30 to –15°C, first incubate the tissue overnight in the reagent at 2–8°C. Then transfer the tissue, in the reagent, to –30 to –15°C for storage.

   For archival storage at –90 to –65°C, first incubate the tissue overnight in the reagent at 2–8°C. Then remove the tissue from the reagent, and transfer it to –90 to –65°C for storage.

   Note: Lower temperatures are recommended for longer storage (e.g., 2–8°C for up to 4 weeks instead of 37°C or room temperature; –30 to –15°C or –90 to –65°C for longer storage).

Tissues stored in RNAprotect Tissue Reagent at –30 to –15°C may not freeze. The low temperature may cause the formation of crystals or a precipitate in the reagent. This will not affect subsequent RNA purification. There is no need to redissolve the precipitate.

RNAprotect-stabilized tissues stored at –30 to –15°C or –90 to –65°C can be thawed at room temperature and frozen again for up to 20 freeze–thaw cycles without affecting RNA quality or yield.

If transporting tissue samples in RNAprotect Tissue Reagent, ensure that the tissues always remain submerged in the reagent. Either keep the tubes upright during transport or fill the tubes completely with RNAprotect Tissue Reagent.

6. After storage, continue with "Protocol: Purification of Total RNA from Animal Tissues" (page 44).

Protocol: Purification of Total RNA from Animal Tissues

Determining the correct amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. A maximum amount of 30 mg fresh or frozen tissue or 15–20 mg RNAprotect stabilized tissue (which is partially dehydrated) can generally be processed. For most tissues, the RNA binding capacity of the RNeasy spin column and the lysing capacity of Buffer RLT will not be exceeded by these amounts. Average RNA yields from various tissues are given in Table 2 (page 21).

Some tissues, such as spleen, parts of brain, lung, and thymus, are more difficult to lyse or tend to form precipitates during RNA purification. The volume of Buffer RLT may need to be increased to facilitate complete homogenization and to avoid significantly reduced RNA yields, DNA contamination, or clogging of the RNeasy spin column. See the procedure below for details.

RNA yields from fibrous tissues, such as skeletal muscle, heart, and skin, may be low due to the abundance of contractile proteins, connective tissue, and collagen. For maximum RNA yields from these tissues, we recommend using the RNeasy Fibrous Tissue Mini Kit instead. See page 86 for Ordering Information.

Greater RNA yields from fatty tissues, such as brain and adipose tissue, can be achieved using the RNeasy Plus Universal Mini Kit, which uses QIAzol Lysis Reagent for optimal tissue lysis. See page 86 for Ordering Information.

If there is no information about the nature of your starting material, we recommend starting with no more than 10 mg tissue. Depending on RNA yield and purity, it may be possible to use up to 30 mg tissue in subsequent preparations.

Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and quality.

Weighing tissue is the most accurate way to quantitate the amount of starting material. As a guide, a 3 mm cube (27 mm^3) of most animal tissues weighs 30–35 mg.

Important points before starting

- If using the QIAwave RNA Mini Kit for the first time, read "Important Notes" (page 17). If working with RNA for the first time, read Appendix A (page 68).
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see protocol on page 40). Tissues can be stored in the reagent for up to 1 day at 37°C, 7 days at 15–25°C, or 4 weeks at 2–8°C, or archived at –30 to –15°C or –90 to –65°C.
- Fresh, frozen, or RNAprotect-stabilized tissues can be used. Tissues can be stored at –90 to –65°C for several months. Flash-freeze tissues in liquid nitrogen, and immediately transfer to –90 to –65°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized tissue lysates from step 4 can also be stored at –90 to –65°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 5. Avoid prolonged incubation, which may compromise RNA integrity.
- If desired, more than 30 mg tissue can be disrupted and homogenized at the start of the procedure (increase the volume of Buffer RLT proportionately). Use a portion of the homogenate corresponding to no more than 30 mg tissue for RNA purification, and store the rest at –90 to –65°C.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.

- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-ME must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.
- Alternatively, add 20 µl of 2 M DTT per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Preparation of Buffer RPE/C: Transfer the entire volume of Buffer RPE/C from the 15 ml bottle into a glass bottle larger than 325 ml, either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (5 liters, cat. no. 129117; 1000 ml, cat. no. 129115)and 260 ml ethanol (96–100%) to obtain a final volume of 325 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- Preassemble RNeasy Mini spin columns with Waste Tubes.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 77).

Procedure

1. Excise the tissue sample from the animal or remove it from storage. Remove RNAprotect-stabilized tissues from the reagent using forceps. Determine the amount of tissue. Do not use more than 30 mg.

   Weighing tissue is the most accurate way to determine the amount.

