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QIAwave RNA Plus Mini Handbook

For purification of total RNA from animal cells and easy-to-lyse animal tissues using gDNA Eliminator columns

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Kit Contents

Kit Catalog no. Number of preps	(50) 74634 50	(250) 74636 250
gDNA Eliminator Mini Spin Columns (uncolored) (each in a 2 mL Collection Tube)	50	250
RNeasy® Mini Spin Columns (pink)	50	250
Waste Tubes (2 mL)*	50	250
Buffer RLT Plus*†	45 mL	220 mL
Buffer RW1†	45 mL	220 mL
Buffer RPE/C‡ (concentrate)	1 mL	5 mL
RNase-Free Water	10 mL	30 mL

* Also available separately. See page 61 for Ordering Information.

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

‡ Before using for the first time, mix with ultrapure water and ethanol (96–100%) according to the instructions given in the protocols section and/or on the label.

Shipping and Storage

The QIAwave RNA Plus Mini Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions, if not otherwise stated on the label.

Intended Use

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

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

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

Safety Information

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

<p>CAUTION</p> 	<p>Do not add bleach or acidic solutions directly to the sample-preparation waste.</p>
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Buffer RLT Plus contains guanidine thiocyanate and Buffer RW1 contains a small amount of guanidine thiocyanate. This chemical can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The QIAwave RNA Plus Mini Kit is designed to purify RNA from small amounts of animal cells or tissues. The kit is compatible with a wide range of cultured cells and with easy-to-lyse tissues. Genomic DNA contamination is effectively removed using a specially designed gDNA Eliminator spin column. The purified RNA is ready to use and is ideally suited for downstream applications that are sensitive to low amounts of DNA contamination, such as quantitative, real-time RT-PCR. The purified RNA can also be used in other applications, including:

- Next-generation sequencing
- RT-PCR and digital PCR
- Differential display
- cDNA synthesis
- Northern, dot, and slot blot analyses
- Primer extension
- Poly A+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

The QIAwave RNA Plus Mini Kit allows the parallel processing of multiple samples in less than 25 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 QIAwave RNA Plus procedure.

Principle and procedure

The QIAwave RNA Plus procedure integrates QIAGEN's patented technology for selective removal of double-stranded DNA with well-established RNeasy technology. Efficient purification of high-quality RNA is guaranteed, without the need for additional DNase digestion.

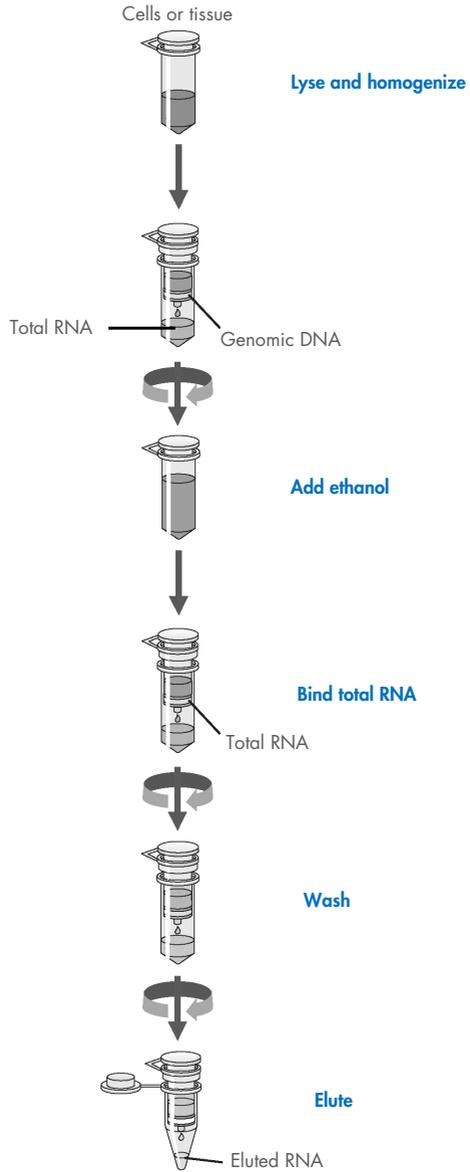
Biological samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate-containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. The lysate is then passed through a gDNA Eliminator spin column. This column, in combination with the optimized high-salt buffer, allows efficient removal of genomic DNA.

