Third Edition

RNeasy® Mini Handbook

RNeasy Mini Kit
RNeasy Plant Mini Kit
For total RNA isolation from
- animal cells
- animal tissues
- bacteria
- yeast
- plants
- filamentous fungi
- and for RNA cleanup

RNeasy Protect Mini Kit
For stabilization and protection of RNA in tissue
For isolation of total RNA from stabilized tissue

June 2001

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Contents

Kit Contents

RNeasy® Mini Kit Catalog No.	(20) 74103	(50) 74104	(250) 74106
Preparations per kit	20	50	250
RNeasy mini spin columns (pink)	20	50	250
Collection tubes (1.5 ml)	20	50	250
Collection tubes (2 ml)	20	50	250
Buffer RLT*†	18 ml	45 ml	220 ml
Buffer RW1†	18 ml	45 ml	220 ml
Buffer RPE‡	5 ml	11 ml	55 ml
RNase-free water	10 ml	10 ml	50 ml
Handbook	1	1	1

RNeasy Protect Mini Kit Catalog No.	(50) 74124	(250) 74126
Preparations per kit	50	250
RNA*later*™ RNA Stabilization Reagent§	50 ml	250 ml
RNeasy mini spin columns (pink)	50	250
Collection tubes (1.5 ml)	50	250
Collection tubes (2 ml)	50	250
Buffer RLT*†	45 ml	220 ml
Buffer RW1†	45 ml	220 ml
Buffer RPE‡	11 ml	55 ml
RNase-free water	10 ml	50 ml
Handbook	1	1

* *Buffer RLT is also available separately. See ordering information (page 108).*

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

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

§ *RNA*later *RNA Stabilization Reagent is also available separately. See ordering information (page 107).*

RNeasy Plant Mini Kits Catalog No.	(20) 74903	(50) 74904
Preparations per kit	20	50
RNeasy mini spin columns (pink)	20	50
QIAshredder spin columns (lilac)	20	50
Collection tubes (1.5 ml)	20	50
Collection tubes (2 ml)	20	50
Buffer RLT*†	18 ml	45 ml
Buffer RLC†	18 ml	45 ml
Buffer RW1†	18 ml	45 ml
Buffer RPE‡	5 ml	11 ml
RNase-free water	10 ml	10 ml
Handbook	1	1

* *Buffer RLT is also available separately. See ordering information (page 108).*

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

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

Storage Conditions

RNeasy Kits, RNeasy Protect Kits, and RNA*later* RNA Stabilization Reagent should be stored dry at room temperature (15 to 25°C) and are stable for at least 9 months under these conditions.

Storage of RNA*later* RNA Stabilization Reagent at lower temperatures may cause precipitation. The precipitate can be redissolved by heating to 37°C with agitation. Redissolve any precipitate before using.

Product Use Limitations

RNeasy and RNeasy Protect Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of RNeasy and RNeasy Protect Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. RNeasy and RNeasy Protect Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

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

Product Warranty and Satisfaction Guarantee

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

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

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding RNeasy Kits, RNA*later* RNA Stabilization Reagent, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside front cover).

Safety Information

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

The following risk and safety phrases apply to components of the RNeasy Mini Kit, the RNeasy Protect Mini Kit, and/or the RNeasy Plant Mini Kit:

Buffer RLT

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

Buffer RLC

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

Buffer RW1

Contains ethanol: flammable. Risk phrase:* R10

** R10: Flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36/38: Irritating to eyes and skin; S13: Keep away from food, drink and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show the container or label.*

Introduction

RNA isolation

RNeasy Kits are designed to isolate total RNA from small quantities of starting material. They provide a fast and simple method for the preparation of up to 100 µg total RNA from animal cells and tissues, bacteria, and yeast (**RNeasy Mini Kits**) or plant cells and tissues and filamentous fungi (**RNeasy Plant Mini Kits**). **RNeasy Protect Mini Kits** provide stabilization of RNA in animal tissues and cells and purification of up to 100 µg total RNA from these sources.

RNeasy Mini Kits and RNeasy Plant Mini Kits make multiple, simultaneous processing of a wide variety of biological samples possible in less than 30 minutes. Time-consuming and tedious methods, such as CsCl step-gradient ultracentrifugation and alcohol precipitation steps, or methods involving the use of toxic substances such as phenol and/or chloroform, are replaced by the RNeasy procedure. The purified RNA is ready for use in standard downstream applications such as:

- RT-PCR
- Poly A$^+$ RNA selection
- RNase and S1 nuclease protection
- Differential display
- Northern, dot, and slot blotting
- Primer extension
- cDNA synthesis
- Expression-array and expression-chip analysis

In addition, RNeasy Kits can be used to desalt or purify RNA from enzymatic reactions such as DNase digestions, proteinase digestions, RNA ligation, and labeling reactions.

A list of references describing the use of RNeasy Kits in a variety of applications can be found on page 86.

RNA stabilization in tissue

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

The RNeasy Protect Mini Kit and RNA*later* RNA Stabilization Reagent represent a new technology enabling rapid and reliable preservation of gene-expression patterns in biological material so as to provide reliable gene-expression analysis. RNA*later* technology is designed for stabilization and protection of cellular RNA in animal tissues (in addition,

RNA*later* technology can be used for cell-culture cells and white blood cells). The samples are harvested and immediately submerged in RNA*later* RNA Stabilization Reagent for storage for:

- 1 day at 37°C
- 7 days at 18 to 25°C
- 4 weeks at 2 to 8°C
- archival storage at –20°C or –80°C

During storage in RNA*later* RNA Stabilization Reagent, even at elevated temperatures (e.g., at room temperature or 37°C), the cellular RNA remains intact and undegraded. RNA*later* technology replaces current inconvenient, dangerous and equipment-intensive methods like snap-freezing of samples in liquid nitrogen, storage at –80°C, cutting and weighing on dry ice, or immediate processing of the harvested samples.

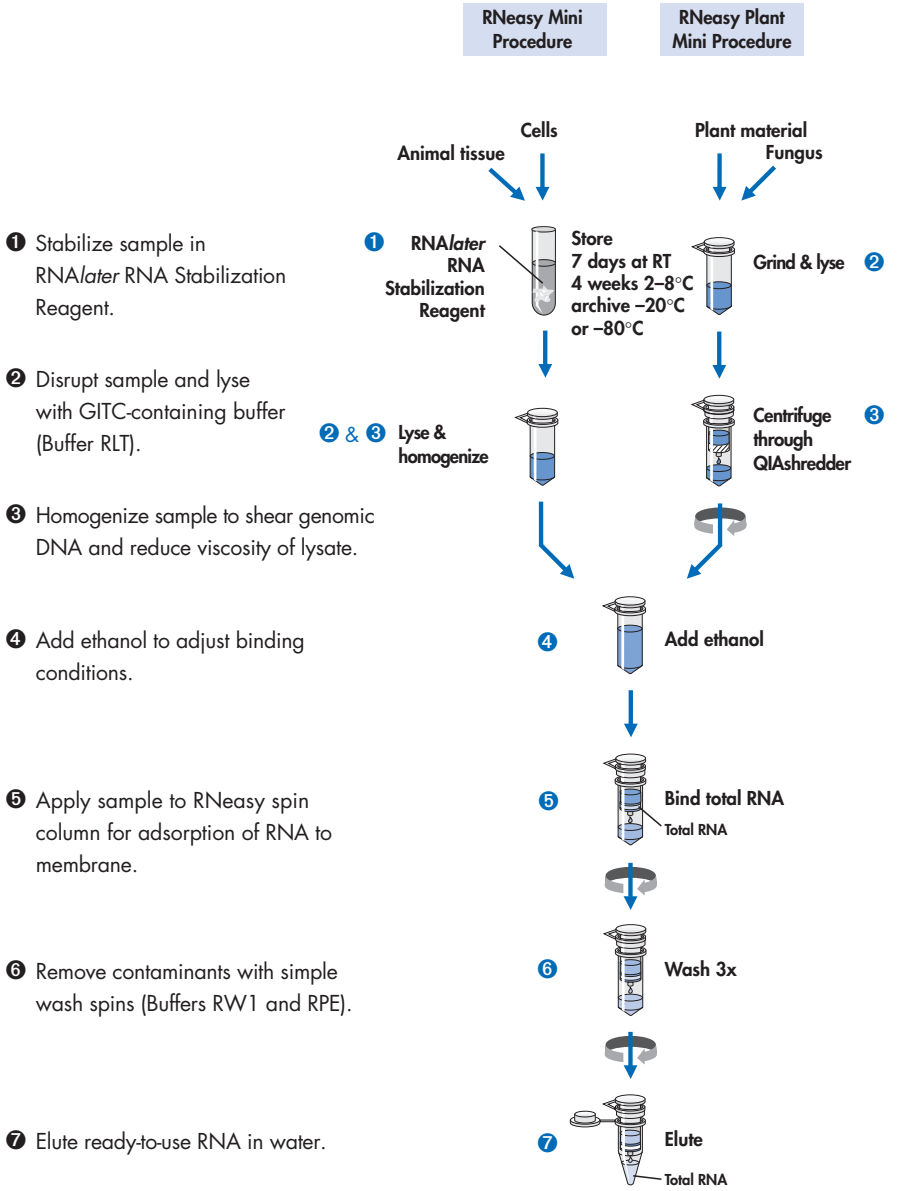
RNeasy Protect Kits and RNA*later* RNA Stabilization Reagent cannot be used for stabilization of RNA in whole blood, plasma, or serum.

The RNeasy principle and procedure

The RNeasy procedure represents a novel technology for RNA isolation. This technology combines the selective binding properties of a silica-gel-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-gel membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine isothiocyanate (GITC)-containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNeasy mini column where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30 µl, or more, of water.

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

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 flowchart).


RNeasy Mini Procedure
RNeasy Plant Mini Procedure
Cells
Animal tissue
Plant material
Fungus
❶ Stabilize sample in RNA*later* RNA Stabilization Reagent.
❶ RNA*later* RNA Stabilization Reagent
Store
7 days at RT
4 weeks 2–8°C
archive –20°C
or –80°C
Grind & lyse ❷
❷ Disrupt sample and lyse with GITC-containing buffer (Buffer RLT).
❷ & ❸ Lyse & homogenize
Centrifuge through QIAshredder ❸
❸ Homogenize sample to shear genomic DNA and reduce viscosity of lysate.
❹ Add ethanol to adjust binding conditions.
❹ Add ethanol
❺ Apply sample to RNeasy spin column for adsorption of RNA to membrane.
❺ Bind total RNA
Total RNA
❻ Remove contaminants with simple wash spins (Buffers RW1 and RPE).
❻ Wash 3x
❼ Elute ready-to-use RNA in water.
❼ Elute
Total RNA

The RNA*later* principle and procedure

RNA*later* RNA Stabilization Reagent, included in RNeasy Protect Kits, represents a novel technology for RNA protection in animal tissues and other biological samples, allowing transport and storage at ambient temperatures. Large numbers of samples can be easily processed without the need for freezing with liquid nitrogen or storage on dry ice. RNA*later* RNA Stabilization Reagent rapidly permeates tissue and single cells to stabilize and protect cellular RNA in situ. For RNA protection and sample storage, the biological sample is submerged in an appropriate volume of RNA*later* RNA Stabilization Reagent immediately after harvesting the material. The reagent preserves RNA for up to 1 day at 37°C, 7 days at 18 to 25°C, or 4 weeks at 2 to 8°C, allowing transportation, storage, and shipping of samples without ice or dry ice. Alternatively, the samples can also be placed at –20°C or –80°C for archival storage.

Description of protocols

Isolation of Total RNA from Animal Cells

Samples (maximum 1 x 10^7 cells using the spin protocol; 1 x 10^6 cells using the vacuum protocol; amounts depend on cell line used) are disrupted in buffer containing guanidine isothiocyanate and homogenized. An overview of disruption and homogenization methods is given on pages 20–24. Ethanol is then added to the lysate, creating conditions that promote selective binding of RNA to the RNeasy silica-gel membrane. The sample is then applied to the RNeasy mini column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water. Using the **Spin Protocol** (pages 31–35), all bind, wash, and elution steps are performed by centrifugation in a microcentrifuge. Using the **Vacuum Protocol** (pages 36–41), bind and wash steps are performed on a QIAvac 24 or QIAvac 6S vacuum manifold, and the final elution steps are performed by centrifugation in a microcentrifuge.

Isolation of Cytoplasmic RNA from Animal Cells

This protocol is particularly advantageous in applications where unspliced or partially spliced RNA is not desirable, since the cytoplasm contains RNA in its mature form. Cytoplasmic RNA accounts for 85% of total cellular RNA. This protocol is also optimal in applications where the absence of DNA contamination is critical, since the intact nuclei are removed. Homogenization to shear genomic DNA is not required. Cultured cells are lysed in a buffer containing a nonionic detergent, which lyses the cell plasma membrane. Nuclei remain intact during the lysis procedure and are removed by centrifugation. GITC-containing lysis buffer and ethanol are added to the supernatant to provide optimal conditions for selective binding of the RNA to the RNeasy silica-gel membrane. The sample is then applied to the RNeasy mini column. Cytoplasmic RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

RNA Stabilization in Tissues with RNA*later* RNA Stabilization Reagent

This handbook provides a detailed protocol for stabilization of RNA in animal tissues. Purification of total RNA from the stabilized tissues can subsequently be carried out using RNeasy Protect Kits or RNeasy Kits following the Protocol for Isolation of Total RNA from Animal Tissues (page 50). In addition, RNA*later* technology can be used for RNA stabilization in other sample material, such as eukaryotic cell-culture cells or white blood cells (see "Guidelines for Other RNAlater Applications" on page 102).

Isolation of Total RNA from Animal Tissues

Fresh, frozen, or RNA*later* stabilized tissue samples (maximum 30 mg; amounts depend on tissue used) are disrupted in buffer containing guanidine isothiocyanate and homogenized. An overview of disruption and homogenization methods is given on pages 20–24. Ethanol is then added to the lysate, creating conditions that promote selective binding of RNA to the RNeasy silica-gel membrane. The sample is then applied to the RNeasy mini column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

Isolation of Total RNA from Bacteria

Bacteria (maximum 1 x 10^9 bacteria) are incubated in a buffer containing lysozyme to digest the bacterial cell wall prior to lysis. After addition of GITC-containing lysis buffer and ethanol, the sample is loaded onto an RNeasy mini column. Total RNA binds to the RNeasy silica-gel membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in RNase-free water.