   Note: If the tissues were stored in RNAprotect Reagent at –30 to –15°C, be sure to remove any crystals that may have formed.

2. Follow either step 2a or 2b.

2a. For RNAprotect-stabilized tissues:
If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed to step 3.

If using only a portion of the tissue, cut it on a clean surface. Weigh the piece to be used, and place it into a suitably sized vessel for disruption and homogenization. Proceed to step 3.

RNA in RNAprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect Tissue Reagent. Previously stabilized tissues can be stored at –90 to –65°C without the reagent.

2b. For unstabilized fresh or frozen tissues:
If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately to step 3.

If using only a portion of the tissue, weigh the piece to be used, and place it into a suitably sized vessel for disruption and homogenization. Proceed immediately to step 3.

RNA in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent, flash-frozen, or disrupted and homogenized in step 3. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

Note: The remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA (see protocol on page 36). However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

3. Disrupt the tissue and homogenize the lysate in Buffer RLT (do not use more than 30 mg tissue) according to step 3a, 3b, 3c, or 3d.

   See "Disrupting and homogenizing starting material", page 22, for more details on disruption and homogenization.

   Note: Ensure that β-ME is added to Buffer RLT before use (see "Things to do before starting").

   After storage in RNAprotect Tissue Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem. For easier disruption and homogenization, we recommend using 600 µl Buffer RLT.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy spin column. Homogenization with the TissueLyser and rotor–stator homogenizers generally results in higher RNA yields than with other methods.

Table 8. Volumes of Buffer RLT for Tissue Disruption and Homogenization

Amount of starting material (mg)	Volume of Buffer RLT (µl)
<20	350 or 600*
20–30	600

* Use 600 µl Buffer RLT for tissues stabilized in RNAprotect Tissue Reagent or for difficult-to-lyse tissues.

3a. Disruption and homogenization using a rotor–stator homogenizer:
Place the weighed (fresh, frozen, or RNAprotect stabilized) tissue in a suitably sized vessel. Add the appropriate volume of Buffer RLT (see Table 8). Immediately disrupt and homogenize the tissue using a conventional rotor–stator homogenizer until it is uniformly homogeneous (usually 20–40 s). Proceed to step 4.

3b. Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer:
Immediately place the weighed (fresh, frozen, or RNAprotect-stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue

powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add the appropriate volume of Buffer RLT (see Table 8). Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Proceed to step 4.

3c. Disruption using a mortar and pestle followed by homogenization using a needle and syringe:
Immediately place the weighed (fresh, frozen, or RNAprotect-stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add the appropriate volume of Buffer RLT (see Table 8), and homogenize by passing the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-free syringe. Proceed to step 4.

3d. Disruption and homogenization using the TissueLyser: See the *TissueLyser Handbook*. Then proceed to step 4.

4. Centrifuge the lysate for 3 min at full speed. Carefully remove the supernatant by pipetting, and transfer it to a new microcentrifuge tube (not supplied). Use only this supernatant (lysate) in subsequent steps.

   In some preparations, very small amounts of insoluble material will be present after the 3 min centrifugation, making the pellet invisible.

5. Add 1 volume of 70% ethanol* to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Proceed immediately to step 6.

   Note: The volume of lysate may be less than 350 or 600 µl due to loss during homogenization and centrifugation in steps 3 and 4.

* Using 50% ethanol (instead of 70% ethanol) may increase RNA yields from liver samples.

Note: Precipitates may be visible after addition of ethanol. This does not affect the procedure.

6. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml Waste Tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and reuse the Waste Tube in step 7.*

   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.*

   Optional: If performing optional on-column DNase digestion (see "Eliminating genomic DNA contamination", page 26), follow Appendix D (page 77), steps 1–4, after performing this step.

7. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step 8.*

   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

   Skip this step if performing optional on-column DNase digestion (page 69).

8. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step 9.

   Note: Buffer RPE/C is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see "Things to do before starting").

9. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.

* The flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

10. **Optional**: Discard the flow-through and reuse the Waste Tube. Close the lid gently, and centrifuge at full speed for 1 min.

    Perform this step to eliminate any possible carryover of Buffer RPE/C, or if residual flow-through remains on the outside of the RNeasy spin column after step 9.

11. Place the RNeasy spin column in a new 1.5 ml collection tube (not supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

12. If the expected RNA yield is >30 µg, repeat step 11 using another 30–50 µl RNase-free water, or using the eluate from step 11 (if high RNA concentration is required). Reuse the collection tube from step 11.