Ethanol is added to the flow-through to provide appropriate binding conditions for RNA, and the sample is then applied to the RNeasy spin column, where total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in ≥ 30 μL of water.

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

In this handbook, different protocols are provided for different starting materials. The protocols differ primarily in the lysis and homogenization of the sample. Once the sample is applied to the gDNA Eliminator spin column, the protocols are similar (see the flowchart in the next page).

QIAwave RNA Plus Procedure



Automated purification of RNA on QIAcube Instruments

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

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

The QIAwave RNA Plus Mini Kit can be automated on QIAcube instruments using the RNeasy Plus Mini Kit protocol.



QIAcube Connect.

Equipment and Reagents to Be Supplied by User

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

For all protocols

- Ultrapure Water
- Glassware for reconstitution of buffers (see “Important Notes” for more information)
- 14.3 M β -mercaptoethanol (β -ME) (commercially available solutions are usually 14.3 M), alternatively dithiothreitol (DTT, 2 M stock solution)
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 mL tubes)
- Microcentrifuge tube for elution (1.5 mL or 2 mL)
- **Optional:** Waste Tubes (2 mL) (cat. no. 19211), in case the user prefers to use a new Waste Tube for each washing step. We recommend reusing the same Waste Tube throughout the procedure to reduce plastic consumption.
- 96–100% ethanol*
- 70% ethanol* in water
- Disposable gloves
- For tissue samples: RNAprotect[®] Tissue Reagent (see Ordering Information, page 61) or liquid nitrogen

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

- Equipment for sample disruption and homogenization (see page 20–21). Depending on the method chosen, one or more of the following are required:
 - Trypsin and PBS
 - QIAshredder homogenizer (see Ordering Information, page 61)
 - Blunt-ended needle and syringe
 - Mortar and pestle
 - TissueLyser II, TissueLyser III or TissueLyser LT (see Ordering Information, page 61)
 - TissueRuptor® II homogenizer (see Ordering Information, page 61)

Important Notes

Working with QIAwave products

Preparation of functional buffers

Preparation of final buffer from buffer concentrates

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water	Ethanol (96-100%)	Final volume
74634	RPE	RPE/C	12 mL	52 mL	65 mL
74636	RPE	RPE/C	60 mL	260 mL	325 mL

* Use entire volume

Selected buffers are provided as concentrates in either 15 mL bottles or 2 mL tubes to shrink buffer bottles and reduce the amount of plastics used. Before using the kit for the first time, concentrates have to be reconstituted to receive the functional buffer. This is done with either water or water and ethanol. To reconstitute, the entire volume of the buffer concentrate should be transferred from the 15 mL bottle or 2 mL tube into a suitably sized glass bottle, either by using a pipette or by pouring. Subsequently, the appropriate volume of water or water and ethanol should be added as indicated in the table above. Afterwards, the glass bottle should be capped tightly and the reconstituted buffer mixed thoroughly by inverting.

Water quality used for preparation of functional buffers

We recommend using highly pure water for reconstitution. Ultrapure water (also known as type 1 water) with a resistivity of 18.2 M Ω -cm at 25°C (such as the one from a MilliQ system) works well. In case the users do not have access to type 1 water, QIAGEN offers Nuclease-

Free Water (5 liters, cat. no. 129117)*; and Nuclease-Free Water (1000 mL, cat. no. 129115).*

Important: Avoid using tap water as this can have detrimental impact on the extraction of the target analyte.

Glassware

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

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

Labeling of functional buffers in glass bottles

Reconstituted buffers from buffer concentrates can be labelled with the additional label supplied with the kit. Use the enclosed label and transfer it onto the glass bottle containing the functional buffer prepared before using the kit for the first time.