Isolation of Total RNA from Yeast

Two different protocols are provided for the isolation of total RNA from yeast (maximum 5 x 10^7 yeast cells). The protocols differ primarily in the way the yeast cell walls are broken. In general, the protocols function equally well. For some applications the Enzymatic Lysis Protocol might be preferable since no additional laboratory equipment is required. The Mechanical Disruption Protocol, however, is well-suited for time-course experiments where enzymatic digestion incubations are not practical.

Enzymatic Lysis Protocol (standard and abbreviated versions)

This protocol uses zymolase or lyticase digestion of the cell walls to convert cells to spheroplasts, which are processed using the RNeasy Mini Kit. In the standard protocol, spheroplasts are separated from the digestion mixture before lysis by centrifugation. In the abbreviated version of this protocol, for use with up to 2 x 10^7 yeast cells, the digestion mixture is used directly in the RNeasy procedure without prior separation of the spheroplasts. After addition of GITC-containing lysis buffer and ethanol, samples are loaded onto the RNeasy mini column. Total RNA binds to the RNeasy silica-gel membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Mechanical Disruption Protocol

Using this protocol, yeast cells are lysed and homogenized by mechanical disruption during high-speed agitation in a bead-mill homogenizer in the presence of glass beads and GITC-containing lysis buffer. Ethanol is added to the lysate creating conditions that promote selective binding of RNA to the RNeasy silica-gel membrane. The sample is then applied to the RNeasy mini column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi

In the RNeasy Plant and Fungi Protocol, samples (maximum 100 mg) are first ground in liquid nitrogen and then lysed under highly denaturing conditions. The RNeasy Plant Mini Kit includes a choice of lysis buffers, Buffer RLT and Buffer RLC, which contain GITC or guanidine hydrochloride (GuHCl), respectively. The higher cell disruption and denaturing properties of Buffer RLT frequently make it the buffer of choice. However some tissues, such as milky endosperm of maize or mycelia of filamentous fungi, solidify in this buffer making the extraction of RNA impossible. In these cases Buffer RLC should be used. After lysis with either buffer, samples are centrifuged through a QIAshredder™ homogenizer, which is supplied with the RNeasy Plant Mini Kit. This simultaneously removes insoluble material and reduces the viscosity of the lysates by disrupting gelatinous material often formed in plant and fungal lysates. Ethanol is added to the cleared lysate, creating conditions which promote selective binding of RNA to the RNeasy silica-gel membrane. The sample is then applied to the RNeasy mini column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

RNeasy Plant Mini Kits can also be used for total RNA minipreparation from animal cells and tissues, bacteria, and yeast.

RNA Cleanup

The RNeasy Mini Kit, RNeasy Protect Mini Kit, and RNeasy Plant Mini Kit can be used to purify RNA from enzymatic reactions (e.g., DNase digestion, RNA labeling) or for desalting RNA samples (maximum 100 µg RNA). GITC-containing lysis buffer 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 column. RNA binds to the RNeasy silica-gel membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Specialized protocols

In addition to the standard protocols in this handbook, a specialized **RNeasy Mini Protocol for Isolation of Total RNA from Heart, Muscle, and Skin Tissue** is included in Appendix C (page 93). This protocol includes a proteinase digestion and optimized procedure for these tissues, which have an abundance of contractile proteins, connective tissues, and collagen.

Optional On-Column DNase Digestion with the RNase-Free DNase Set

Generally, DNase digestion is not required with RNeasy Mini Kits since the 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-abundant target). In these cases, residual DNA can be removed using the RNase-Free DNase Set (cat. no. 79254) for optional on-column DNase digestion (see Appendix D, page 99). The DNase is efficiently removed in subsequent wash steps. Alternatively, residual DNA can be removing by a DNase digestion after RNA isolation. The DNase digestion can then be cleaned up, if desired, using the RNeasy Mini Protocol for RNA Cleanup.

Important Points before Using RNeasy Kits

Determining the amount of starting material

Using the correct amount of starting material is essential in order to obtain high yield and pure RNA with RNeasy columns. The maximum amount that can be used is limited by:

- The volume of Buffer RLT required for efficient lysis and the maximum loading volume of the RNeasy column
- The RNA binding capacity of the RNeasy column (100 µg)

For samples that contain high amounts of RNA, this means that less than the absolute maximum amounts of starting material listed in Table 1 should be used in order not to exceed the RNA binding capacity of the column. For samples which contain average or low amounts of RNA, the maximum amount of starting material can be used. In these cases, even though the RNA binding capacity of the column may not be reached, the maximum amount of starting material must not be exceeded or lysis will be incomplete, resulting in lower yield and purity. The maximum amount of a specific biological sample that can be processed depends on the type of tissue or cells being processed and the corresponding RNA content. More information for calculating starting amounts of material is given in each protocol. Typical amounts of starting materials for use with RNeasy columns are shown in Table 1. Table 2 gives examples of expected RNA yields from various sources.

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

Table 1. RNeasy mini 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 tissue	30 mg*
Bacteria	1×10^9*
Yeast	5×10^7*
Plant tissue	100 mg
Filamentous fungi	100 mg

* *For larger amounts, RNeasy Kits and RNeasy Protect Kits are available in midi and maxi formats. Please call our Technical Service Group or see ordering information on pages 108–109.*

Table 2. Yields of total RNA with RNeasy Mini or RNeasy Plant Mini Kits

Source	Average 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
Brain	8
Heart	10†
Kidney	35
Liver	40
Spleen	35
Thymus	45
Lung	10
Bacteria (1 x 10^9 cells)	
E. coli	55
B. subtilis	33
Yeast (1 x 10^7 cells)	
S. cerevisiae	25
Plant (100 mg leaves)	
Arabidopsis	35
Maize	25
Tomato	65
Tobacco	60

* *Amounts can vary due to species, developmental stage, growth conditions used, etc. 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.*

† *Using the specialized RNeasy Mini Protocol for Isolation of Total RNA from Heart, Muscle, and Skin Tissue. See Appendix C, page 93.*

Counting cells or weighing tissue are the most accurate ways to quantitate the amount of starting material. However, the following may be used as a guide.

Animal cells

The number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 3.

Table 3. Growth area and number of HeLa cells in various culture dishes

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 (approximate 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.

Animal tissue

A 3 mm cube (27 mm^3) of most animal tissues weighs 30–35 mg.

Bacteria and yeast

Bacterial and yeast growth are usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relations between OD values and cell numbers in bacterial and 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 used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g. see 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 and the dilution factor used in calculating the number of cells per ml.

The following calculation can be considered as a rough guide that may be helpful. An *E. coli* culture of 1 x 10^9 cells per ml, diluted 1 in 4, gives OD_{600} values of 0.25 measured using a Beckman DU®-7400 or 0.125 using a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1.0 or 0.5 respectively for 1 x 10^9 cells per ml. The same OD_{600} values of 1.0 or 0.5 respectively are obtained for a *S. cerevisiae* culture with a cell density of 1–2 x 10^7 cells per ml.

Plant tissue

A 1.5 cm diameter leaf disc weighs 25–75 mg.

Handling and storage of starting material

RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents.

For stabilization in RNA*later* RNA Stabilization Reagent, included in RNeasy Protect Kits, samples should be submerged in the appropriate volume of the reagent **immediately** after harvesting the material. This prevents unwanted changes in the gene-expression pattern due to RNA degradation or new induction of genes. The relevant procedures for harvesting and stabilization should be carried out as quickly as possible. See "Important Notes before Using RNeasy Protect Kits and RNA*later* RNA Stabilization Reagent", page 25.

Alternatively, samples can be immediately flash frozen in liquid nitrogen and stored at –70°C as soon as they are harvested or excised. Frozen animal or plant tissue should not be allowed to thaw during handling or weighing, but cell pellets can partially thaw enough to allow them to be dislodged by flicking. The relevant procedures should be carried out as quickly as possible.

Samples can also be stored at –70°C in lysis buffer (Buffer RLT) after disruption and homogenization. Frozen samples are stable for months.

Note: Only freshly harvested samples can be used for enzymatic lysis of yeast cells or isolation of cytoplasmic RNA from animal cells.

Disruption and homogenization of starting materials

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

Disruption: Complete disruption of cells 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 yields.

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

Some disruption methods simultaneously homogenize the sample while others require an additional homogenization step. Table 4 (pages 22–23) gives an overview of different disruption and homogenization methods suitable for various starting materials. It can be used as a guide to choose the appropriate method for the starting material with which you are working. The different disruption and homogenization methods listed in Table 4 are described in more detail below.

Note: After storage in RNA*later* RNA Stabilization Reagent, tissues become slightly harder than fresh or thawed tissues. Disruption and homogenization of this tissue, however, is usually not a problem.

Disruption and homogenization using rotor–stator homogenizers

Rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize, in the presence of lysis buffer, animal tissues in 5–90 seconds depending on the toughness 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, by keeping the tip of the homogenizer submerged and holding the immersed tip to one side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes. Probes with diameters of 5 mm and 7 mm are suitable for volumes up to 300 µl and can be used for homogenization in microfuge tubes. Probes with a diameter of 10 mm or above require larger tubes. See page 106 for a list of suppliers of rotor–stator homogenizers.

Disruption and homogenization using the Mixer Mill MM 300 and other bead mills

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 agitator
- disintegration time

The optimal beads to use are is 0.1 mm (mean diameter) glass beads for bacteria, 0.5 mm glass beads for yeast and unicellular animal cells, and 3–7 mm stainless steel beads for animal tissues. It is essential that glass beads are pretreated by washing in concentrated nitric acid. All other disruption parameters must be determined empirically for each application. A protocol for mechanical disruption of yeast cells with glass beads is included in this handbook (page 71) since this is the most widespread application for bead-milling. In addition, Appendix E (page 101) contains guidelines for disruption and homogenization of RNA*later* stabilized tissues using the Mixer Mill MM 300 and stainless steel beads. Please refer to suppliers' guidelines for further details.

Plant tissues can be disrupted in the Mixer Mill MM 300 (see page 108 for ordering information) and other bead mills using stainless steel or tungsten carbide beads. In this case, plant material, beads, and disruption vessels must all be precooled in liquid nitrogen, and disruption is performed without lysis buffer.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the sample 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 methods below.

Note: Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization must be performed separately before proceeding with the RNeasy protocol.

Table 4. Guide to methods of disruption and homogenization of samples

Starting material	Disruption method
Cultured animal cells	Addition of lysis buffer
Animal tissue	Rotor–stator homogenizer Mortar and pestle Mixer Mill MM 300 (for RNA*later* stabilized tissues)
Bacteria	Enzymatic (lysozyme) digestion followed by addition of lysis buffer
Yeast	Enzymatic (lyticase/zymolase) digestion of cell wall followed by lysis of spheroplasts by addition of lysis buffer Glass beads in a bead mill with lysis buffer
Plants and filamentous fungi	Mortar and pestle

* *QIAshredder homogenizers are supplied with the RNeasy Plant Mini Kit and can be purchased separately for use with the RNeasy Mini Kit. See page 108 for ordering information.*

Homogenization method	Comments
Rotor–stator homogenizer or QIAshredder homogenizer* or syringe and needle	If ≤1 x 10^5 cells are processed, lysate can be homogenized by vortexing. No homogenization needed for cytoplasmic RNA protocol
Rotor–stator homogenizer	Simultaneously disrupts and homogenizes.
QIAshredder homogenizer* or syringe and needle	Rotor–stator homogenizer usually gives higher yields than mortar and pestle
Mixer Mill MM 300	The Mixer Mill MM 300 gives results comparable to using a rotor–stator homogenizer
Vortex	If more than 5 x 10^8 cells are being processed further homogenization using QIAshredder homogenizer* or a syringe and needle may increase yield.
Vortex	
Glass beads in a bead mill with lysis buffer	Bead-milling simultaneously disrupts and homogenizes; bead-milling cannot be replaced by vortexing.
QIAshredder homogenizer*	Mortar and pestle cannot be replaced by rotor–stator homogenizer.

Homogenization using QIAshredder homogenizers

Use of QIAshredder modules is a fast and efficient way to homogenize cell and tissue lysates without cross contamination of the samples. The lysate (maximum volume 700 µl) is loaded onto the QIAshredder spin column sitting in a 2 ml collection tube, spun for 2 min at maximum speed in a microfuge and the homogenized lysate collected. QIAshredder spin columns are supplied in the RNeasy Plant Mini Kit and can be purchased separately for use with RNeasy Mini Kits. Call our Technical Service Group for further details or see ordering information on page 108.

Homogenization using a syringe and needle

Cell and tissue lysates can be homogenized using a syringe and needle. High-molecular-weight DNA can be sheared by passing the lysate 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.

Important Points before Using RNeasy Protect Kits and RNA*later* RNA Stabilization Reagent

Handling of the starting material prior to stabilization

RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent. Samples should be submerged in the appropriate volume of the reagent **immediately** after harvesting the material. This prevents unwanted changes in the gene-expression pattern due to RNA degradation or new induction of genes. The relevant procedures for harvesting and stabilization should be carried out as quickly as possible.

RNA*later* RNA Stabilization Reagent stabilizes RNA in freshly harvested samples. Previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

Maximum tissue size for effective stabilization

Tissue size is critical for successful RNA stabilization with RNA*later* technology.

RNA*later* RNA Stabilization Reagent penetrates the sample by diffusion and protects the cellular RNA. Immediately upon contact, the reagent diffuses into individual cells and into the surface layer and outer portions of solid tissues. In order 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 so long as one dimension of the sample is <0.5 cm.

Small organs such as rat kidney and spleen or most mouse organs (except liver) can be processed whole with RNA*later* RNA Stabilization Reagent. Larger samples should simply be cut into slices less than 0.5 cm thick.

If tissue slices are thicker than 0.5 cm, diffusion of the reagent into the sample will be too slow for reliable RNA stabilization in the interior. RNA degradation will occur in tissue slices that are too thick.

Determining the volume of RNA*later* RNA Stabilization Reagent for RNA stabilization in animal tissues

RNA is protected in animal tissues in situ only after the sample is completely submerged in a sufficient volume of RNA*later* RNA Stabilization Reagent. An appropriate volume of RNA*later* RNA Stabilization Reagent is essential for reliable RNA stabilization in various tissue types.

The tissue should be placed in **at least 10 volumes of RNA*later* RNA Stabilization Reagent (or approximately 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.

The tissue should be completely submerged in the RNA*later* RNA Stabilization Reagent. Storage containers should be wide enough so that the reagent is able to cover the entire tissue. Storage containers or tubes with large diameters may require more of the reagent to completely cover the tissue.