    If using the eluate from step 11, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Protocol: Purification of Total RNA from Yeast

Disrupting yeast cells

This protocol for purifying total RNA from yeast provides 2 alternative methods of disrupting the walls of yeast cells:

- **Enzymatic lysis**: This method requires digestion of the cell wall with zymolyase or lyticase to convert cells to spheroplasts. For samples of up to 5 x 10^7 yeast cells, spheroplasts are separated from the digestion mixture by centrifugation before being lysed. For samples of up to 2 x 10^7 yeast cells, the digestion mixture is used directly in the QIAwave RNA procedure without prior separation of the spheroplasts.
- **Mechanical disruption**: This method uses high-speed agitation in the TissueLyser or other bead mill in the presence of glass beads and Buffer RLT to lyse yeast cells and release RNA.

In general, both methods function equally well. For some applications, enzymatic lysis might be preferable since no additional laboratory equipment is required. Mechanical disruption, however, is well suited for timecourse experiments where enzymatic digestion incubations are not practical.

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. The maximum amount depends on:

- The RNA binding capacity of the RNeasy spin column (100 µg RNA)
- The volume of Buffer RLT required for efficient lysis (the maximum volume of Buffer RLT that can be used limits the maximum amount of starting material to 5 x 10^7 yeast cells)

When processing cultures containing high amounts of RNA, fewer cells should be used, so that the RNA binding capacity of the RNeasy spin column is not exceeded. When processing cultures containing lower amounts of RNA, the maximum number of cells can be used. However, even though the RNA binding capacity of the RNeasy spin column is not reached, using more cells would lead to incomplete lysis, resulting in lower RNA yield and purity.

Usually 2 x 10^6 to 5 x 10^7 yeast cells can be processed. Depending on the strain and growth conditions, 30–100 µg RNA can be expected from 4 x 10^7 cells.

If there is no information about the RNA content of your starting material, we recommend starting with no more than 2 x 10^7 yeast cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and purity.

Yeast growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relationship between OD values and cell numbers in yeast cultures. Cell density is influenced by a variety of factors (e.g., species, media, and shaker speed), and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between the sample and the detector and therefore readings vary between different types of spectrophotometer. In addition, different species show different OD values at defined wavelengths (e.g., 600 or 436 nm).

We therefore recommend calibrating the spectrophotometer by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., Ausubel, F.M. et al., eds. [1991] Current Protocols in Molecular Biology. New York: Wiley Interscience). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range; the dilution factor should then be used to calculate the number of cells per milliliter.

The following values may be used as a rough guide. An *S. cerevisiae* culture containing 1–2 x 10^7 cells per milliliter, diluted 1 in 4, gives an OD600 value of approximately 0.25 with a Beckman DU®-7400 spectrophotometer or 0.125 with a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1 or 0.5, respectively, for 1–2 x 10^7 yeast cells per milliliter.

Important points before starting

- If using the QIAwave RNA Kit for the first time, read "Important Notes" (page 17). If working with RNA for the first time, read Appendix A (page 68).
- Yeast cells should be harvested in log-phase growth.
- **Important**: If performing enzymatic lysis (step 1a or 1b), use only freshly harvested cells.
- If performing mechanical disruption, cell pellets can be stored at –90 to –65°C for later use or used directly in the procedure. Homogenized cell lysates from step 1c can be stored at –90 to –65°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. Proceed to step 2.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- After enzymatic lysis or mechanical disruption, perform all steps of the procedure at room temperature. During the procedure, work quickly.
- After harvesting the cells, perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-ME must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.

- Preparation of Buffer RPE/C: Transfer the entire volume of Buffer RPE/C from the 15 ml bottle into a glass bottle larger than 325 ml , either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (5 liters, cat. no. 129117; 1000 ml, cat. no. 129115) and 260 ml ethanol (96–100%) to obtain a final volume of 325 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- Preassemble RNeasy Mini spin columns with Waste Tubes.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 77).
- **If performing enzymatic lysis (step 1a or 1b)**, prepare Buffer Y1 as follows. Prepare a solution containing 1 M sorbitol and 0.1 M EDTA, pH 7.4. Just before use, add 0.1% β-ME and lyticase/zymolyase (final concentration of 50 U per 1 x 10^7 cells).
- Depending on the yeast strain and enzyme used, the incubation time, enzyme concentration, and composition of Buffer Y1 may vary. Please follow the guidelines of the enzyme supplier.
- **If performing mechanical disruption (step 1c)**, prepare acid-washed glass beads, 0.45–0.55 mm diameter, by soaking in concentrated nitric acid for 1 h in a fume hood, washing extensively with deionized water, and drying in a baking oven.

Procedure

1. Prepare yeast lysate according to step 1a (enzymatic lysis for ≤5 x 10^7 cells), step 1b (enzymatic lysis for ≤2 x 10^7 cells), or step 1c (mechanical disruption).