Waste Tubes

The newly introduced Waste Tube is made of recycled plastic recovered from post-consumer plastic waste. Due to the slight composition differences of the raw material, the color may

* This item needs to be purchased separately.

differ from lot to lot. However, this has no effect on its intended use to collect the flow-through from sample binding and membrane washing. After each step, the flow-through is discarded and the Waste Tube can be reused. The Waste Tube is only used for process waste and should never come into direct contact with the analyte of interest. For detailed instructions, watch our instructional video at www.qiagen.com/qiawavewastetube

Elution tubes

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

Recycling information

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

Determining the amount of starting material

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

- The type of sample, and its DNA and RNA content
- The volume of Buffer RLT Plus required for efficient lysis
- The maximum loading volume of the RNeasy spin column
- The DNA removal capacity of the gDNA Eliminator spin column
- The RNA binding capacity of the RNeasy spin column

When processing samples containing high amounts of DNA or RNA, less than the maximum amount of starting material shown in Table 1 should be used. This is to ensure that the DNA removal capacity of the gDNA Eliminator spin column, and the RNA binding capacity of the RNeasy spin column, will not be exceeded.

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

More information on using the correct amount of starting material is given in each protocol. Table 2 shows expected RNA yields from various cells and tissues.

Note: If the DNA removal capacity of the gDNA Eliminator spin column is exceeded, the purified RNA will be contaminated with DNA. Although the gDNA Eliminator spin column can bind more than 100 µg DNA, we recommend using samples containing less than 20 µg DNA to ensure removal of virtually all genomic DNA.

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

Table 1. RNeasy Mini spin column specifications

Specifications	Amount
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 x 10 ⁷ cells
Animal tissues	30 mg

Table 2. Yields of total RNA with the QIAwave RNA Plus Mini Kit

Cell cultures (1 x 10 ⁶ cells)	Average yield of total RNA* (µg)	Mouse/rat tissues (10 mg)	Average yield of total RNA* (µg)
NIH/3T3	10	Embryo (13 day)	25
HeLa	15	Brain	5–10
COS-7	35	Heart	4–8
LMH	12	Kidney	20–30
Huh	15	Liver	40–60
		Lung	10–20

* Amounts can vary due to different factors such as species, developmental stage, and growth conditions. Because the QIAwave RNA Plus 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.

Counting cells or weighing tissue is the most accurate way 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 vessels after confluent growth is given in Table 3.

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

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

A 3 mm cube (27 mm³) of most animal tissues weighs 30–35 mg.

Handling and storing starting material

RNA in harvested tissues is not protected until the sample is treated with RNAprotect Tissue Reagent, undergo flash-freezing, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur.

Tissue samples should be immediately frozen in liquid nitrogen and stored at -70°C , or immediately immersed in RNAprotect Tissue Reagent. The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RLT Plus (lysis buffer), samples can be stored at -70°C for months.

Disrupting and homogenizing starting material

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

- **Disruption:** Complete disruption of plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced RNA yields.
- **Homogenization:** Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Incomplete homogenization results in inefficient binding of RNA to the RNeasy spin column membrane and therefore significantly reduced RNA yields.

Disruption and homogenization of tissue samples can be carried out rapidly and efficiently using either a TissueLyser system, the TissueLyser III or TissueLyser II, (for processing multiple samples simultaneously) or the TissueRuptor II (for processing samples individually). Disruption and homogenization with TissueLyser systems and TissueRuptor generally results in higher RNA yields than with other methods.

Disruption and homogenization using TissueLyser systems

In bead milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. Different bead mills are available from QIAGEN: the TissueLyser III and TissueLyser II, for medium- to high-throughput disruption; and the TissueLyser LT, for low- to medium-throughput disruption.

The TissueLyser III and the TissueLyser II disrupt and homogenize up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24, which holds 48 x 2 mL microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. For guidelines, please refer to the respective handbooks. If other bead mills are being used for sample disruption and homogenization, refer to suppliers' guidelines.

The TissueLyser III and TissueLyser II can also disrupt and homogenize up to 192 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 96, which holds 192 x 1.2 mL microtubes containing stainless steel beads of 5 mm mean diameter. In this case, we recommend using the RNeasy 96 Universal Tissue Kit, which provides high-throughput RNA purification from all types of tissue in 96-well format. For Ordering Information, see page 61.