If the tissue samples are transported in RNA*later* RNA Stabilization Reagent make sure that the tissue remains submerged in the liquid during transport. Make sure that the tubes remain upright during transport, or fill the tube completely with RNA*later* RNA Stabilization Reagent.

The following may be used as a guide for determination the amount of tissue for 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 RNA*later* RNA Stabilization Reagent.

A 3 mm cube ([3 mm]^3 = 27mm^3 = 27 µl) of most animal tissues weighs 30–35 mg and requires at least 300–350 µl of RNA*later* RNA Stabilization 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. RNA in tissues weighing, for example, 200 mg can be stabilized in 2 ml of RNA*later* RNA Stabilization Reagent (10 µl/1 mg tissue). For tissue pieces weighing more than 200 mg, more of the reagent must be used in order to have 10 volumes of RNA*later* RNA Stabilization Reagent. For tissue pieces weighing less than 200 mg, less RNA*later* RNA Stabilization Reagent can be used (although it is always possible to add more).

Average weights of various entire adult mouse organs and the corresponding amounts of RNA*later* RNA Stabilization Reagent to use are given in Table 5.

Table 5. Tissue weights and amounts of RNA*later* RNA Stabilization Reagent

Mouse organ	Weight	Amount of RNA*later* RNA Stabilization Reagent
Kidney	180–250 mg	≥2.5 ml
Spleen	100–160 mg	≥1.6 ml
Lung	190–210 mg	≥2.1 ml
Heart	100–170 mg	≥1.7 ml
Liver	1.0–1.8 g	≥18 ml

Reagents and Equipment to Be Supplied by User

See page 106 for a list of suppliers of disruption and homogenization equipment and enzymes.

For all protocols

14.3 M β-mercaptoethanol (β-ME)* (commercially available solutions are usually 14.3 M)

Sterile, RNase-free pipet tips

Microcentrifuge (with rotor for 2 ml tubes)

Equipment for disruption and homogenization (see pages 20–24)

Ethanol (96–100%)

Disposable gloves

For animal cell and animal tissue protocols

Ethanol (70%)

For animal cell protocols

For isolation of total RNA using vacuum technology

- QIAvac 24 (cat. no. 19403); or QIAvac 6S (cat. no. 19503) 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
- Vacuum source capable of generating a vacuum pressure of –800 to –900 mbar. We recommend using a vacuum pump with a capacity of 18–20 liter/min (e.g., as supplied by KNF Neuberger). Use of insufficient vacuum pressure may reduce RNA yield and purity.

  Note: This protocol requires higher vacuum pressures than those used for other protocols on the QIAvac. 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 columns are used on the vacuum manifold. Vacuum pressures exceeding –900 mbar should be avoided. The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 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.

** β-ME must be added to Buffer RLT and Buffer RLC before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl of β-ME per 1 ml of Buffer RLT or RLC. The solution is stable for 1 month after the addition of β-ME.*

- Optional: VacConnectors (cat. no. 19407); these disposable connectors fit between the RNeasy mini columns and the luer extensions on the QIAvac 24 or the QIAvac Luer Adapters on the QIAvac 6S. They prevent direct contact between the RNeasy mini columns and luer connectors during RNA purification, avoiding any cross contamination between samples. VacConnectors are discarded after single use.

For isolation of cytoplasmic RNA

Buffer RLN*	50 mM	Tris·Cl, pH 8.0
	140 mM	NaCl
	1.5 mM	$MgCl_2$
	0.5%	Nonidet P-40†
	Just before use add:	
	1000 U/ml	RNase inhibitor (optional)
	1 mM	DTT (optional)

For bacterial protocol

TE buffer† (10 mM Tris·Cl, 1 mM EDTA pH 8.0)

Lysozyme (20 mg/ml in water for Gram-negative or 50 mg/ml in water for Gram-positive bacteria respectively). Store lysozyme stock solution in single-use aliquots at –20°C. Add lysozyme stock solution to TE buffer just before use.

For Gram-negative bacteria:
Add 2 µl of 20 mg/ml lysozyme stock solution per 100 µl of TE buffer.

For Gram-positive bacteria:
Add 6 µl of 50 mg/ml lysozyme stock solution per 100 µl of TE buffer.

** The use of molecular-biology-grade reagents is recommended.*

† Nonidet P-40 is no longer manufactured. It can be replaced with Nonidet P-40 Substitute (Fluka, cat. no. 74385) or Igepal® CA-630 (SIGMA, cat. no. I 3021).

For yeast protocols

Enzymatic protocol

Ethanol (70%)

Buffer for enzymatic lysis:

Depending on the yeast strain and enzyme used, the amount of enzyme and composition of this buffer may vary. Please adhere to guidelines of enzyme supplier. However, in most cases Buffer Y1 (see below) can be used.

Buffer Y1* 1 M sorbitol
0.1 M EDTA, pH 7.4
Just before use add:
0.1% β-ME
50 U lyticase/zymolase per 10^7 cells

Mechanical disruption protocol

Acid-washed glass beads, 0.5 mm diameter:

Prepare acid-washed glass beads (0.45–0.55 mm diameter) by soaking in concentrated nitric acid† for 1 hour, washing extensively with deionized water, and drying in a baking oven.

Mixer Mill MM 300 or other bead mill

For plant and fungi protocol

Liquid nitrogen‡

Mortar and pestle (alternatively: bead mill)

** The use of molecular-biology-grade reagents is recommended.*

† Nitric acid is extremely carrosive and can cause severe burns. Use in a fume hood and take appropriate safety measures.

‡ Liquid nitrogen can cause severe burns. Take appropriate safety measures.

RNeasy Mini Protocols for Isolation of Total RNA from Animal Cells

Determining the correct amount of starting material

It is essential to use the correct number of cells in order to obtain optimal RNA yield and purity with RNeasy columns. A minimum of 100 cells can generally be processed with RNeasy mini columns.* The maximum number of cells that can be used depends on the specific RNA content of the cell line used, which varies greatly between cell types. Two main criteria limit the maximum number of cells to use:

- The binding capacity of the RNeasy mini column (100 µg RNA)
- The volume of Buffer RLT required for efficient lysis. The maximum volume of Buffer RLT that can be used in the RNeasy procedure limits the amount of starting material to an absolute maximum of 1 x 10^7 cells.

The following examples illustrate how to determine the correct maximum amount of starting material:

COS cells:	High RNA content (approximately 35 µg RNA per 10^6 cells) No more than 3 x 10^6 cells can be used. Otherwise the binding capacity of the RNeasy mini column will be exceeded
HeLa cells:	Average RNA content (approximately 15 µg RNA per 10^6 cells) No more than 7 x 10^6 cells can be used. Otherwise the binding capacity of the RNeasy mini column will be exceeded
NIH/3T3 cells:	Low RNA content (approximately 10 µg RNA per 10^6 cells) The maximum number of cells (1 x 10^7) that can be processed with the RNeasy mini column can be used.

If the cell type used is not shown in Table 2 (page 17) and you have no information about the RNA content of your starting material, we recommend starting with no more than 3–4 x 10^6 cells. Depending on yield and purity, it may be possible to increase the cell number in subsequent preparations.

Do not overload the column. Overloading will significantly reduce yield and purity.

The numbers of HeLa cells expected in certain cell culture vessels are shown in Table 3 (page 18).

** Please call QIAGEN Technical Services for guidelines to purify RNA from smaller numbers of cells.*

RNeasy Mini Protocol for Isolation of Total RNA from Animal Cells

I. Spin Protocol

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- RNA in cells can be stabilized in RNA*later* RNA Stabilization Reagent for later use. See Appendix F (page 102) for details.
- Cell pellets can be stored at –70°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 cell pellets can be dislodged by flicking in step 2. Homogenized cell lysates (in Buffer RLT, step 3) can be stored at –70°C for several months. To process frozen lysates, thaw samples for 15–20 min at 37°C in a water bath to dissolve salts. 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.
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- All steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.

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

1. Harvest cells.

a. 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 for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and continue with step 2 of the protocol.

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

b. Cells grown in a monolayer (Do not use more than 1 x 10^7 cells.)

Cells grown in a monolayer in cell-culture vessels can either be lysed directly in the culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

To lyse cells directly in culture dish: Determine the number of cells. (Table 3 on page 18 gives approximate cell numbers for various cell-culture vessels.) Completely aspirate cell-culture medium, and continue immediately with step 2 of the protocol.

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

To trypsinize cells: Determine the number of cells. (Table 3 on page 18 gives approximate cell numbers for various cell-culture vessels.) Aspirate medium, and wash cells with PBS. Aspirate PBS and add 0.10–0.25% trypsin in PBS to trypsinize the cells. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and pellet by centrifugation at 300 x *g* for 5 min. Completely aspirate supernatant, and continue with step 2 of the protocol.

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

2. Disrupt cells by addition of Buffer RLT.

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

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields. Ensure that β-ME is added to Buffer RLT before use (see "Important notes before starting").

Number of pelleted cells	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 below) to the cell-culture dish. Collect cell lysate with a rubber policeman. Pipet 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.

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

Dish diameter (cm)	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 sample.

Three alternative methods (a, b, or c) may be used to homogenize the sample. After homogenization, proceed with step 4. See "Disruption and homogenization of starting material", pages 20–24, for a more detailed description of homogenization methods.

If ≤1 x 10^5 cells are processed, the cells can be homogenized by vortexing for 1 min.

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

a. Pipet the lysate directly onto a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed.

or

b. Homogenize cells for 30 s using a rotor–stator homogenizer.

or

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

4. **Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.**

   If some lysate is lost during homogenization, adjust volume of ethanol accordingly.

   Note: Visible precipitates may form after the addition of ethanol when preparing RNA from certain cell lines, but this will not affect the RNeasy procedure.

5. **Apply up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy mini column placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

   Reuse the collection tube in step 6.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

6. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 8.

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

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

8. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 9, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 8a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

8a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

9. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

10. **If the expected RNA yield is >30 µg, repeat the elution step (step 9) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 9). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocol for Isolation of Total RNA from Animal Cells

II. Vacuum Protocol

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- RNA in cells can be stabilized in RNA*later* RNA Stabilization Reagent for later use. See Appendix F (page 102) for details.
- Cell pellets can be stored at –70°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 cell pellets can be dislodged by flicking in step 2. Homogenized cell lysates (in Buffer RLT, step 3) can be stored at –70°C for several months. To process frozen lysates, thaw samples for 15–20 min at 37°C in a water bath to dissolve salts. 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.
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Do not use more than **10^6 cells per prep**. The cell numbers in each prep should be similar (no more than a twofold difference between the highest and lowest) to allow uniform flow rates on the vacuum manifold.
- Between loading steps, the vacuum must be switched off and the manifold ventilated to maintain uniform conditions for each sample. This can be done with a vacuum regulator 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 columns open while applying vacuum.
- Prepare the vacuum manifold before use.
    - **QIAvac 24:** Place the QIAvac 24 lid on top of the QIAvac 24 base. Make sure that the gasket fits tightly in the groove of the QIAvac 24 lid. Insert up to 24 RNeasy mini columns into the luer extensions of the QIAvac 24. VacConnectors (optional) can be inserted between each RNeasy mini column and luer extension to avoid any cross contamination between samples. Close unused positions with luer caps provided with the QIAvac 24. Connect the QIAvac 24 to an appropriate vacuum source.
    - **QIAvac 6S:** Open the QIAvac 6S lid. Place QIAvac Luer Adapters (or blanks to seal unused slots) into the slots of the QIAvac top plate, and close the lid. Place the waste tray inside the QIAvac base, and place the top plate squarely over the base. Insert each RNeasy mini column into a luer connector on the Luer Adapter(s) in the vacuum manifold. VacConnectors (optional) can be inserted between each RNeasy mini column and luer connector to avoid any cross contamination between samples. Seal unused luer connectors with plugs provided in the QIAvac Luer Adapter Set. Connect the QIAvac 6S to an appropriate vacuum source.
    - **Other vacuum manifolds:** Follow the supplier's instructions. Insert each RNeasy mini column into a luer connector.
- The flow-through from each vacuum step is collected in the QIAvac 24 base or in the QIAvac 6S waste tray. The capacity of each is sufficient for 24 samples to be processed without needing to empty the waste. After sample processing is complete, discard the liquid waste* and clean the vacuum manifold components as described in the *QIAvac Handbook*. With other vacuum manifolds, follow supplier's instructions.
- All steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- All centrifugation steps are performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

** Buffers RLT and RW1 in the liquid waste contain a guanidine salt, which is an irritant and not compatible with disinfecting agents containing bleach. Take appropriate safety measures and wear gloves when handling.*

1. **Harvest cells.**

   a. **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 for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and continue with step 2 of the protocol.

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

   b. **Cells grown in a monolayer (Do not use more than 1 x 10^6 cells.)**

   Cells grown in a monolayer in cell-culture vessels can either be lysed directly in the culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

   To lyse cells directly in culture dish: Determine the number of cells. (Table 3 on page 18 gives approximate cell numbers for various cell-culture vessels.) Completely aspirate cell-culture medium, and continue immediately with step 2 of the protocol.

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

   To trypsinize cells: Determine the number of cells. (Table 3 on page 18 gives approximate cell numbers for various cell-culture vessels.) Aspirate medium, and wash cells with PBS. Aspirate PBS and add 0.10–0.25% trypsin in PBS to trypsinize the cells. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and pellet by centrifugation at 300 x *g* for 5 min. Completely aspirate supernatant, and continue with step 2 of the protocol.

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

2. **Disrupt cells by addition of 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 yields. Ensure that β-ME is added to Buffer RLT before use (see "Important notes before starting").

For direct lysis of cells grown in a monolayer, add the 350 µl Buffer RLT to the cell-culture dish (If 350 µl is not enough to cover the cell-culture dish, use 600 µl Buffer RLT instead. Be sure then also to use 600 µl of 70% ethanol later in step 4). Collect cell lysate with a rubber policeman. Pipet 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.

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

3. Homogenize the sample.

Three alternative methods (a, b, or c) may be used to homogenize the sample. After homogenization, proceed with step 4. See "Disruption and homogenization of starting material", pages 20–24, for a more detailed description of homogenization methods.

If $\leq 1 \times 10^5$ cells are processed, the cells can be homogenized by vortexing for 1 min.

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

a. Pipet the lysate directly onto a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed.

or

b. Homogenize cells for 30 s using a rotor–stator homogenizer.

or

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

4. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.

If some lysate is lost during homogenization, adjust volume of ethanol accordingly.

Note: Visible precipitates may form after the addition of ethanol when preparing RNA from certain cell lines, but this will not affect the RNeasy procedure.