   1a. Enzymatic lysis of ≤5 x 10^7 freshly harvested cells (do not use more than 5 x 10^7 cells):

   - Harvest the cells in a 12 ml or 15 ml centrifuge tube by centrifuging at 1000 x *g* for 5 min at 4°C. Decant the supernatant, and carefully remove any remaining media by aspiration. If the centrifuge will be used later in this procedure, heat it to 20–25°C.

     Note: Incomplete removal of medium will affect digestion of the cell wall.

- Resuspend the cells in 2 ml freshly prepared Buffer Y1 containing lyticase or zymolyase. Incubate for 10–30 min at 30°C with gentle shaking to generate spheroplasts. Spheroplasts must be handled gently.

  Depending on the yeast strain used, the incubation time, amount of enzyme, and composition of Buffer Y1 may vary. For optimal results, follow the guidelines of the enzyme supplier. Complete spheroplasting is essential for efficient lysis.
- Centrifuge for 5 min at 300 x *g* to pellet the spheroplasts. Carefully remove and discard the supernatant.

  Note: Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.
- Add 350 µl Buffer RLT and vortex vigorously to lyse the spheroplasts. If insoluble material is visible, centrifuge for 2 min at full speed, and use only the supernatant in subsequent steps.

  Note: Ensure that β-ME is added to Buffer RLT before use (see "Things to do before starting").
- Add 1 volume (usually 350 µl) of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 2.

  Precipitates may be visible after addition of ethanol. This does not affect the procedure.

1b. Enzymatic lysis of ≤2 x 10^7 freshly harvested cells (do not use more than 2 x 10^7 cells):

- Harvest the cells in a 12 ml or 15 ml centrifuge tube by centrifuging at 1000 x *g* for 5 min at 4°C. Decant the supernatant, and carefully remove any remaining media by aspiration. If the centrifuge will be used later in this procedure, heat it to 20–25°C.

  Note: Incomplete removal of medium will affect digestion of the cell wall.
- Resuspend the cells in 100 µl freshly prepared Buffer Y1 containing lyticase or zymolyase. Incubate for 10–30 min at 30°C with gentle shaking to generate spheroplasts. Spheroplasts must be handled gently.

Depending on the yeast strain used, the incubation time, amount of enzyme, and composition of Buffer Y1 may vary. For optimal results, follow the guidelines of the enzyme supplier. Complete spheroplasting is essential for efficient lysis.

- Add 350 µl Buffer RLT and vortex vigorously to lyse the spheroplasts. If insoluble material is visible, centrifuge for 2 min at full speed, and use only the supernatant in subsequent steps.

  Note: Ensure that β-ME is added to Buffer RLT before use (see "Things to do before starting").
- Add 250 µl ethanol (96–100%) to the homogenized lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 2.
- Precipitates may be visible after addition of ethanol. This does not affect the procedure.

1c. Mechanical disruption of cells (do not use more than 5 x 10^7 cells):

- Add approximately 600 µl of acid-washed glass beads to a tube that fits the TissueLyser or other bead mill (see page 22 for details).
- Harvest the cells by centrifuging at 1000 x *g* for 5 min at 4°C. Decant the supernatant, and carefully remove any remaining media by aspiration. If the centrifuge will be used later in this procedure, heat it to 20–25°C.

  Note: Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy membrane. Both effects may reduce RNA yield.
- Loosen the cell pellet thoroughly by flicking the tube. Add 600 µl Buffer RLT, and vortex to resuspend the cell pellet. Add the sample to the acid-washed glass beads.

  Note: Ensure that β-ME is added to Buffer RLT before use (see "Things to do before starting").
- Vortex and agitate the sample at top speed in the TissueLyser or other bead mill with cooling until cells are completely disrupted.

  Most small-capacity bead mills do not have a cooling mechanism and therefore require the user to stop the bead mill regularly and cool the sample on ice. The

time required for cell disruption and the length and frequency of the cooling intervals may vary depending on the type of bead mill used. Please refer to the supplier's instructions.

Note: Do not replace bead milling with vortexing, as this significantly reduces RNA yield.

- Remove the sample from the TissueLyser or bead mill, and allow the beads to settle. Transfer the lysate (usually 350 µl) to a new microcentrifuge tube (not supplied). Centrifuge for 2 min at full speed, and transfer the supernatant to a new microcentrifuge tube (not supplied). Use only the supernatant in subsequent steps.
- Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge. Proceed to step 2.

Note: The volume of lysate may be less than 350 µl due to loss during homogenization.

Note: Precipitates may be visible after addition of ethanol. This does not affect the procedure.

2. Transfer the sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml Waste Tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and reuse the Waste Tube in step 3.*

   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.*

   Optional: If performing optional on-column DNase digestion (see "Eliminating genomic DNA contamination", page 26), follow Appendix D (page 77), steps 1–4, after performing this step.