The TissueLyser LT disrupts and homogenizes up to 12 samples at the same time. The instrument needs to be used in combination with the TissueLyser LT Adapter, which holds 12 x 2 mL microcentrifuge tubes containing stainless steel beads of 5 or 7 mm mean diameter. For guidelines on using the TissueLyser LT, refer to the *TissueLyser LT Handbook*.

Disruption and homogenization using the TissueRuptor II

The TissueRuptor II is a rotor–stator homogenizer that thoroughly disrupts and simultaneously homogenizes single tissue samples in the presence of lysis buffer in 15–90 seconds, depending on the toughness and size of the sample. The blade of the TissueRuptor disposable probe rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. For guidelines on using the TissueRuptor II, refer to the *TissueRuptor Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.

Protocol: Purification of Total RNA from Animal Cells

Determining the correct amount of starting material

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

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

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

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

If the cell type being processed is not listed in Table 2 (page 18), and if there is no information about its RNA content, we recommend starting with no more than 3 to 4×10^6 cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

Important: Do not overload the gDNA Eliminator spin column, as this will lead to copurification of DNA with RNA. Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and purity.

As a guide, Table 3 (page 19) shows the expected numbers of HeLa cells in different cell-culture vessels.

Important points before starting

- If using the QIAwave RNA Plus Mini Kit for the first time, read the “Important Notes” (page 14).
- If preparing RNA for the first time, read Appendix A (page 43).
- 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 they can be dislodged by flicking the tube in step 2. Homogenized cell lysates from step 3 can be stored at -70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at $3000\text{--}5000 \times g$. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.
- If purifying RNA from cell lines rich in RNases, we recommend adding β -ME to Buffer RLT Plus before use. Add $10 \mu\text{L}$ β -ME per 1 mL Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β -ME can be stored at room temperature for up to 1 month. Alternatively, add $20 \mu\text{L}$ of 2 M DTT per 1 mL Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- Buffer RLT Plus may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature.

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

Things to do before starting

Table 4. Preparation of final buffer from buffer concentrates

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water (mL)	96–100% Ethanol (mL)	Final volume (mL)
74634	RPE	RPE/C	12	52	65
74636	RPE	RPE/C	60	260	325

*Use entire volume

- If purifying RNA from cell lines rich in RNAses, we recommend adding β -ME to Buffer RLT Plus before use. Add 10 μ L β -ME per 1 mL Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β -ME can be stored at room temperature for up to 1 month.
- **Preparation of Buffer RPE (cat. no. 74634):** Transfer the entire volume of Buffer RPE/C from the 2 mL tube into a glass bottle larger than 65 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 52 mL ethanol (96–100%) to obtain a final volume of 65 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer RPE (cat. no. 74636):** Transfer the entire volume of Buffer RPE/C from the 15 mL bottle into a glass bottle larger than 325 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 260 mL ethanol (96–100%) to obtain a final

volume of 325 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.
- Preassemble RNeasy Mini spin columns with Waste Tubes

Procedure

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

1a. **Cells grown in suspension (do not use more than 1×10^7 cells):**

Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at $300 \times g$ in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for DNA removal and RNA purification. Both effects may reduce RNA quality and yield.

1b. **Cells grown in a monolayer (do not use more than 1×10^7 cells):**

Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the 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: Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for DNA removal and RNA purification. Both effects may reduce RNA quality and yield.

To trypsinize and collect cells: Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x g for 5 min. Completely aspirate the supernatant, and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for DNA removal and RNA purification. Both effects may reduce RNA quality and yield.

2. Disrupt the cells by adding Buffer RLT Plus.

2a. **For pelleted cells:**

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

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

Table 5. Volumes of Buffer RLT Plus for lysing pelleted cells

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

2b. **For direct lysis of cells grown in a monolayer:**

Add the appropriate volume of Buffer RLT Plus (see Table 6) to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge

tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

Note: Ensure that β -ME is added to Buffer RLT Plus before use (see “Important points before starting”).

Table 6. Volumes of Buffer RLT Plus for direct cell lysis

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

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

3. Homogenize the lysate according to step 3a, 3b, or 3c. See “Disrupting and homogenizing starting material”, page 20, for more details on homogenization. If processing $\leq 1 \times 10^5$ cells, they can be homogenized by vortexing for 1 min. After homogenization, proceed to step 4.