5. Pipet 700 µl of each sample from step 4, including any precipitate that may have formed, into each RNeasy column on the vacuum manifold.

6. **Switch on vacuum source. Apply vacuum until transfer is complete. Switch off 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 base or the QIAvac 6S waste tray.* If the column clogs, switch off vacuum, ventilate, and try again. If it still clogs, continue with the standard RNeasy Mini Protocol for Isolation of Total RNA from Animal Cells using a microcentrifuge.

   Note: The vacuum must be switched off and the manifold ventilated 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 base or the QIAvac 6S waste tray.*

8. **Pipet 700 µl Buffer RW1 onto each RNeasy column.**

9. **Switch on vacuum source. Apply vacuum until transfer is complete. Switch off vacuum, and ventilate the vacuum manifold.**

   The flow-through is collected in the QIAvac 24 base or the QIAvac 6S waste tray.*

10. **Pipet 750 µl Buffer RPE onto each RNeasy column.**

    Note: Ensure that ethanol is added to Buffer RPE before use (see "Important notes before starting").

11. **Switch on vacuum source. Apply vacuum until transfer is complete. Switch off vacuum, and ventilate the vacuum manifold.**

    The flow-through is collected in the QIAvac 24 base or the QIAvac 6S waste tray.

12. **Remove RNeasy column from vacuum manifold, and place in a 2 ml collection tube (provided).**

13. **Pipet 250 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥10,000 x *g* (≥12,500 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 14, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 13a.**

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

    Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

** Flow-through contains a guanidine salt, which is an irritant and not compatible with disinfecting agents containing bleach. Take appropriate safety measures and wear gloves when handling.*

13a. Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.

14. To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.

15. If the expected RNA yield is >30 µg, repeat the elution step (step 14) as described with a second volume of RNase-free water. Elute into the same collection tube.

To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 14). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocol for Isolation of Cytoplasmic RNA from Animal Cells

Determining the correct amount of starting material

It is essential to use the correct number of cells in order to obtain optimal RNA yield and purity with RNeasy columns. A minimum of 100 cells can generally be processed with RNeasy mini columns.* The maximum number of cells that can be used depends on the specific RNA content of the cell line used, which varies greatly between cell types. Read the detailed information on page 30 to determine the correct number of cells to use.

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- Only use freshly harvested cells. Ice crystals form during freezing and thawing and destroy the nuclear membranes, releasing DNA and other nuclear molecules. After addition of Buffer RLT (step 4), samples can be stored at –70°C for several months. To process, frozen lysates should be thawed and incubated at 37°C for 10 min to ensure that all salts have been dissolved. Vortex vigorously. If any insoluble material remains, centrifuge for 2 min and use the supernatant. Continue with step 5.
- **Prepare Buffer RLN† and precool to 4°C.**

50 mM	Tris·Cl, pH 8.0
140 mM	NaCl
1.5 mM	$MgCl_2$
0.5% (v/v)	Nonidet P-40‡ (1.06 g/ml)

  Just before use, add:

1000 U/ml	RNase inhibitor (optional)
1 mM	DTT (optional)

- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**

* *Please call QIAGEN Technical Services for guidelines to purify RNA from smaller numbers of cells.*

† *The use of molecular biology grade reagents is recommended.*

‡ *Nonidet P-40 is no longer manufactured. It can be replaced with Nonidet P-40 Substitute (Fluka, cat. no. 74385) or Igepal CA-630 (SIGMA, cat. no. I 3021).*

- Using the cytoplasmic protocol, DNase digestion is generally not required: most of the DNA is removed by pelleting the nuclei during the procedure, and the RNeasy silica-membrane technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- Cell lysis is performed on ice and the following centrifugation at 4°C. All subsequent steps of the RNeasy protocol should be performed at room temperature. With the exception of Buffer RLN, buffers should not be precooled.
- After cell harvesting and pelleting of nuclei, all centrifugation steps are performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. Harvest cells.

a. 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 for 5 min at 300 x *g* in a centrifuge tube (not supplied). Decant supernatant. Carefully remove all remaining media by aspiration, and continue with step 2 of the protocol.

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

b. Cells grown in a monolayer (Do not use more than 1 x 10^7 cells.)

Cells grown in a monolayer in cell-culture dishes with a diameter of ≤3.5 cm can either be lysed directly in the culture dish or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks or larger cell-culture dishes should always be trypsinized.

To lyse directly in cell-culture dish (≤3.5 cm diameter): Determine the number of cells. (Table 3 on page 18 gives approximate cell numbers for various cell-culture vessels.) Completely aspirate cell-culture medium, and continue immediately with step 2 of the protocol.

To trypsinize cells: Determine the number of cells. (Table 3 on page 18 gives approximate cell numbers for various cell-culture vessels.) Aspirate medium, and wash cells with PBS. Aspirate PBS, and add 0.10–0.25% trypsin in PBS to trypsinize the cells. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer cells to a centrifuge tube (not supplied), and pellet by centrifugation at 300 x *g* for 5 min. Completely aspirate supernatant, and continue with step 2 of the protocol.

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

2. **Add Buffer RLN to lyse plasma membrane.**

   For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Carefully resuspend cells in 175 µl cold (4°C) Buffer RLN, and incubate on ice for 5 min. Proceed to step 3.

   The suspension should clear rapidly, indicating lysis of the plasma membrane, which occurs almost immediately.

   Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields. For isolation of cytoplasmic RNA, freshly harvested cells must be used.

   For direct lysis of cells in cell-culture dishes (≤3.5 cm diameter), add 175 µl cold Buffer RLN (4°C). Detach cells gently using a rubber policeman, and transfer to a microcentrifuge tube (not supplied). Incubate on ice for 5 min. Proceed to step 3.

   The suspension should clear rapidly, indicating lysis of the plasma membrane, which occurs almost immediately.

3. **Centrifuge lysate at 4°C for 2 min at 300 x *g*. Transfer supernatant to a new centrifuge tube (not supplied), and discard the pellet. After centrifuging, heat the centrifuge to 20–25°C if the same centrifuge is to be used in the following centrifugation steps of the protocol.**

   The supernatant contains the cytoplasmic extract. It is generally slightly cloudy and yellow-white, depending on the cell type used. The pellet contains the nuclei and cell debris. The pellet is white and considerably smaller than the whole cell pellet obtained during harvesting in step 1.

4. **Add 600 µl Buffer RLT to the supernatant. Mix thoroughly by vigorously vortexing.**

   No further homogenization is required since genomic DNA is not released.

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

5. **Add 430 µl ethanol (96–100%) to the homogenized lysate. Mix thoroughly by pipetting. Do not centrifuge.**

   A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. **Apply 700 µl of the sample, including any precipitate that may have formed, to an RNeasy mini column placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Repeat with the remaining sample.**

   Reuse the collection tube in step 7.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Using the cytoplasmic protocol, DNase digestion is generally not required: most of the DNA is removed by pelleting the nuclei during the procedure, and the RNeasy silica-membrane technology efficiently removes nearly all of the remaining small amounts of DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

7. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 9.

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

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

9. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 10, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 9a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

9a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

10. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

11. **If the expected RNA yield is >30 µg, repeat the elution step (step 10) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 10). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

Protocol for RNA Stabilization in Tissues with RNA*later* RNA Stabilization Reagent

This protocol describes the procedure for storing human and animal tissues in RNA*later* RNA Stabilization Reagent, included in RNeasy Protect Kits. For RNA isolation from stabilized tissues using RNeasy Protect Kits or RNeasy Kits, see the RNeasy Mini Protocols for Isolation of Total RNA from Animal Tissues, page 50.

Important notes before starting

- If using RNeasy Protect Kits or RNA*later* RNA Stabilization Reagent for the first time, read "Important Notes before Using RNeasy Protect Kits and RNA*later* RNA Stabilization Reagent" on pages 16 and 26.
- It is important to use sufficient volumes of RNA*later* RNA Stabilization Reagent to effectively preserve the RNA in tissues. Using too little of the reagent will lead to degradation of RNA in the tissues during storage. Read "Determining the volume of RNA*later* RNA Stabilization Reagent for RNA stabilization in animal tissues" on page 25.
- The tissue must be cut into slices less than 0.5 cm thick. If the tissue slice is thicker than 0.5 cm, it will need to be cut into thinner slices before storing in RNA*later* RNA Stabilization Reagent (see step 2 in the protocol, and read "Maximum tissue size for effective stabilization" on pages 25–26). If tissue slices are thicker than 0.5 cm, diffusion of the reagent into the sample may be too slow for reliable RNA stabilization in the interior. RNA degradation will occur in tissue slices that are too thick.
- Only fresh, unfrozen samples can be stabilized using RNA*later* RNA Stabilization Reagent. Previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.
- RNA is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent. Samples should be submerged in the appropriate volume of RNAlater RNA Stabilization Reagent **immediately** after harvesting the material (see steps 1–3 in the protocol below).
- RNA*later* RNA Stabilization Reagent may form a precipitate when stored below room temperature (15 to 25°C). The precipitate can be redissolved by heating to 37°C with agitation. Redissolve any precipitate before using.

1. **Before excising the tissue sample, estimate the volume (or weight) of the tissue piece to be stabilized in RNA*later* RNA Stabilization Reagent.**
2. **Determine the appropriate volume of RNA*later* RNA Stabilization Reagent needed for preserving the tissue to be used. A minimum of 10 volumes of RNA*later* RNA Stabilization Reagent (or approximately 10 µl/1 mg of tissue) is required. Calculate the correct amount, and pipet it into an appropriate collection vessel.**

If the tissue samples are to be transported in RNA*later* RNA Stabilization Reagent make sure that the tissue remains submerged in the liquid during transport (see "Determining the volume of RNA*later* RNA Stabilization Reagent for RNA stabilization in animal tissues" on page 25).

Note: The tissue should be placed in **at least 10 volumes of RNA*later* RNA Stabilization Reagent (or 10 µl reagent per 1 mg tissue)**. Larger volumes can be used if necessary or desired. Smaller volumes will lead to RNA degradation during storage. See "Determining the volume of RNA*later* RNA Stabilization Reagent for RNA stabilization in animal tissues" on page 25.

3. **Excise the tissue sample from the animal, and cut it into slices less than 0.5 cm thick. Perform this step as quickly as possible. Then proceed immediately with step 4.**

   If the tissue is already thinner than 0.5 cm (in at least one dimension), it does not need to be cut into smaller pieces. Proceed immediately with step 4.

   Note: If tissue slices are thicker than 0.5 cm, diffusion of the reagent into the sample will be too slow for reliable RNA stabilization in the interior. RNA degradation will occur in tissue slices that are too thick. Simply cut larger tissue pieces prior to stabilization into slices less than 0.5 cm thick. The slices can be any convenient size so long as one dimension of the sample is <0.5 cm. See "Maximum tissue size for effective stabilization" on pages 25–26.

4. **Completely submerge the tissue piece(s) in the RNA*later* RNA Stabilization Reagent (pipetted into the collection vessel in step 2).**

   Note: RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent. Samples should be submerged in the appropriate volume of the reagent **immediately** after harvesting the material.

   If the tissue samples are to be transported, make sure that the tubes remain upright during transport, or fill the tube completely with RNA*later* RNA Stabilization Reagent.

5. **Store the tissue submerged in RNA*later* RNA Stabilization Reagent for up to 4 weeks at 2 to 8°C, up to 7 days at 18 to 25°C, or up to 1 day at 37°C.**

   For archival storage at –20°C, first incubate the sample overnight in the reagent at 2 to 8°C. Then transfer the tissue, in the reagent, to –20 °C for storage.

   For archival storage at –80 °C, first incubate the sample overnight in the reagent at 2 to 8°C. Then remove the tissue from the RNA*later* RNA Stabilization Reagent, and transfer it to –80°C for storage.

   Samples stored in RNA*later* RNA Stabilization Reagent at –20°C may not freeze. The low temperature may cause the formation of crystals or a precipitate in the storage solution. This will not affect subsequent RNA isolation. There is no need to redissolve the precipitate.

RNA*later* stabilized samples stored at –20°C or –80°C can be thawed at room temperature and frozen again for up to 20 freeze–thaw cycles without affecting RNA quality or yield.

After storage, continue with the RNeasy Mini Protocol for Isolation of Total RNA from Animal Tissues (page 50).

Note: If available, lower temperatures are recommended for longer storage (e.g., 2 to 8°C for up to 4 weeks instead of 37°C or room temperature; –20°C or –80°C for longer storage).

RNeasy Mini Protocol for Isolation of Total RNA from Animal Tissues

Determining the correct amount of starting material

It is essential to begin with the correct amount of tissue in order to obtain optimal RNA yield and purity with RNeasy columns. A maximum amount of 30 mg tissue can generally be processed with RNeasy mini columns. For most tissues, the binding capacity of the column (100 µg RNA) and the lysing capacity of Buffer RLT will not be exceeded by these amounts. Average RNA yields from various sources are given in Table 2 (page 17).

Some tissues such as spleen, parts of brain, lung, and thymus are more difficult to lyse or tend to form precipitates during the procedure. The volume of lysis buffer may need to be increased to facilitate complete homogenization and to avoid significantly reduced yields, DNA contamination, or clogging of the RNeasy column. See protocol for recommended amounts of lysis buffer to use.

Total RNA isolation from skeletal muscle, heart, and skin tissue can be difficult due to the abundance of contractile proteins, connective tissue, and collagen. The specialized protocol in Appendix C (page 93) includes a proteinase digestion and optimized RNA isolation procedure for these tissues.

If you have no information about the nature of your starting material, we recommend starting with no more than 10 mg of tissue. Depending on the yield and purity obtained, it may be possible to increase the amount of tissue to 30 mg.

Do not overload the column. Overloading will significantly reduce yield and quality.

Important notes before starting

- If using RNeasy or RNeasy Protect Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- **For best results, stabilize animal tissues immediately in RNA*later* RNA Stabilization Reagent following the protocol on page 47. Tissues can be stored in RNA*later* RNA Stabilization Reagent for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or for archival storage at –20°C or –80°C.**
- Fresh, frozen, or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen, and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 4) can also be stored at –70°C for several months. To process frozen lysates, thaw samples and incubate for 15–20 min at 37°C in a water bath to dissolve salts. Continue with step 5.
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**

- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- All steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- All centrifugation steps are performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

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

   Weighing tissue is the most accurate way to determine the amount. See page 50 for guidelines to determine the amount of starting material.
3. **For RNA*later* stabilized tissues:**

   If the entire piece of RNA*later* stabilized tissue can be used for RNA isolation, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 4.