* The flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

3. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step 4.*

   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

   Skip this step if performing optional on-column DNase digestion (page 69).

4. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step 5.

   Note: Buffer RPE/C is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see "Things to do before starting").

5. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.

   The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

6. **Optional**: Discard flow-through and reuse the Waste Tube. Close the lid gently, and centrifuge at full speed for 1 min.

   Perform this step to eliminate any possible carryover of Buffer RPE/C, or if the residual flow-through remains on the outside of the RNeasy spin column after step 5.

* The flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

7. Place the RNeasy spin column in a new 1.5 ml collection tube (not supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.
8. If the expected RNA yield is >30 µg, repeat step 7 using another 30–50 µl RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the collection tube from step 7.

   If using the eluate from step 7, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Protocol: RNA Cleanup

The QIAwave RNA Mini Kit can be used to clean up RNA previously isolated by different methods or after enzymatic reactions, such as labeling or DNase digestion.

Determining the correct amount of starting material

A maximum of 100 µg RNA can be cleaned up in this protocol. This amount corresponds to the RNA binding capacity of the RNeasy spin column.

Important points before starting

- If using the QIAwave RNA Mini Kit for the first time, read "Important Notes" (page 17).
- If working with RNA for the first time, read Appendix A (page 68).
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Preparation of Buffer RPE/C: Transfer the entire volume of Buffer RPE/C from the 15 ml bottle into a glass bottle larger than 325 ml, either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (5 liters, cat. no. 129117; 1000 ml, cat. no. 129115) and 260 ml ethanol (96–100%) to obtain a final volume of 325 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.

- Preassemble RNeasy Mini spin columns with Waste Tubes.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 77).

Procedure

1. Adjust the sample to a volume of 100 µl with RNase-free water. Add 350 µl Buffer RLT, and mix well.
2. Add 250 µl ethanol (96–100%) to the diluted RNA, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.
3. Transfer the sample (700 µl) to an RNeasy Mini spin column placed in a 2 ml Waste Tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and reuse the Waste Tube in step 4.*

   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

   Optional: If performing optional on-column DNase digestion (see "Eliminating genomic DNA contamination", page 26), follow Appendix D (page 77), steps 1–4, after performing this step.
4. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step 5.

   Note: Buffer RPE/C is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see "Things to do before starting").

* The flow-through contains Buffer RLT and is therefore not compatible with bleach. See page 6 for Safety Information.

5. Add 500 µl Buffer RPE/C to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.

   The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

   Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

6. **Optional**: Discard the flow-through and reuse the Waste Tube. Close the lid gently, and centrifuge at full speed for 1 min.

   Perform this step to eliminate any possible carryover of Buffer RPE/C, or if the residual flow-through remains on the outside of the RNeasy spin column after step 5.

7. Place the RNeasy spin column in a new 1.5 ml collection tube (not supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

8. If the expected RNA yield is >30 µg, repeat step 7 using another 30–50 µl RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the collection tube from step 7.

   If using the eluate from step 7, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

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**).

Issue	Comments and suggestions
Clogged RNeasy spin column	
a) Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 22) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see protocols) and/or increase the volume of lysis buffer and the homogenization time. If working with tissues rich in proteins, we recommend using the RNeasy Fibrous Tissue Mini Kit (see page 86 for Ordering Information).
b) Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
c) Centrifugation before adding ethanol not performed (protocols for tissues and mechanical disruption of yeast)	Centrifuge the lysate before adding ethanol, and use only this supernatant in subsequent steps (see protocols). Pellets contain cell debris that can clog the RNeasy spin column.
d) Centrifugation temperature too low	The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the RNeasy spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring it to the RNeasy spin column.

Issue	Comments and suggestions
Low RNA yield	
a) Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 22) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see protocols) and/or increase the volume of lysis buffer and the homogenization time. If working with tissues rich in proteins, we recommend using the RNeasy Fibrous Tissue Mini Kit (see page 86 for Ordering Information).
b) Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
c) RNA still bound to RNeasy spin column membrane	Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifuging.
d) Ethanol carryover	During the second wash with Buffer RPE/C, be sure to centrifuge at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. To eliminate any chance of possible ethanol carryover, place the RNeasy spin column in a new 2 ml collection tube and perform the optional 1-min centrifugation step as described in the protocols.
e) Incomplete removal of cell-culture medium (cell samples)	When processing cultured cells, ensure complete removal of the cell-culture medium after harvesting cells (see protocols).
Low A_{260}/A_{280} value	
Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mm Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 71).
RNA degraded	
a) Harvested animal tissue not immediately stabilized	Submerge the tissue in the appropriate volume of RNAprotect Tissue Reagent immediately after harvesting.
b) Too much animal tissue for proper stabilization	Reduce the amount of tissue or increase the amount of RNAprotect Tissue Reagent used for stabilization (see protocol on page 40).
c) Animal tissue too thick for stabilization	Cut large samples into slices less than 0.5 cm thick for stabilization in RNAprotect Tissue Reagent.