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

- 3a. Pipet the lysate directly into a QIAshredder spin column placed in a 2 mL collection tube, and centrifuge for 2 min at maximum speed. Proceed to step 4.
 - 3b. Homogenize the lysate for 30 s using the TissueRuptor II. Proceed to step 4.
 - 3c. Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.
4. Transfer the homogenized lysate to a gDNA Eliminator spin column placed in a 2 mL collection tube (supplied). Centrifuge for 30 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the column, and save the flow-through.

Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

5. Add 1 volume (usually 350 or 600 μL) of 70% ethanol to the flow-through, and mix well by pipetting. Do not centrifuge. If some lysate was lost during homogenization and DNA removal, adjust the volume of ethanol accordingly.

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

6. Transfer up to 700 μL of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 mL Waste Tube (both supplied). Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through*. Reuse the Waste Tube in step 7. If the sample volume exceeds 700 μL , centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

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

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

8. Add 500 μL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through. Reuse the Waste Tube in step 9.

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

9. Add 500 μL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. The long

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

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

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

Optional: Discard the flow-through and reuse the Waste Tube. Close the lid gently, and centrifuge at full speed for 1 min. Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 9.

10. Place the RNeasy spin column in a new 1.5 mL microcentrifuge tube (not supplied). Add 30–50 μ L RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute the RNA.
11. If the expected RNA yield is $>30 \mu\text{g}$, repeat step 10 using another 30–50 μ L of RNase-free water, or using the eluate from step 10 (if high RNA concentration is required). Reuse the collection tube from step 10.

If using the eluate from step 10, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Protocol: Purification of Total RNA from Animal Tissues

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. A maximum amount of 30 mg fresh or frozen tissue or 15–20 mg RNAprotect-stabilized tissue (which is partially dehydrated) can generally be processed. For most tissues, the DNA removal capacity of the gDNA Eliminator spin column, the RNA binding capacity of the RNeasy spin column, and the lysing capacity of Buffer RLT Plus will not be exceeded by these amounts. However, smaller amounts may allow more efficient DNA removal. Average RNA yields from various tissues are given in Table 2 (page 18).

For maximum RNA yields from liver, 50% ethanol (instead of 70% ethanol) should be used in step 5 of the procedure.

Some tissues, such as spleen and thymus, contain very high amounts of DNA, which will overload the gDNA Eliminator spin column. For these tissues, we recommend using the QIAWave RNA Mini Kit in combination with the RNase-Free DNase Set (see page 61 for Ordering Information).

RNA yields from fibrous tissues, such as skeletal muscle, heart, and skin, may be low due to the abundance of contractile proteins, connective tissue, and collagen. Proteinase K digestion is not compatible with the QIAwave RNA Plus Mini Kit. For maximum RNA yields from these tissues, we recommend using RNeasy Fibrous Tissue Kits (see page 61 for Ordering Information).

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

Important: Do not overload the gDNA Eliminator spin column, as this will lead to copurification of DNA with RNA. Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and quality.

Important points before starting

- If using the QIAwave RNA Plus Mini Kit for the first time, read the “Important Notes” (page 14).
- If preparing RNA for the first time, read Appendix A (page 43).
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Handbook*). Tissues can be stored in the reagent for up to 1 day at 37°C, 7 days at 18–25°C, or 4 weeks at 2–8°C; or archived at –30°C to –15°C (or at –80°C).
- Fresh, frozen, or RNAprotect-stabilized tissues can be used. Tissues can be stored at –70°C for several months. Flash-freeze tissues in liquid nitrogen, and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT Plus. Homogenized tissue lysates from step 3 can also be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.

Note: If desired, more than 30 mg tissue can be disrupted and homogenized at the start of the procedure (increase the volume of Buffer RLT Plus proportionately). Use a portion of the homogenate corresponding to no more than 30 mg tissue for RNA purification, and store the rest at –80°C.

- β -ME must be added to Buffer RLT Plus before use. Add 10 μ L β -ME per 1 mL Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus

is stable at room temperature for 1 month after addition of β -ME. Alternatively, add 20 μ L 2 M DTT per 1 mL Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.