   If only a portion of the RNA*later* stabilized tissue is to be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed with step 4.

   RNA in the RNA*later* treated tissue is still protected while the tissue is processed at 18 to 25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent for further storage. Previously stabilized tissues can be stored at –80°C without the reagent.

For unstabilized fresh or frozen tissues:

If the entire piece of tissue can be used for RNA isolation, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately with step 4.

If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 4.

RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash frozen, or disrupted and homogenized in protocol step 4. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

Note: The remaining fresh tissue can be placed into RNA*later* RNA Stabilization Reagent for stabilization (see Protocol for RNA Stabilization in Tissues with RNA*later* RNA Stabilization Reagent, page 47). However, previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

4. Disrupt tissue and homogenize lysate in Buffer RLT. (Do not use more than 30 mg tissue.)

Disruption and homogenization of animal tissue can be performed by 4 alternative methods (a, b, c, or d). See pages 20–24 for a more detailed description of disruption and homogenization methods.

After storage in RNA*later* RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem. For easier disruption and homogenization it is recommended to increase the volume of lysis Buffer RLT to 600 µl as recommended for tissues that are difficult to lyse.

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

a. Rotor–stator homogenization:

Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a suitably sized vessel for the homogenizer. Add the appropriate volume of Buffer RLT (see below). Homogenize immediately using a conventional rotor–stator homogenizer until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 5.

Rotor–stator homogenization simultaneously disrupts and homogenizes the sample.

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

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

** Use 600 µl Buffer RLT if preparing RNA from tissues that have been stabilized in RNA*later *RNA Stabilization Reagent or that are difficult to lyse.*

b. Mortar and pestle with QIAshredder homogenization:

Immediately place the weighed (fresh, frozen, or RNA*later* 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 below). Pipet the lysate directly onto a QIAshredder spin column placed in 2 ml collection tube, and centrifuge for 2 min at maximum speed. Continue the protocol with step 5.

Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization is carried out by centrifugation through the QIAshredder spin column.

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

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

** Use 600 µl Buffer RLT if preparing RNA from tissues that have been stabilized in RNA*later *RNA Stabilization Reagent or that are difficult to lyse.*

c. Mortar and pestle with needle and syringe homogenization:

Immediately place the weighed (fresh, frozen, or RNA*later* 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 below), and homogenize by passing lysate at least 5 times through a 20-gauge needle fitted to an RNase-free syringe. Continue the protocol with step 5.

Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization is carried out with the needle and syringe.

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

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

** Use 600 µl Buffer RLT if preparing RNA from tissues that have been stabilized in RNA*later *RNA Stabilization Reagent or that are difficult to lyse.*

d. Mixer Mill MM 300:

See "Appendix E: Disruption and Homogenization of RNA*later* Stabilized Tissues Using the Mixer Mill MM 300" (page 101) for guidelines.

The Mixer Mill MM 300 simultaneously disrupts and homogenizes the sample.

5. Centrifuge the tissue lysate for 3 min at maximum speed in a microcentrifuge. Carefully transfer the supernatant to a new microcentrifuge tube (not supplied) by pipetting. Use only this supernatant (lysate) in subsequent steps.

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

6. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Continue without delay with step 7.

If some lysate is lost during steps 4 and 5, adjust volume of ethanol accordingly.

A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

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

Reuse the collection tube in step 8.

If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.[†]

Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

[†] *Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

8. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 10.

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

10. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 11, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 10a.**

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

    Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

10a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

11. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

12. **If the expected RNA yield is >30 µg, repeat the elution step (step 11) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 11). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

RNeasy Mini Protocol for Isolation of Total RNA from Bacteria

Determining the correct amount of starting material

It is essential to use the correct number of bacteria in order to obtain optimal RNA yield and purity with RNeasy columns. Two main criteria limit the maximum number of bacteria to use:

- The RNA binding capacity of the RNeasy mini column (100 µg RNA)
- The volume of Buffer RLT required for efficient lysis. The maximum volume of Buffer RLT that can be used in the RNeasy procedure limits the amount of starting material to an absolute maximum of 1 x 10^9 bacteria.

For bacterial cultures containing high levels of RNA, fewer bacteria should be used in order not to exceed the RNA binding capacity of the RNeasy mini column. For bacterial cultures containing lower levels of RNA, the maximum number of bacteria can be used. In these cases, even though the RNA binding capacity of the column may not be reached, use of more cells would lead to incomplete lysis, resulting in lower RNA yield and purity. Average RNA yields from some bacterial species are given in Table 2 (page 17) and can be used as a guide for calculating the number of bacteria to use.

If the bacterial species used is not shown in Table 2 (page 17) and you have no information about the RNA content of your starting material, we recommend starting with no more than 5 x 10^8 bacteria. Depending on yield and purity, it may be possible to increase the number of bacteria in subsequent preparations.

Do not overload the column. Overloading will significantly reduce yield and purity.

Bacterial growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relations between OD values and cell number in cultures. Cell density is influenced by a variety of factors (e.g., species, media, incubation time, 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. Therefore readings vary between different types of spectrophotometers. In addition, different bacterial species show different OD values at the same wavelength.

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see 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 in calculating the number of cells per milliliter.

The following values may be used as a rough guide. An *E. coli* culture containing 1 x 10^9 cells per milliliter, diluted 1 in 4, gives an OD_{600} 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 1.0 or 0.5, respectively, for 1 x 10^9 bacteria per milliliter.

The RNA content also varies greatly between bacterial strains and under different growth conditions: 1 x 10^9 bacteria corresponds to approximately 20–160 µg total RNA.

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- For RNA isolation, bacteria should be harvested in log-phase growth.
- Bacterial pellets can be stored at –70°C for later use, or used directly in the procedure. Frozen bacterial pellets should be thawed slightly to allow pellets to be dislodged by flicking in step 2. Homogenized cell lysates (in Buffer RLT, step 3) can be stored at –70°C for several months. To process frozen lysates, thaw samples for 15–20 min at 37°C in a water bath to dissolve salts. Continue with step 4.
- **Prepare TE buffer, pH 8.0, with 400 µg/ml lysozyme for Gram-negative bacteria or 3 mg/ml lysozyme for Gram-positive bacteria. TE buffer and lysozyme are not supplied with the kit. See page 28 for details**
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.

- The bacterial culture should be harvested at 4°C. All subsequent steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- After harvesting the cells, all centrifugation steps should be performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. **Harvest bacteria by centrifuging at 5000 x *g* for 5 min at 4°C. (Do not use more than 1 x 10^9 bacteria.) Decant supernatant, and carefully remove all remaining media by aspiration. After centrifuging, heat the centrifuge to 20–25°C if the same centrifuge is to be used in the following centrifugation steps of the protocol.**

   Note: Incomplete removal of the supernatant will inhibit cell-wall digestion in step 2.

2. **Loosen the bacterial pellet by flicking the bottom of the tube. Resuspend the bacteria thoroughly in 100 µl of lysozyme-containing TE buffer (see table below) by vortexing. Incubate at room temperature for the time indicated below.**

	Lysozyme concentration in TE buffer	Incubation time (room temperature)
Gram-negative bacteria	400 µg/ml	3–5 min
Gram-positive bacteria	3 mg/ml	5–10 min

   Depending on the bacterial strain used, the amount of enzyme required and/or the incubation time may vary. For best results, follow the guidelines of the lysozyme supplier. Complete digestion of the cell wall is essential for efficient lysis.

3. **Add 350 µl Buffer RLT to the sample. Mix thoroughly by vortexing vigorously. If insoluble material is visible, centrifuge for 2 min in a microcentrifuge at maximum speed, and use only the supernatant in subsequent steps.**

   If more than 5 x 10^8 bacteria are processed, additional homogenization with a QIAshredder homogenizer or a syringe and needle may increase RNA yield (see pages 20–24).

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

4. **Add 250 µl ethanol (96–100%) to the lysate. Mix thoroughly by pipetting. Do not centrifuge.**

   A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

5. **Apply the sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy mini column placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

   Reuse the collection tube in step 6.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

6. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 8.

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

8. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x g (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 9, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 8a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

8a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

9. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

10. **If the expected RNA yield is >30 µg, repeat the elution step (step 9) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 9). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocols for Isolation of Total RNA from Yeast

There are two different types of protocol for the isolation of total RNA from yeast.

The **Enzymatic Lysis Protocols** (standard and abbreviated versions) require digestion of the cell wall with zymolase or lyticase to convert cells to spheroplasts. In the standard protocol, spheroplasts are separated from the digestion mixture by centrifugation before lysis by centrifugation. In the abbreviated version, for use with up to 2 x 10^7 cells, the digestion mixture is used directly in the RNeasy procedure without prior separation of the spheroplasts.

The **Mechanical Disruption Protocol** uses high-speed agitation in a bead mill in the presence of glass beads and lysis buffer to lyse the cells and release the RNA.

The two types of protocol differ primarily in the way the yeast cell walls are disrupted. In general, the protocols function equally well. For some applications, the Enzymatic Lysis Protocols may be preferable as no additional laboratory equipment is required. However, the Mechanical Disruption Protocol can be used in time-course experiments where enzymatic incubation steps cannot be tolerated.

Determining the correct amount of starting material

It is essential to use the correct number of yeast cells to obtain optimal RNA yield and purity with RNeasy columns. Two main criteria limit the maximum number of yeast cells to use:

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

For yeast cultures containing high levels of RNA, fewer cells should be used in order not to exceed the RNA binding capacity of the RNeasy mini column. For yeast cultures containing lower levels of RNA, the maximum number of cells can be used. In these cases, even though the RNA binding capacity of the column may not be reached, use of more cells would lead to incomplete lysis, resulting in lower RNA yield and purity.

Usually 2 x 10^6 – 5 x 10^7 yeast cells can be processed. Depending on the strain and growth conditions used, 30–100 µg RNA can be expected from 4.0 x 10^7 yeast cells.

If you have 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 yield and purity it may be possible to increase the cell number in subsequent preparations.

Do not overload the column. Overloading will significantly reduce yield and purity.

Yeast growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relations between OD values and cell number in cultures. Cell density is influenced by a variety of factors (e.g., species, media, incubation time, 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. Therefore readings vary between different types of spectrophotometers. In addition, different species show different OD values at the same wavelength.

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see 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 in calculating 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 OD_{600} 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 1.0 or 0.5, respectively, for 1–2 x 10^7 yeast cells per milliliter.

RNeasy Mini Protocol for Isolation of Total RNA from Yeast

I. Enzymatic Lysis Protocol — standard version

Use an appropriate number of yeast cells (see page 61).

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- For RNA isolation from yeast, cells should be harvested in log-phase growth. Use only freshly harvested cells for the enzymatic lysis protocol.
- **Prepare Buffer Y1 (see page 29 for details).**

  Buffer Y1 1 M sorbitol
  0.1 M EDTA, pH 7.4

  Just before use, add:
  0.1% β-mercaptoethanol
  50 U lyticase/zymolase 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 adhere to the guidelines of the enzyme supplier.

- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.

** The use of molecular biology grade reagents is recommended.*

- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- After enzymatic lysis, all steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- After harvesting the cells, all centrifugation steps should be performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. **Harvest yeast cells in a 12 ml or 15 ml centrifuge tube by centrifuging at 1000 x *g* for 5 min at 4°C. (Do not use more than 5 x 10^7 yeast cells.) Decant supernatant, and carefully remove remaining media by aspiration. After centrifuging, heat the centrifuge to 20–25°C if the same centrifuge is to be used in the following centrifugation steps of the protocol.**

   Incomplete removal of the supernatant will affect digestion of the cell wall in step 2.

   Note: Freshly harvested cells must be used.

2. **Resuspend cells in 2 ml freshly prepared Buffer Y1 containing lyticase or zymolase. 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 best results, follow the guidelines of lyticase/zymolase supplier. Complete spheroplasting is essential for efficient lysis.

   Note: Freshly harvested cells must be used for preparation of spheroplasts.

3. **Centrifuge for 5 min at 300 x *g* to pellet 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 silica-gel membrane. Both effects may reduce RNA yield.

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

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

5. **Add 1 volume (usually 350 µl) of 70% ethanol to the homogenized lysate, and mix thoroughly by pipetting. Do not centrifuge. Continue immediately with step 6.**

   A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. **Apply the sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy mini column placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

   Reuse the collection tube in step 7.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

7. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 9.

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

9. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 10, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 9a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

9a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

10. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**
11. **If the expected RNA yield is >30 µg, repeat the elution step (step 10) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 10). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocol for Isolation of Total RNA from Yeast

II. Enzymatic Lysis Protocol — abbreviated version (for up to 2 x 10^7 cells)

Use an appropriate number of yeast cells (see page 61). Do not use more than 2 x 10^7 cells in this protocol.

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page16).
- If working with RNA for the first time, read Appendix A (page 88).
- For RNA isolation from yeast, cells should be harvested in log-phase growth. Use only freshly harvested cells for the enzymatic lysis protocol.
- **Prepare Buffer Y1 (see page 29 for details).**

  Buffer Y1* 1 M sorbitol

  0.1 M EDTA, pH 7.4

  Just before use, add:

  0.1% β-mercaptoethanol

  50 U lyticase/zymolase 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 adhere to the guidelines of the enzyme supplier.
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.

** The use of molecular biology grade reagents is recommended.*

- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- After enzymatic lysis, all steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- After harvesting the cells, all centrifugation steps should be performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. **Harvest yeast cells in a 12 ml or 15 ml centrifuge tube by centrifuging at 1000 x *g* for 5 min at 4°C. (Do not use more than 2 x 10^7 yeast cells.) Decant supernatant, and carefully remove remaining media by aspiration. After centrifuging, heat the centrifuge to 20–25°C if the same centrifuge is to be used in the following centrifugation steps of the protocol.**

   Incomplete removal of the supernatant will affect digestion of the cell wall in step 2.

   Note: Freshly harvested cells must be used.

2. **Resuspend cells in 100 µl freshly prepared Buffer Y1 containing lyticase or zymolase. 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 best results, follow the guidelines of lyticase/zymolase supplier. Complete spheroplasting is essential for efficient lysis.

   Note: Freshly harvested cells must be used for preparation of spheroplasts.

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

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

4. **Add 250 µl ethanol (96–100%) to the homogenized lysate, and mix thoroughly by pipetting. Do not centrifuge. Continue immediately with step 5.**

   A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

5. **Apply the sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy mini column placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

   Reuse the collection tube in step 6.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

6. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 8.