Issue	Comments and suggestions
d) Frozen animal tissue used for stabilization	Use only fresh, unfrozen tissue for stabilization in RNAprotect Tissue Reagent.
e) Storage duration in RNAprotect Tissue Reagent exceeded	RNAprotect-stabilized tissue can be stored for up to 1 day at 37°C, up to 7 days at 15–25°C, or up to 4 weeks at 2–8°C, and can be archived at –30 to –15°C or –90 to –65°C.
f) Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –90 to –65°C. Perform the RNeasy procedure quickly, especially the first few steps. See Appendix A (page 68), "Handling and storing starting material" (page 20), and the RNAprotect protocol (page 40).
g) RNase contamination	Although all QIAwave RNA buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the RNeasy procedure or later handling. See Appendix A (page 68) for general remarks on handling RNA. Do not put RNA samples into a vacuum dryer that has been used in DNA preparations where RNases may have been used.
DNA contamination in downstream experiments	
a) Optimal procedure not used (cell samples)	For animal cells, we recommend purifying cytoplasmic RNA for applications where the absence of DNA contamination is critical, since intact nuclei are removed at the start of the procedure. The protocol can be downloaded at **www.qiagen.com/resources/RNeasyMini**.
b) No incubation with Buffer RW1	In subsequent preparations, incubate the RNeasy spin column for 5 min at room temperature after addition of Buffer RW1 and before centrifuging.
c) No DNase treatment	Perform optional on-column DNase digestion using the RNase-Free DNase Set (see Appendix D, page 77) at the point indicated in the individual protocols. Alternatively, after the RNeasy procedure, DNase digest the RNA eluate. After inactivating the DNase by heat treatment, the RNA can be either used directly in the downstream application without further treatment, or repurified using the RNA cleanup protocol (page 61).

Issue	Comments and suggestions
RNA does not perform well in downstream experiments	
a) Salt carryover during elution	Ensure that Buffer RPE/C is at 20–30°C. When reusing collection tubes between washing steps, remove the residual flow-through from the rim by blotting on clean paper towels.
b) Ethanol carryover	During the second wash with Buffer RPE/C, be sure to centrifuge at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. To eliminate any chance of possible ethanol carryover, place the RNeasy spin column in a new 2 ml collection tube and perform the optional 1 min centrifugation step as described in the protocols.

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.

Disposable plasticware

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

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mm EDTA* followed by RNase-free water (see "Solutions", page 70). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC.* Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 h) at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.

Electrophoresis tanks

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

* 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.

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

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 min to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 min.

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

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

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

Storage of RNA

Purified RNA may be stored at –30 to –15°C or –90 to –65°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 accurately quantified using an Agilent® 2100 bioanalyzer, quantitative RT-PCR, or fluorometric quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260} = 1 → 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 72), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

* 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.

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 70). 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 ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.† For accurate values, we recommend measuring

* 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.

† 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.

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 71).

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. While RNeasy kits will remove the vast majority of cellular DNA, trace amounts 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 ABI PRISM® and LightCycler instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Assays from QIAGEN are designed for real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible. For real-time RT-PCR assays where amplification of genomic 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 86).

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

For other sensitive applications, DNase digestion of the purified RNA with RNase-free DNase is recommended. A protocol for optional on-column DNase digestion using the RNase-Free DNase Set is provided in Appendix D (page 77). The DNase is efficiently washed away in subsequent wash steps. Alternatively, after the RNeasy procedure, the RNA eluate can be treated with DNase. The RNA can then be repurified according to the RNA cleanup protocol (page 61), or after heat inactivation of the DNase, the RNA can be used directly in downstream applications.

The protocol for purification of cytoplasmic RNA from animal cells (available at **www.qiagen.com/resources/RNeasyMini**) is particularly advantageous in applications where the absence of DNA contamination is critical since intact nuclei are removed. Using this protocol, DNase digestion is generally not required – most of the DNA is removed with the nuclei, and RNeasy technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment. However, even further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundance target). Using the cytoplasmic RNA protocol with optional DNase digestion results in undetectable levels of DNA, even in sensitive quantitative RT-PCR analyses.