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

Things to do before starting

Table 7. Preparation of final buffer from buffer concentrates

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water (mL)	96–100% Ethanol (mL)	Final volume (mL)
74634	RPE	RPE/C	12	52	65
74636	RPE	RPE/C	60	260	325

- If purifying RNA from cell lines rich in RNases, we recommend adding β -ME to Buffer RLT Plus before use. Add 10 μ L β -ME per 1 mL Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β -ME can be stored at room temperature for up to 1 month.
- **Preparation of Buffer RPE (cat. no. 74634):** Transfer the entire volume of Buffer RPE/C from the 2 mL tube into a glass bottle larger than 65 mL, either by using a pipet or by pouring. Add 12 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 52 mL ethanol (96–100%) to obtain a final volume of 65 mL. Cap the glass bottle tightly and mix by inverting the bottle several

times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- **Preparation of Buffer RPE (cat. no. 74636):** Transfer the entire volume of Buffer RPE/C from the 15 mL bottle into a glass bottle larger than 325 mL, either by using a pipet or by pouring. Add 60 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 260 mL ethanol (96–100%) to obtain a final volume of 325 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.
- Preassemble RNeasy Mini spin columns with Waste Tubes.

Procedure

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

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

2. Follow either step 2a or 2b.

2a. **For RNAprotect-stabilized tissues:**

If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed to step 3.

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

RNA in RNAprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (18–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in

RNAprotect Tissue Reagent. Previously stabilized tissues can be stored at -80°C without the reagent.

2b. **For unstabilized fresh or frozen tissues:**

If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately to step 3.

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

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

Note: Remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA (see the *RNAprotect Handbook*). However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

3. Disrupt the tissue and homogenize the lysate in Buffer RLT Plus (do not use more than 30 mg tissue) according to step 3a, 3b, 3c, or 3d. See “Disrupting and homogenizing starting material”, page 20, for more details on disruption and homogenization.

Note: Ensure that β -ME is added to Buffer RLT Plus before use (see “Important points before starting”).

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

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

Table 8. Volumes of Buffer RLT Plus for tissue disruption and homogenization

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

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

3a. Disruption and homogenization using the TissueRuptor II:

Place the weighed (fresh, frozen, or RNAprotect-stabilized) tissue in a suitably sized vessel. Add the appropriate volume of Buffer RLT Plus (see Table 8). Immediately disrupt and homogenize the tissue until it is uniformly homogeneous (usually approx. 20–40 s); see the *TissueRuptor Handbook*. Proceed to step 4.

3b. Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer:

Immediately place the weighed (fresh, frozen, or RNAprotect-stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen-cooled, 2 mL microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

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

3c. Disruption using a mortar and pestle followed by homogenization using a needle and syringe:

Immediately place the weighed (fresh, frozen, or RNAprotect-stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen-cooled, 2 mL microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

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

3d. **Disruption and homogenization using the TissueLyser II, TissueLyser III or TissueLyser LT:**

See the *TissueLyser Handbook* or the *TissueLyser LT Handbook*. Then proceed to step 4.

4. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting, and transfer it to a gDNA Eliminator spin column placed in a 2 mL collection tube (supplied). Centrifuge for 30 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the column, and save the flow-through.

Important: This step removes insoluble material that could clog the gDNA Eliminator spin column and interfere with DNA removal. In some preparations, very small amounts of insoluble material will be present after the 3 min centrifugation, making the pellet invisible.

Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

5. Add 1 volume (usually 350 μ L or 600 μ L) of 70% ethanol to the flow-through, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 6.
If some lysate was lost during homogenization and DNA removal, adjust the volume of ethanol accordingly.

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

Note: For maximum RNA yields from liver, use 50% ethanol instead of 70% ethanol.

6. Transfer up to 700 μ L of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 mL Waste Tube (both supplied). Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through*. Reuse the Waste Tube in step 7. If the sample volume exceeds 700 μ L, centrifuge

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

successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

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

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

8. Add 500 μL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through. Reuse the Waste Tube in step 9.

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

9. Add 500 μL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

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

Optional: Discard the flow-through and reuse the Waste Tube. Close the lid gently, and centrifuge at full speed for 1 min. Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 9.