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

8. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 9, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 8a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

8a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

9. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

10. **If the expected RNA yield is >30 µg, repeat the elution step (step 9) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 9). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocol for Isolation of Total RNA from Yeast

III. Mechanical Disruption Protocol

Use an appropriate number of cells (see page 61 for detailed information).

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- For RNA isolation from yeast, cells should be harvested in log-phase growth. Cell pellets can be stored at –70°C for later use or used directly in the procedure. Cell lysates (in Buffer RLT, step 5) can be stored at –70°C for several months. To process frozen lysates, thaw samples for 15–20 min at 37°C in a water bath to dissolve salts. Continue with step 6.
- **Prepare acid-washed glass beads, 0.45–0.55 mm diameter, by soaking in concentrated nitric acid* for 1 hour, washing extensively with deionized water, and drying in a baking oven.**
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.

** Nitric acid is extremely corrosive and can cause severe burns. Use in a fume hood and take appropriate safety measures.*

- After disruption, all steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- After harvesting the cells, all centrifugation steps should be performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. **Add approximately 600 µl of acid-washed glass beads to a tube that fits the bead mill (see page 21 for details).**
2. **Harvest yeast cells by centrifuging at 1000 x *g* for 5 min at 4°C. (Do not use more than 5 x 10^7 yeast cells.) Decant supernatant, and carefully remove remaining media by aspiration. After centrifuging, heat the centrifuge to 20–25°C if the same centrifuge is to be used in the following centrifugation steps of the protocol.**

   Note: Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy silica-gel membrane. Both effects may reduce RNA yield.
3. **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 glass beads prepared in step 1.**

   Note: Ensure that β-ME is added to Buffer RLT before use (see "Important notes before starting").
4. **Vortex and agitate at top speed in a bead-mill homogenizer 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.
5. **Remove the sample from the bead mill, and allow beads to settle. Transfer lysate (usually 350 µl) to a new microcentrifuge tube (not supplied). Centrifuge for 2 min in a microcentrifuge at maximum speed, and transfer the supernatant to a new microcentrifuge tube (not supplied). Use only the supernatant in subsequent steps.**
6. **Add 1 volume (usually 350 µl) of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.**

   If some lysate is lost during homogenization, adjust volume of ethanol accordingly.

   Note: Visible precipitates may form after the addition of ethanol, but this will not affect the RNeasy procedure.

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

   Reuse the collection tube in step 8.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages99–100 after performing this step.

8. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

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

   Reuse the collection tube in step 10.

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

10. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 11, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 10a.**

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

    Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

10a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

11. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

12. **If the expected RNA yield is >30 µg, repeat the elution step (step 11) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 11). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi

Total RNA isolation from plant material and filamentous fungi requires the RNeasy Plant Mini Kit and cannot be performed with the RNeasy Mini Kit or the RNeasy Protect Mini Kit.

Determining the correct amount of starting material

It is essential to begin with the correct amount of plant material in order to obtain optimal RNA yield and purity with RNeasy columns. A maximum of 100 mg plant material or 1 x 10^7 cells can generally be processed with RNeasy mini columns. For most plant material, the binding capacity of the column (100 µg RNA) and the lysing capacity of Buffer RLT will not be exceeded by these amounts. Average RNA yields from various sources are given in Table 2 (page 17).

If you have no information about the nature of your starting material, we recommend starting with no more than 50 mg of plant material or 3–4 x 10^6 cells. Depending on the yield and purity obtained, it may be possible to increase the amount of plant material to 100 mg or to increase the cell number to 1 x 10^7 in subsequent preparations.

Do not overload the column. Overloading will significantly reduce yield and quality.

Important notes before starting

- If using the RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- Fresh or frozen tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen, and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized lysates (in Buffer RLT, step 4) can also be stored at –70°C for several months. To process frozen lysates, thaw samples and incubate for 15–20 min at 37°C in a water bath to dissolve salts. Continue with step 5.
- The RNeasy Plant Mini Kit provides two different lysis buffers, Buffer RLT and Buffer RLC, which contain guanidine isothiocyanate (GITC) and guanidine hydrochloride, respectively. In most cases, Buffer RLT is the lysis buffer of choice due to the greater cell disruption and denaturation properties of GITC. However, depending on the amount and type of secondary metabolites in some tissues (such as milky endosperm of maize or mycelia of filamentous fungi), GITC can cause solidification of the sample, making extraction of RNA impossible. In these cases, Buffer RLC should be used.
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT or Buffer RLC before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**

- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). In these cases, the small residual amounts of DNA remaining can be removed using the RNase-Free DNase Set (cat. no. 79254) for the optional on-column DNase digestion (see page 99) or by a DNase digestion after RNA isolation. For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT, Buffer RLC, and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- All steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- All centrifugation steps are performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. **Determine the amount of plant material. Do not use more than 100 mg.**

   Weighing tissue is the most accurate way to determine the amount. See page 75 for guidelines to determine the amount of starting material.

2. **Immediately place the weighed sample in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant 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. Continue immediately with step 3.**

   RNA in plant material is not protected after harvesting until the sample is flash frozen in liquid nitrogen. Frozen tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. **Add 450 µl Buffer RLT or Buffer RLC (see "Important notes before starting") to a maximum of 100 mg tissue powder. Vortex vigorously.**

   A short (1–3 min) incubation at 56°C may help to disrupt the tissue. However, for samples with a high starch content, incubation at elevated temperatures should be omitted in order to prevent swelling of the starting material.

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

4. **Pipet the lysate directly onto a QIAshredder spin column (lilac) placed in 2 ml collection tube, and centrifuge for 2 min at maximum speed. Carefully transfer the supernatant of the flow-through fraction to a new microcentrifuge tube (not supplied) without disturbing the cell-debris pellet in the collection tube. Use only this supernatant in subsequent steps.**

   It may be necessary to cut the end off the pipet tip in order to pipet the lysate onto the QIAshredder spin column. Centrifugation through the QIAshredder spin column removes cell debris and simultaneously homogenizes the lysate. While most of the cell debris is retained on the QIAshredder spin column, a very small amount of cell debris will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet when transferring the lysate to a new microcentrifuge tube (not supplied).

5. **Add 0.5 volume (usually 225 µl) ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Continue without delay with step 6.**

   If some lysate is lost during step 4, adjust volume of ethanol accordingly.

   A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. **Apply sample (usually 650 µl), including any precipitate that may have formed, to an RNeasy mini column (pink) placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

   Reuse the collection tube in step 7.

   If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

7. **Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the column. Discard the flow-through and collection tube.***

   Skip this step if performing the optional on-column DNase digestion (page 99).

** Flow-through contains Buffer RLT, Buffer RLC, or Buffer RW1 and is therefore not compatible with bleach.*

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

   Reuse the collection tube in step 9.

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

9. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 10, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 9a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

9a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

10. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

11. **If the expected RNA yield is >20 µg, repeat the elution step (step 10) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 10). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

RNeasy Mini Protocol for RNA Cleanup

RNeasy Kits 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 used in the RNA cleanup protocol. This amount corresponds to the binding capacity of the RNeasy mini columns.

Important notes before starting

- If using RNeasy Kits for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- Generally, DNase digestion is not required since the 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-abundant target). We recommend a DNase digestion of the reaction mixture before starting the procedure. The DNase is then removed during the cleanup procedure. Alternatively, the RNase-Free DNase Set (cat. no. 79254) can be used for the optional on-column DNase digestion (see page 99). For on-column DNase digestion with the RNase-Free DNase Set, prepare the DNase I stock solution as described on page 99 before beginning the procedure.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- All steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.
- All centrifugation steps are performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

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

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

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

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

   Optional: QIAGEN offers the RNase-Free DNase Set (cat. no. 79254) for convenient on-column DNase digestion during RNA purification. Generally, DNase digestion is not required since the 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-abundant target). If using the RNase-Free DNase Set, follow the steps shown on pages 99–100 after performing this step.

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

   Reuse the collection tube in step 5.

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

5. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 6, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 5a.**

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

   Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

5a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

** Flow-through contains Buffer RLT and is therefore not compatible with bleach.*

6. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

7. **If the expected RNA yield is >30 µg, repeat the elution step (step 6) as described with a second volume of RNase-free water. Elute into the same collection tube.**

   To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 6). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see inside front cover for contact information).

	Comments and suggestions
Clogged RNeasy column	
a) Inefficient disruption and/or homogenization	See "Disruption and homogenization of starting material" (pages 20–24) for a detailed description of homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see pages 16–19 and protocols) and/or increase the volume of lysis buffer and the homogenization time. If working with tissues rich in proteins, the RNeasy Mini Protocol for Isolation of Total RNA from Heart, Muscle, and Skin Tissue (Appendix C, page 93) may provide better results than the standard tissue protocol.
b) Too much starting material	In subsequent preparations, reduce amounts of starting material. It is essential to use the correct amount of starting material (see pages 16–19 and protocols).
c) Tissues, yeast (mechanical disruption protocol) and bacteria: centrifugation before adding ethanol not performed	Centrifuge lysate before adding ethanol, and use only this supernatant in subsequent steps (see protocols). Pellets contain cell debris that can clog the RNeasy 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 precipitates to form that can clog the RNeasy column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring to the RNeasy column.

Low RNA yield

a) Insufficient disruption and homogenization	See "Disruption and homogenization of starting material" (pages 20–24) for a detailed description of homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see pages 16–19 and protocols) and/or increase the volume of lysis buffer and the homogenization time. If working with tissues rich in proteins, the RNeasy Mini Protocol for Isolation of Total RNA from Heart, Muscle, and Skin Tissue (Appendix C, page 93) may provide better results than the standard tissue protocol.
b) Too much starting material	In subsequent preparations, reduce amounts of starting material. It is essential to use the correct amount of starting material (see pages 16–19 and protocols).
c) RNA still bound to the membrane	Repeat elution, but incubate the RNeasy column on the benchtop for 10 min with RNase-free water before centrifuging.
d) Ethanol carryover	During the second Buffer RPE wash, be sure to dry the RNeasy silica-gel membrane by centrifugation at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C. Following the centrifugation, remove the RNeasy column from the centrifuge tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol. To eliminate any chance of possible ethanol carryover, transfer the RNeasy column to a new 2 ml collection tube and perform the optional 1 min centrifugation step as described in the protocol.
e) Cells: incomplete removal of cell-culture medium	When processing cultured cells, ensure complete removal of the cell-culture medium after harvesting cells (see protocols).

	Comments and suggestions
f) RNA*later* stabilized cells: not pelleted completely or efficiently	Since RNA*later* RNA Stabilization Reagent has a higher density than most cell-culture media, higher centrifugal forces may be necessary to pellet the cells. Initially, try to pellet cells by centrifugation at 3000 x *g*. If necessary, increase the *g*-force in increments of 2000 x *g* until a cell pellet is obtained that is similar to the size of the cell pellet before stabilization. See Appendix F: Guidelines for Other RNA*later* Applications, page 102.
Low A_{260}/A_{280} value	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, pages 90–92).
RNA degraded	
a) Starting material not immediately stabilized	Submerge the sample in the appropriate volume of the RNA*later* RNA Stabilization Reagent immediately after harvesting the material.
b) Too much starting material for proper stabilization	Reduce the amount of starting material or increase the amount of RNA*later* RNA Stabilization Reagent used for stabilization (see "Important Points before Using RNeasy Kits" on pages 25–26).
c) Sample material too thick for stabilization	Cut large samples into slices less than 0.5 cm thick for stabilization in RNA*later* RNA Stabilization Reagent.
d) Frozen samples used for stabilization	Use only fresh, unfrozen material for stabilization.
e) Storage duration exceeded	Storage of RNA*later* stabilized material is possible for up to 1 day at 37°C, up to 7 days at 18 to 25°C, and up to 4 weeks at 2 to 8°C. Store at –20°C or –80°C for archival storage.
f) Sample inappropriately handled	Ensure that samples are properly stabilized and stored in RNA*later* RNA Stabilization Reagent. For frozen cell pellets or tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the protocol quickly, especially the first few steps. See Appendix A (page 88), "Handling and storage of starting material" (page 19), and "Handling of the starting material prior to stabilization" (page 25).
g) RNase contamination	Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be

certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 88).

Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.

DNA contamination in downstream experiments

a) Cells: optimal procedure not used

For animal cells, the cytoplasmic RNA protocol is recommended for applications where the absence of DNA contamination is critical since the intact nuclei are removed at the start of the procedure. Follow the protocol for isolation of cytoplasmic RNA (see page 12 and RNeasy Mini Protocol for Isolation of Cytoplasmic RNA from Animal Cells, page 42).

b) No incubation with Buffer RW1

In subsequent preparations, incubate the RNeasy column for 5 min at room temperature after addition of Buffer RW1 and before centrifuging.

c) No DNase treatment

Follow the optional on-column DNase digestion using the RNase-Free DNase Set (Appendix D, page 99) at the point indicated in the individual protocols.

Alternatively after the RNeasy procedure, DNase digest the eluate containing the RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment, or repurified using the RNA cleanup protocol (page 79).

RNA does not perform well in downstream experiments

a) Salt carryover during elution

Ensure that Buffer RPE is at 20–30°C.

b) Ethanol carryover

During the second Buffer RPE wash, be sure to dry the RNeasy silica-gel membrane by centrifugation at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C. Following the centrifugation, remove the RNeasy column from the centrifuge tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

To eliminate any chance of possible ethanol carryover, transfer the RNeasy column to a new 2 ml collection tube and perform the optional 1 min centrifugation step as described in the protocol.

RNeasy References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, and/or more.

This is a partial list of papers citing RNeasy Kits for total RNA isolation and cleanup. For a complete list of references, visit the QIAGEN Reference database online at **www.qiagen.com/RefDB/search.asp** or contact QIAGEN Technical Services or your local distributor.