Integrity of RNA

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

* 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, J., et al. [1989] *Molecular Cloning — A Laboratory Manual*. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

FA gel preparation

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

- 1.2 g agarose*
- 10 ml 10x FA gel buffer (see composition below)
- Add RNase-free water to 100 ml

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

Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde* and 1 µl of a 10 mg/ml ethidium bromide* stock solution. Mix thoroughly and pour onto gel support. Prior to running the gel, equilibrate in 1x FA gel running buffer (see composition below) for at least 30 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 (e.g., 10 µl of loading buffer and 40 µl of RNA) and mix.

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

* 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.

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 10 x FA gel buffer
- RNase-free water to 10 ml

Stability: approximately 3 months at 4°C

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

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

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

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

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

Lysis and homogenization of the sample and binding of RNA to the RNeasy 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 RNeasy membrane. The DNase I is removed by a second wash with Buffer RW1. Washing with Buffer RPE/C and elution of RNA are then performed according to the standard protocols.

Important points before starting

- Generally, DNase digestion is not required since RNeasy technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundant 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. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –30 to –15°C °C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Prepare and load samples onto the RNeasy spin column as indicated in the individual protocols. Instead of performing the first wash step, follow steps 1–4 below.

1. Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and reuse the Waste Tube in step D4.*

   Reuse the collection tube in step D4.
2. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube. Centrifuge briefly to collect residual liquid from the sides of 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.

* The flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

3. Add the DNase I incubation mix (80 µl) directly to the RNeasy spin column membrane, and place on the benchtop (20–30°C) for 15 min.

   Note: Be sure to add the DNase I incubation mix directly to the RNeasy spin column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

4. Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and reuse the Waste Tube.* Continue with the first Buffer RPE/C wash step in the relevant protocol.

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

Appendix E: DNase Digestion of RNA before RNA Cleanup

This protocol describes how to use the RNase-Free DNase Set (cat. no. 79254) to digest contaminating DNA in RNA solutions prior to RNA cleanup. DNase digestion can alternatively be carried out during RNA cleanup (see Appendix D, page 77). For samples highly contaminated with DNA, we recommend DNase digestion in solution, as it is more efficient than on-column DNase digestion.

Important points before starting

- Generally, DNase digestion is not required since RNeasy technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundant target).
- **Important**: 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. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –30 to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Mix the following in a microcentrifuge tube:

   ≤87.5 µl RNA solution (contaminated with genomic DNA)
   10 µl Buffer RDD
   2.5 µl DNase I stock solution
   Make the volume up to 100 µl with RNase-free water.

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

2. Incubate on the benchtop (20–25°C) for 10 min.
3. Clean up the RNA according to “Protocol: RNA Cleanup” on page 61.

Appendix F: Acetone Precipitation of Protein from Buffer RLT Lysates

This protocol is designed for acetone precipitation of protein from cell lysates prepared using Buffer RLT. The precipitated, denatured protein is suitable for applications such as SDS-PAGE, western blotting, and 2D gel electrophoresis.

Equipment and reagents to be supplied by user*

- Ice
- Benchtop centrifuge Acetone
- **Optional**: Ethanol
- Buffer for downstream application (e.g., loading buffer for SDS-PAGE gel)

Important point before starting

- **Important**: Do not use trichloroacetic acid (TCA) to precipitate protein from Buffer RLT lysates.
- This buffer contains guanidine thiocyanate, which can form highly reactive compounds when combined with acidic solutions.

Procedure

1. Prepare cell lysate and centrifuge it through an RNeasy spin column, as described in the protocols in this handbook.
2. Add 4 volumes of ice-cold acetone to the flow-through from the RNeasy spin column.
3. Incubate for 30 min on ice or at –30 to –15°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.

4. Centrifuge for 10 min at maximum speed in a benchtop centrifuge. Discard the supernatant and air-dry the pellet.*
5. **Optional**: Wash the pellet with 100 µl ice-cold ethanol and air-dry.

   Do not overdry the pellet as this may make resuspension more difficult.
6. Resuspend the pellet in the buffer for your downstream application.

   Sodium dodecyl sulfate (SDS) causes guanidine salts to precipitate. In case the pellet contains traces of guanidine thiocyanate, load the sample onto an SDS-PAGE gel immediately after heating for 7 min at 95°C.

* Supernatant contains guanidine thiocyanate and is therefore not compatible with bleach. See page 6 for Safety Information.