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RNeasy purified RNA from dendritic cells for **quantitative RT-PCR analysis on the LightCycler system**

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Chen, X.-P. et al. (2000) Herpes simplex virus type 1 ICP0 protein does not accumulate in the nucleus of primary neurons in culture. J. Virol. ***74****, 10,132.*
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Gonzalez, P., Zigler, J.S., Jr., Epstein, D.L., and Borrás, T. (1999) Identification and isolation of differentially expressed genes from very small tissue samples. BioTechniques ***26****, 884.*
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Gu, L., Tseng, S., Horner, R.M., Tam, C., Loda, M., and Rollins, B.J. (2000) Control of T_H2 polarization by the chemokine monocyte chemoattractant protein-1. Nature ***404****, 407.*
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Lepik, D., Ilves, I., Kristjuhan, A., Maimets, T., and Ustav, T. (1998) p53 protein is a suppressor of papillomavirus DNA amplificational replication. J. Virol. ***72****, 6882.*
Total RNA from **transfected CHO** cells for northern-blot analysis

Lewin, A.S. et al. (1998) Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. Nature Med. **4***, 967.*
Total RNA from **rat retinae** for RT-PCR

Mahadevappa, M. and Warrington, J.A. (1999) A high-density probe array sample preparation method using 10- to 100-fold fewer cells. Nat. Biotechnol. ***17****, 1134.*
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Motlik, J., Carnwath, J.W., Herrmann, D., Terletski, V., Anger, M., and Niemann, H. (1998) Automated recording of RNA differential display patterns from pig granulosa cells. BioTechniques ***24****, 148.*
Total RNA from **pig granulosa cells** for differential-display RT-PCR

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Total RNA from **human liver carcinoma biopsy samples** for RT-PCR

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Microarray analysis of **genome-wide variation of gene expression in human cancer cell lines**

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Differential display of RNeasy purified RNA for **cDNA microarray probes**

Appendix A: General Remarks on Handling RNA

Handling RNA

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

General handling

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

Disposable plasticware

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

Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water (see "Solutions", page 89). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

Glassware

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

* *DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.*

Electrophoresis tanks

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

Solutions

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

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

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

† *DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.*

Appendix B: Storage, Quantitation, and Determination of Quality of Total RNA

Storage of RNA

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

Quantitation of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. To ensure significance, readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml (A_{260} = 1 ⇒ 40 µg/ml). This relation is valid only for measurements in water. Therefore, if it is necessary to dilute the RNA sample, this should be done in water. As discussed below (see "Purity of RNA"), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1M NaOH, 1 mM EDTA followed by washing with RNase-free water (see "Solutions", page 89). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

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

Volume of RNA sample = 100 µl

Dilution = 10 µl of RNA sample + 490 µl distilled water (1/50 dilution).

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

A_{260} = 0.23

Concentration of RNA sample	= 40 x A_{260} x dilution factor = 40 x 0.23 x 50 = 460 µg/ml
Total yield	= concentration x volume of sample in milliliters = 460 µg/ml x 0.1 ml = 46 µg

Purity of RNA

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

* *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.*

measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1* in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution.

For determination of RNA concentration, however, we still recommend dilution of the sample in water since the relationship between absorbance and concentration (A_{260} reading of 1 = 40 µg/ml RNA) is based on an extinction coefficient calculated for RNA in water (see "Quantitation of RNA").

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. To prevent any interference by DNA in RT-PCR applications, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. Alternatively, DNA contamination can be detected on agarose gels following RT-PCR by performing control experiments in which no reverse transcriptase is added prior to the PCR step or by using intron-spanning primers. For sensitive applications, such as differential display, or if it is not practical to use splice-junction primers, 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 99). The DNase is efficiently washed away in the subsequent wash steps. Alternatively, after the RNeasy procedure, the eluate containing the RNA can be treated with DNase. The RNA can then be repurified with the RNeasy cleanup protocol (page 79), or after heat inactivation of the DNase, the RNA can be used directly in downstream applications.

The RNeasy Mini Protocol for Isolation of Cytoplasmic RNA from Animal Cells (page 42) is particularly advantageous in applications where the absence of DNA contamination is critical, since the intact nuclei are removed. Using the cytoplasmic protocol, DNase digestion is generally not required: most of the DNA is removed with the nuclei, and the RNeasy silica-membrane 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-abundant target). Using the cytoplasmic protocol with the optional DNase digestion results in undetectable levels of DNA, even by 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 (see "Appendix H: Protocol for Formaldehyde Agarose Gel Electrophoresis", page 104). The respective ribosomal bands (Table 6) should appear as sharp bands on the stained gel.

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

28S ribosomal RNA bands should be present with an intensity approximately twice that of the 18S RNA band (Figure 1). If the ribosomal bands in a given lane are not sharp, but appear as a smear of smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

Table 6. Size of ribosomal RNAs from various sources

Source	rRNA	Size (kb)
E. coli	16S	1.5
	23S	2.9
S. cerevisiae	18S	2.0
	26S	3.8
Mouse	18S	1.9
	28S	4.7
Human	18S	1.9
	28S	5.0

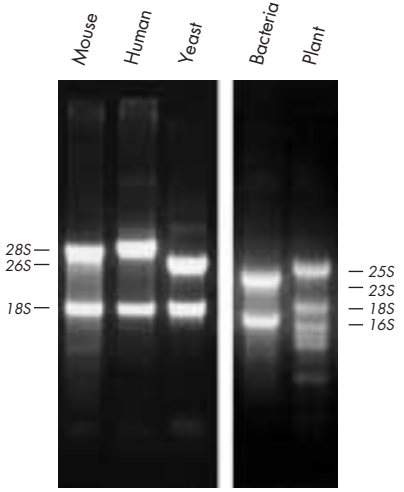

Figure 1. *Formaldehyde agarose gel of total RNA isolated from the indicated sources using RNeasy Kits. 10 µg RNA was loaded per lane.*

Appendix C: RNeasy Mini Protocol for Isolation of Total RNA from Heart, Muscle, and Skin Tissue

Total RNA isolation from skeletal muscle, heart, and skin tissue can be difficult due to the abundance of contractile proteins, connective tissue, and collagen. In order to remove these proteins, which can interfere with the procedure, the standard RNeasy Mini Protocol for Isolation of Total RNA from Animal Tissues has been adapted to include a proteinase K digest. Samples are lysed in a guanidine-isothiocyanate–containing lysis buffer (Buffer RLT). After dilution of the lysate, the sample is treated with proteinase K. Debris is pelleted by centrifugation. Ethanol is then added to the cleared lysate and RNA is bound to the RNeasy silica-gel membrane. Traces of DNA that may copurify are removed by a DNase treatment on the RNeasy mini column. DNase and any contaminants are washed away, and total RNA is eluted in RNase-free water.

The procedure has been used successfully for isolation of total RNA from heart, muscle, and skin tissue. For other tissues, the standard RNeasy Mini Protocol for Isolation of Total RNA from Animal Tissues is generally the method of choice. If working with other tissues rich in proteins where a proteinase K digestion might be desired, we recommend performing a comparison of the two protocols. Since the RNase-inactivating Buffer RLT must be diluted to permit proteinase K digestion, this protocol should not be used for tissues rich in RNases, such as spleen or intestine.

Additional reagents to be supplied by user

- QIAGEN Proteinase K, >600 mAU/ml (cat. no. 19131 or 19133). Proteinase K must be used in the procedure. If using proteinase K from another supplier, use a 20 mg/ml solution in water.
- RNase-Free DNase Set (cat. no. 79254) for on-column DNase treatment
- Double-distilled water

Determining the correct amount of starting material

It is essential to begin with the correct amount of tissue in order to obtain optimal RNA yield and purity with RNeasy columns. A maximum of 30 mg tissue can generally be processed with RNeasy mini columns. For most tissues, the binding capacity of the column (100 µg RNA) and the lysing capacity of Buffer RLT will not be exceeded by these amounts. Average RNA yields from various sources are given in Table 2 (page 17).

If you have no information about the nature of your starting material, we recommend starting with no more than 10 mg of tissue. Depending on the yield and purity obtained, it may be possible to increase the amount of tissue to 30 mg.

Do not overload the column. Overloading will significantly reduce yield and quality.

Important notes before starting

- If using the RNeasy Mini Kit for the first time, read "Important Points before Using RNeasy Kits" (page 16).
- If working with RNA for the first time, read Appendix A (page 88).
- **For best results, stabilize animal tissues immediately in RNA*later* RNA Stabilization Reagent following the protocol on page 47. Tissues can be stored in RNA*later* RNA Stabilization Reagent for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or for archival storage at –20°C or –80°C.**
- Fresh, frozen, and RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen, and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 4) can also be stored at –70°C for several months. To process frozen lysates, thaw samples and incubate for 15–20 min at 37°C in a water bath to dissolve salts. Continue with step 5.
- **Heat a water bath or heating block to 55°C for proteinase K digestion in step 6.**
- **β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.**
- **Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.**
- **Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. Do not vortex.**
- **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.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
- 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. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at 20–25°C.

1. **Excise the tissue sample from the animal or remove it from storage. Remove RNA*later* stabilized tissues from the reagent using forceps.**

2. **Determine the amount of tissue. Do not use more than 30 mg.**

   Weighing tissue is the most accurate way to determine the amount. See page 50 for guidelines to determine the amount of starting material.

3. **For RNA*later* stabilized tissues:**

   If the entire piece of RNA*later* stabilized tissue can be used for RNA isolation, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 4.

   If only a portion of the RNA*later* stabilized tissue is to be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed with step 4.

   RNA in the RNA*later* treated tissue is still protected while the tissue is processed at 18 to 25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent for further storage. Previously stabilized tissues can be stored at –80°C without the reagent.

   For unstabilized fresh or frozen tissues:

   If the entire piece of tissue can be used for RNA isolation, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately with step 4.

   If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 4.

   RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash frozen, or disrupted and homogenized in protocol step 4. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

   Note: The remaining fresh tissue can be placed into RNA*later* RNA Stabilization Reagent for stabilization (see Protocol for RNA Stabilization in Tissues with RNA*later* RNA Stabilization Reagent, page 47). However, previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

4. **Disrupt tissue and homogenize lysate.**

   Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a suitably sized vessel for the homogenizer. Add 300 µl Buffer RLT, and homogenize immediately using a conventional rotor–stator homogenizer until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 5.

Rotor–stator homogenization simultaneously disrupts and homogenizes the sample.

After storage in RNA*later* RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem.

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

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

Alternative method for disruption and homogenization: The sample can alternatively be disrupted using a mortar and pestle and homogenized using QIAshredder homogenizers (cat. no. 79654 or 79656) or a needle and syringe. These methods, however, generally result in lower RNA yields. Homogenization with rotor–stator homogenizers is the method of choice for heart, muscle, or skin tissue.

Immediately place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into a liquid-nitrogen–cooled tube. Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Add 300 µl Buffer RLT.

Pipet the lysate directly onto a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed. Alternatively, homogenize by passing the lysate at least 5–10 times through a 20-gauge needle (0.9 mm diameter) fitted to a syringe. Continue the protocol with step 5.

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

5. **Add 590 µl double-distilled water to the homogenate. Then add 10 µl QIAGEN Proteinase K solution and mix thoroughly by pipetting.**

   Due to the rigorous denaturing conditions of the homogenate, the water added in this step does not need to be RNase-free. Do not use the RNase-free water provided in the RNeasy Mini Kit or the RNase-Free DNase Set for this step.

6. **Incubate at 55°C for 10 min.**

7. **Centrifuge for 3 min at 10,000 x *g* at 20 to 25°C.**

   A small pellet of tissue debris will form, sometimes accompanied by a thin layer or film on top of the supernatant.

8. **Pipet the supernatant (approximately 900 µl) into a new tube (not provided).**

   Avoid transferring any of the pellet. If unavoidable, however, a small amount of pelleted debris may be carried over without affecting the RNeasy procedure. Hold the pipet tip under the thin layer or film on top of the supernatant, if present. This layer will usually adhere to the outside of the pipet tip and should not be transferred.

9. **Add 0.5 volumes (usually 450 µl) of ethanol (96–100%) to the cleared lysate. Mix well by pipetting. Do not centrifuge.**

   A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

10. **Pipet 700 µl of the sample, including any precipitate that may have formed, into an RNeasy mini column placed in a 2 ml collection tube. Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

    Reuse collection tube in step 11.

11. **Repeat step 10, using the remainder of the sample. Discard the flow-through.***

    Reuse collection tube in step 12.

12. **Pipet 350 µl Buffer RW1 into the RNeasy mini column, and centrifuge for 15 s at ≥8000 x *g* to wash. Discard flow-through.***

    and reuse collection tube.

    Optional: If on-column DNase treatment using the RNase-Free DNase Set is not desired, increase the amount of Buffer RW1 in this step to 700 µl, centrifuge for 15 s at ≥8000 x *g* to wash, and discard flow-through* and collection tube. Continue the protocol with step 16.

13. **Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube.**

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

14. **Pipet the DNase I incubation mix (80 µl) directly onto the RNeasy silica-gel membrane, and place on the benchtop (20° to 30°C) for 15 min.**

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

15. **Pipet 350 µl Buffer RW1 into the RNeasy mini column, and centrifuge for 15 s at ≥8000 x *g*. Discard flow-through* and collection tube.**

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

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

    Reuse the collection tube in step 17.

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

17. **Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 18, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 17a.**

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

    Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

17a. **Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.**

18. **To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute.**

19. **If the expected RNA yield is >30 µg, repeat the elution step (step 18) as described with a second volume of RNase-free water. Elute into the same collection tube.**

    To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 18). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

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-membrane DNase digestion. Use of other buffers may affect the binding of the RNA to the RNeasy silica-gel membrane, reducing the yield and integrity of the RNA.

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

- Generally, DNase digestion is not required since the 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-abundant target). DNA can also be removed by a DNase digestion following RNA isolation.
- **Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. Do not vortex.**
- **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.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Carry out lysis, homogenization, and loading onto the RNeasy mini column as indicated in the individual protocols. Instead of continuing with the Buffer RW1 step, follow steps D1–D4 below.

D1. Pipet 350 µl Buffer RW1 into the RNeasy mini column, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash. Discard the flow-through.

Reuse the collection tube in step D3.

D2. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube.

Buffer RDD is supplied with the RNase-Free DNase Set.

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

D3. Pipet the DNase I incubation mix (80 µl) directly onto the RNeasy silica-gel membrane, and place on the benchtop (20–30°C) for 15 min.

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

D4. Pipet 350 µl Buffer RW1 into the RNeasy mini column, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.* Continue with the first Buffer RPE wash step in the relevant protocol.

Note: In most of the protocols, the immediately following BufferRW1 wash step is skipped (as indicated in the protocol). Continue with the first Buffer RPE wash step.

** Flow-through contains Buffer RW1 and is therefore not compatible with bleach.*

Appendix E: Disruption and Homogenization of RNA*later* Stabilized Tissues Using the Mixer Mill MM 300

The Mixer Mill MM 300 allows high-throughput, rapid, and effective disruption of 48 biological samples in 2–4 minutes. Homogenization and disruption with the Mixer Mill MM 300 gives results comparable to using rotor–stator homogenization.

The following guidelines can be used for disruption and homogenization of RNA*later* stabilized tissues using the Mixer Mill MM 300.

M1. Stabilize tissue in RNA*later* RNA Stabilization Reagent as described in the Protocol for RNA Stabilization in Tissues with RNA*later* RNA Stabilization Reagent (page 47).

M2. Pipet 600 µl RNA lysis Buffer RLT into a 2 ml collection tube.

M3. Add one stainless steel bead* to each tube. For best results, we recommend using a 5 mm (mean diameter) stainless steel bead.

M4. Add up to 30 mg tissue (stabilized in RNA*later* RNA Stabilization Reagent) per tube.

M5. Homogenize on the Mixer Mill MM 300 for 2 min at 20 Hz. Homogenization time depends on the tissue used and can be extended until the tissue is completely homogenized.

M6. Rotate the Mixer Mill rack to allow even homogenization, and homogenize for another 2 min at 20 Hz.

M7. Centrifuge the tissue sample (including the bead) for 3 min at maximum speed in a microcentrifuge. Carefully transfer the supernatant to a new microcentrifuge tube (not supplied) by pipetting. Use only this supernatatnt (lysate) in subsequent steps. Do not reuse the stainless steel bead.

M8. Proceed with step 6 of the RNeasy Mini Protocol for Isolation of Total RNA from Animal Tissue (page 54).

** Stainless steel beads for the Mixer Mill MM 300 are available from F. Kurt Retsch GmbH & Co. KG, Haan, Germany or their local distributors (see www.retsch.de).*

Appendix F: Guidelines for Other RNA*later* Applications

RNA stabilization in cell-culture cells

For RNA stabilization:

Pellet the cells by centrifugation at 300 x *g* for 5 min. Discard supernatant and wash cells (e.g., with PBS) to remove all medium. Resuspend the cells in a small volume of PBS (e. g., 50 µl to 100 µl PBS for 1 x 10^6 cells). Add 5 to 10 volumes of RNA*later* RNA Stabilization Reagent.

Note: Do not add the RNA*later* RNA Stabilization Reagent directly to the cell pellet. Always resuspend the cells first in a small volume of PBS.

For RNA isolation:

Pellet the cells, in the RNA*later* RNA Stabilization Reagent, by centrifugation. Remove the supernatant completely and continue with step 2 of the RNeasy Mini Protocol for Isolation of Total RNA from Animal Cells, following either the spin protocol (page 32) or the vacuum protocol (page 38).

Since RNA*later* RNA Stabilization Reagent has a higher density than most cell-culture media, higher centrifugal forces may be necessary for pelleting the cells. It is recommended to use small volumes of cells in the reagent (e. g., up to 500 µl) since smaller volumes of cells pellet efficiently with lower centrifugal force. For example, 500 µl suspensions of HeLa cells or macrophages in RNA*later* RNA Stabilization Reagent will pellet efficiently at 3000 x *g*.

RNA stabilization in white blood cells

RNA*later* RNA Stabilization Reagent can be used to stabilize total RNA in white blood cells. It cannot be used, however, for stabilization of RNA in whole blood, plasma, or sera. These materials will form an insoluble precipitate upon contact with the RNA*later* RNA Stabilization Reagent. White blood cells must be separated from the red blood cells and sera prior to adding the reagent. RNA can be stabilized in the separated white blood cells following the guidelines “RNA stabilization in cell-culture cells” given above.

Appendix G: Guidelines for RT-PCR

To perform PCR using RNA as a starting template, the RNA must first be reverse transcribed into cDNA in a reverse transcription (RT) reaction. RT and PCR can be carried out either sequentially in the same tube (1-step RT-PCR) or separately (2-step RT-PCR).

One-step RT-PCR requires gene-specific primers. For this application, QIAGEN offers the QIAGEN OneStep RT-PCR Kit (see page 111 for ordering information). Two-step RT-PCR is generally carried out using oligo-dT primers in the RT step and gene-specific primers in the PCR step (see Table 7).

Table 7. General guidelines for performing 2-step RT-PCR

Reverse transcription:	QIAGEN offers Omniscript™ and Sensiscript RT Kits for reverse transcription (see page 111 for ordering information). Omniscript RT is specially designed for all reverse transcription with any amount of RNA from 50 ng to 2 µg per reaction. Sensiscript RT is optimized for use with very small amounts of RNA (1 pg – 50 ng). Follow the detailed protocol in the accompanying handbook, or, when using an enzyme from another supplier, follow the supplier's instructions. The following guidelines may be helpful. Mix the following reagents in a microcentrifuge tube: 2.0 µl 10x Buffer RT 2.0 µl dNTP Mix (5 mM each dNTP) 2.0 µl oligo-dT primer (10 µM) 1.0 µl RNase inhibitor (10 units/µl) 1.0 µl Omniscript or Sensiscript RT x µl template RNA (up to 2 µg with Omniscript RT or up to 50 ng with Sensiscript RT) Add RNase-free water to a final volume of 20 µl. • Incubate at 37°C for 60 min.*
PCR:	• Add an aliquot of the finished reverse-transcription reaction to the PCR mix. (No more than 1/5 of the final PCR volume should derive from the finished reverse-transcription reaction.) • Carry out PCR with *Taq* DNA polymerase as recommended by the supplier. (We have consistently obtained excellent results using *Taq* DNA Polymerase or HotStarTaq DNA Polymerase from QIAGEN. See page 111 for ordering information.)

** For other enzymes, refer to supplier's instructions.*

Appendix H: Protocol for Formaldehyde Agarose Gel Electrophoresis

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

1.2% 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 loading buffer (see composition below) per 4 volumes of RNA sample (for example 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.

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

** Toxic and/or mutagenic. Take appropriate safety measures.*

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
3084 µl	formamide
4 ml	10 x FA gel buffer

RNase-free water to 10 ml

Stability: Approximately 3 months at 4°C

* *Toxic and/or mutagenic. Take appropriate safety measures.*

† *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 I: Equipment and Reagent Suppliers*

Rotor–stator homogenizers can be purchased from:

- Kinematica AG; sold by Brinkmann Instruments, Westbury, NY (Polytron® Homogenizers); worldwide, http://www.kinematica.ch
- IKA Works, Cincinnati, OH
- Silverson Machines, Bay Village, OH
- VirTis Company, Gardiner, NY
- IKA Analysentechnik GmbH (Ultra Turrax®)

Bead-mill homogenizers can be purchased from:

- QIAGEN (Mixer Mill MM 200, see ordering information, page 108)

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

- Retsch, Haan, Germany

Lyticase/zymolase can be purchased from:

- Roche Molecular Biochemicals (formerly Boehringer Mannheim Corp.)
- SIGMA
- Medac

Lysozyme can be purchased from:

- Roche Molecular Biochemicals (formerly Boehringer Mannheim Corp.)
- SIGMA
- SERVA

Substitutes for Nonidet P-40 can be purchased from:

- Fluka (Nonidet P-40 Substitute, cat. no. 74385)
- SIGMA (Igepal CA-630, cat. no. I 3021)

Vacuum pumps (18–20 liter/min) can be purchased from:

- In the USA: KNF Neuberger Inc., 2 Black Forest Road, Edgebrook Park, Trenton, NJ 08691-9428
  Telephone: 1-609-890-8889
- In Germany: KNF Neuberger GmbH, Alter Weg 3, D-79112 Freiburg
  Telephone: (49) 7664-5909-0

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

Ordering Information

Product	Contents	Cat. No.
RNeasy Mini Kit — for isolation of up to 100 µg total RNA from animal cells or tissues, yeast, and bacteria		
RNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Mini Kit (250)	250 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74106
RNeasy Protect Mini Kit — for stabilization and purification of RNA from biological samples		
RNeasy Protect Mini Kit (50)	RNA*later* RNA Stabilization Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74124
RNeasy Protect Mini Kit (250)	RNA*later* RNA Stabilization Reagent (250 ml), 250 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74126
RNeasy Plant Mini Kit — for isolation of up to 100 µg total RNA from plants and fungi		
RNeasy Plant Mini Kit (20)	20 RNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74903
RNeasy Plant Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74904
RNA*later* RNA Stabilization Reagent — for stabilization of RNA in animal tissues		
RNA*later* RNA Stabilization Reagent (50 ml)	50 ml RNA*later* RNA Stabilization Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNA*later* RNA Stabilization Reagent (250 ml)	250 ml RNA*later* RNA Stabilization Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106

Ordering Information

Product	Contents	Cat. No.
Accessories		
RNase-Free DNase Set — for DNase digestion during RNA purification		
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer, and RNase-free water for 50 RNA minipreps	79254
QIAshredder Homogenizers — for simple and rapid homogenization of cell and tissue lysates		
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid minipreparation, caps	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers for use in nucleic acid minipreparation, caps	79656
Mixer Mill MM 300 — for simultaneous, rapid, and effective disruption of up to 192 biological samples		
Mixer Mill MM 300	Universal laboratory mixer mill	Inquire
Buffers and collection tubes		
Buffer RLT (220 ml)	220 ml RNeasy Lysis Buffer	79216
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
Related products		
RNeasy Midi Kit — for isolation of up to 1 mg total RNA from animal cells or tissues, yeast, and bacteria		
RNeasy Midi Kit (10)	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Midi Kit (50)	50 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75144
RNeasy Maxi Kit — for isolation of up to 6 mg total RNA from animal cells or tissues, yeast, and bacteria		
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75162

Ordering Information

Product	Contents	Cat. No.
RNeasy Protect Kits — for stabilization and purification of RNA from biological samples		
RNeasy Protect Midi Kit — for isolation of up to 1 mg total RNA		
RNeasy Protect Midi Kit (10)	RNA*later* RNA Stabilization Reagent (20 ml), 10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75152
RNeasy Protect Midi Kit (50)	RNA*later* RNA Stabilization Reagent (100 ml), 50 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75154
RNeasy Protect Maxi Kit — for isolation of up to 6 mg total RNA		
RNeasy Protect Maxi Kit (12)	RNA*later* RNA Stabilization Reagent (100 ml), 12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75182
RNeasy 96 Kit — for high-throughput RNA isolation from animal cells		
RNeasy 96 Kit (4)	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes (1.2 ml), Caps, RNase-free Reagents and Buffers	74181
RNeasy 96 Kit (24)*	For 24 x 96 total RNA preps: 24 RNeasy 96 Plates, Collection Microtubes (1.2 ml), Caps, RNase-free Reagents and Buffers	74183
RNeasy 96 BioRobot® Kit (24)†	For 24 x 96 total and cytoplasmic RNA preps: 24 RNeasy 96 Plates, Collection Microtubes (1.2 ml), Caps, Square-Well Blocks, RNase-free Reagents and Buffers	967143

* *Requires use of either QIAvac 96 or the QIAGEN 96-Well-Plate Centrifugation System.*

† *For use on the BioRobot 9604, configuration C; please inquire.*

Ordering Information

Product	Contents	Cat. No.
QIAamp RNA Blood Mini Kit* — for isolation of total RNA from up to 1.5 ml whole blood		
QIAamp RNA Blood Mini Kit (20)†	For 20 RNA preps: 20 QIAamp Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52303
Oligotex mRNA Kits‡ — for purification of mRNA from total RNA		
Oligotex mRNA Mini Kit (12)	For 12 mRNA minipreps: 200 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	70022
Oligotex mRNA Midi Kit (12)	For 12 mRNA midipreps: 700 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	70042
Oligotex mRNA Maxi Kit (6)	For 6 mRNA maxipreps: 700 µl Oligotex Suspension, Large Spin Columns, Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	70061
Oligotex Suspension‡		
Oligotex Suspension (0.5 ml)	0.5 ml for mRNA isolation from up to 8 mg of total RNA	79000
QIAGEN RNA/DNA Kits — for parallel RNA and DNA, total, and low-molecular-weight RNA isolation		
QIAGEN RNA/DNA Mini Kit (25)	25 QIAGEN-tip 20, RNase-free Reagents and Buffers	14123
QIAGEN RNA/DNA Midi Kit (10)	10 QIAGEN-tip 100, RNase-free Reagents and Buffers	14142
QIAGEN RNA/DNA Maxi Kit (10)	10 QIAGEN-tip 500, RNase-free Reagents and Buffers	14162

* *QIAamp Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of QIAamp Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. QIAamp Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.*

† *Larger kit sizes available; please inquire.*

‡ *Not available in Japan*

Ordering Information

Product	Contents	Cat. No.
Omniscript RT Kit — for reverse transcription using ≥50 ng RNA		
Omniscript RT Kit (50)*	For 50 reverse-transcription reactions: 200 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,† RNase-free water	205111
Sensiscript RT Kit — for reverse transcription using <50 ng RNA		
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,† RNase-free water	205211
QIAGEN OneStep RT-PCR Kit — for fast and efficient one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit (25)*	For 25 reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,‡ dNTP Mix,§ 5x Q-Solution, RNase-free water	210210
HotStarTaq DNA Polymerase — for hot-start PCR		
HotStarTaq DNA Polymerase (250 U)*	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,¶ 5x Q-Solution, 25 mM $MgCl_2$	203203
***Taq* DNA Polymerase — for standard PCR**		
Taq DNA Polymerase (250 U)*	250 units *Taq* DNA Polymerase, 10x PCR Buffer,¶ 5x Q-Solution, 25 mM $MgCl_2$	201203
RNampliFire Kit — for rapid isothermal RNA amplification		
RNampliFire Kit (100)	For 100 RNampliFire reactions: RNampliFire Enzyme Mix, RNase-free NTPs, Reagents, and Buffers	215142
ProofStart™ DNA Polymerase — for high-fidelity PCR		
ProofStart DNA Polymerase (100 U)	100 units ProofStart DNA Polymerase, 10x ProofStart PCR Buffer,** 5x Q-Solution, 25 mM $MgSO_4$	202203

* *Other kit sizes and/or formats available; please inquire.*
† *Contains 5 mM each dNTP*
‡ *Contains 12.5 mM $MgCl_2$*
§ *Contains 10 mM each dNTP*
¶ *Contains 15 mM $MgCl_2$*
** *Contains 15 mM $MgSO_4$*

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RNampliFire products are developed, designed and sold for research and development purposes only. They are not to be used for commercial purposes nor for diagnostic or drug purposes and shall not be administered to humans. All due care and attention should be exercised in the handling of many of the materials described in this text.

RNampliFire products utilize the "NASBA" technology which is based on isothermal transcription-based enzymatic nucleic acid amplification, covered by the following patents and their foreign counterparts: US Patents 5,130,238, 5,409,818, and 5,554,517; European Patents 329,822 and 487,628; and which technology is licensed by QIAGEN from Organon Teknika.

Notes

Notes

QIAGEN Companies

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

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