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

RT-PCR

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

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

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

- Omniscript® RT Kit — for cDNA synthesis using 50 ng – 2 µg RNA per reaction
  Sensiscript® RT Kit — for cDNA synthesis using less than 50 ng RNA per reaction
- For the PCR step, QIAGEN offers enzymes that minimize PCR optimization:
- Taq DNA Polymerase — for PCR without a hot start HotStarTaq® DNA Polymerase — for PCR with a hot start
- HotStarTaq Plus DNA Polymerase — for PCR with a hot start and a fast 5 min enzyme activation time

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

Real-time RT-PCR

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

Ordering Information

Product	Contents	Cat. no.
QIAwave RNA Mini Kit (250)	250 RNeasy Mini Spin Columns, Waste Tubes (2 ml), RNase-free Reagents and Buffers	74536
QIAwave DNA Blood & Tissue Kit (250)	250 DNeasy® Mini Spin Columns, Waste Tubes (2 ml), Proteinase K, Buffers,	69556
QIAwave Plasmid Miniprep Kit (250)	250 QIAprep® 2.0 Spin Columns, Waste Tubes (2 ml), Reagents	27206
Accessories		
Buffer RLT (220 ml)	220 ml Buffer RLT	79216
Nuclease-Free Water (1000 ml)	1000 ml nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); provided in a plastic bottle	129115
Nuclease-Free Water (5 liters)	5 liters nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); Provided in five 1 liter bottles, delivered in a cardboard box	129117
Waste Tubes (2 ml)	1000 Waste Tubes (2 ml)	19211
RNAprotect Tissue Reagent (250 ml)	For stabilization of RNA in 125 x 200 mg tissue samples: 250 ml RNAprotect RNA Stabilization Reagent	76106

Product	Contents	Cat. no.
RNAprotect Tissue Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml 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
RNAprotect Bacteria Reagent	RNAprotect Bacteria Reagent (2 x 100 ml)	76506
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
Vacuum Pump (115 V, 60 Hz)	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84010
Vacuum Pump (230 V, 50 Hz)	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84020
Vacuum Regulator	For use with QIAvac manifolds	19530
VacConnectors (500)	500 disposable connectors for use with QIAGEN spin columns on luer connectors	19407

Product	Contents	Cat. no.
QIAshredder (250)	250 disposable cell-lysate homogenizers	79656
TissueLyser II*	Universal laboratory mixer-mill disruptor	Inquire
RNase-Free DNase Set (250)	For 250 RNA minipreps: 5 x1500 units RNase-Free DNase I, RNase-Free Buffer RDD, and RNase-Free Water	79256
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect†	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µl filter-tips (1024); 1000 µl filter-tips (1024); 30 ml reagent bottles (12); rotor adapters (240); rotor adapter holder	990395
Related products for downstream applications		
Omniscript RT Kit — for reverse transcription using 50 ng to 2 µg RNA per reaction		
Omniscript RT Kit (50)‡	For 50 x 20 µl reactions: Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix, RNase-Free Water	205111

* Visit **www.qiagen.com/products/accessories** for details about the TissueLyser and accessories.

† All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

‡ Larger kit size available; see **www.qiagen.com/products/pcr**.

Product	Contents	Cat. no.
Sensiscript RT Kit — for reverse transcription using less than 50 ng RNA per reaction		
Sensiscript RT Kit (50)*	For 50 x 20 µl reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix, RNase-Free Water	205211
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, OneStep RT-PCR Buffer, dNTP Mix, 5x Q-Solution, RNase-Free Water	210210
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, RNase-Free Water	205311
QuantiTect SYBR Green PCR Kit — for quantitative, real-time, two-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204143
QuantiTect SYBR Green RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green RT-PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204243

* Larger kit size available; see **www.qiagen.com/products/pcr**.

† Visit **www.qiagen.com/GeneGlobe** to search for and order primer sets or primer–probe sets.

Product	Contents	Cat. no.
QuantiTect Probe PCR Kit — for quantitative, real-time, 2-step RT-PCR using sequence-specific probes		
QuantiTect Probe PCR Kit (200) *†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204343
QuantiTect Probe RT-PCR Kit — for quantitative, real-time, 1-step RT-PCR using sequence-specific probes		
QuantiTect Probe RT-PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204443
QuantiTect Multiplex PCR Kits — for quantitative, multiplex, real-time, two-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex PCR Kit (200)*†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water	204543
QuantiTect Multiplex PCR NoROX Kit (200)*†§	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains no ROX dye), 2 x 2 ml RNase-Free Water	204743

* Larger kit size available; see **www.qiagen.com/products/pcr**.

† Visit **www.qiagen.com/GeneGlobe** to search for and order primer sets or primer–probe sets.

‡ Recommended for ABI PRISM and Applied Biosystems® cyclers.

§ Recommended for all other cyclers.

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

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.

* Larger kit size available; see **www.qiagen.com/products/pcr**.
† Visit **www.qiagen.com/GeneGlobe** to search for and order primer sets or primer–probe sets.
‡ Recommended for ABI PRISM and Applied Biosystems cyclers.
§ Recommended for all other cyclers.

Document Revision History

Date	Changes
01/2022	Initial release

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QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.

Notes

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