10. Place the RNeasy spin column in a new 1.5 mL microcentrifuge tube (not supplied). Add 30–50 μL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute the RNA.

11. If the expected RNA yield is >30 µg, repeat step 10 using another 30–50 µL of RNase-free water, or using the eluate from step 10 (if high RNA concentration is required). Reuse the collection tube from step 10.
If using the eluate from step 10, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Troubleshooting Guide

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

Comments and suggestions

Clogged gDNA Eliminator spin column

- | | |
|---|--|
| a) Inefficient disruption and/or homogenization | See “Disrupting and homogenizing starting material” (page 20) for details on disruption and homogenization methods.
Increase <i>g</i> force and centrifugation time if necessary.
In subsequent preparations, reduce the amount of starting material (see the protocols, pages 22 and 30) and/or increase the homogenization time. |
| b) Too much starting material | Reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 16). |
| c) Centrifugation temperature too low | The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the gDNA Eliminator spin column. If this happens, set the centrifugation temperature to 25°C. Warm the lysate to 37°C before transferring it to the gDNA Eliminator spin column. |

Low RNA yield

- | | |
|---|--|
| a) Insufficient disruption and homogenization | See “Disrupting and homogenizing starting material” (page 20) for details on disruption and homogenization methods.
In subsequent preparations, reduce the amount of starting material (see the protocols, pages 22 and 30) and/or increase the volume of lysis buffer and the homogenization time. |
| b) Too much starting material | Overloading the RNeasy spin column significantly reduces RNA yield. Reduce the amount of starting material (see page 16). |
| c) Ethanol added to lysate before DNA removal | Pass the lysate through the gDNA Eliminator spin column before adding ethanol to it. |

Comments and suggestions

- | | |
|---|---|
| d) RNA still bound to RNeasy spin column membrane | Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifuging. |
| e) Ethanol carryover | During the second wash with Buffer RPE, be sure to centrifuge at $\geq 8000 \times g$ ($\geq 10,000$ rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane.
Perform the optional centrifugation to dry the RNeasy spin column membrane if any flow-through is present on the outside of the column (step 10 of the protocols). |
| f) Incomplete removal of cell-culture medium (cell samples) | When processing cultured cells, ensure complete removal of cell-culture medium after harvesting cells (see the protocol on page 22). |
| g) Buffer RPE prepared incorrectly | Make sure that ultrapure water and ethanol has been added to Buffer RPE/C before use (see “Things to do before starting”, pages 24 and 32). |

Low A_{260}/A_{280} value

Water used to dilute RNA for A_{260}/A_{280} measurement

Use 10 mM Tris-Cl, * pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 46).

RNA degraded

- | | |
|--|--|
| a) Inappropriate handling of starting material | Ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent.
For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the RNeasy procedure quickly, especially the first few steps.
See Appendix A (page 43) and “Handling and storing starting material” (page 20). |
| b) RNase contamination | Although all RNeasy buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the RNeasy procedure or later handling. See Appendix A (page 43) for general remarks on handling RNA. |

DNA contamination in downstream experiments

- | | |
|-------------------------|--|
| a) Cell number too high | For some cell types, the efficiency of DNA removal may be reduced when processing very high cell numbers (containing more than 20 μg genomic DNA). If the eluted RNA contains substantial DNA contamination, try processing smaller cell numbers. |
|-------------------------|--|

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

Comments and suggestions

- | | |
|---|--|
| b) Incomplete removal of cell-culture medium or stabilization reagent | Be sure to remove any excess cell-culture medium or stabilization reagent to prevent significant dilution of the lysis buffer. The gDNA Eliminator spin column will not work effectively if the lysis buffer is diluted. |
| c) Tissue has high DNA content | For certain tissues with extremely high DNA content (e.g., thymus), DNA may not be completely removed. Try using smaller samples (containing less than 20 µg genomic DNA), or perform DNase digestion of the eluted RNA followed by RNA cleanup. |

RNA concentration too low

- | | |
|-------------------------|--|
| Elution volume too high | Elute RNA with less than 2 x 50 µL of water. Do not use less than 1 x 30 µL of water. Although eluting with less than 2 x 50 µL of water results in increased RNA concentrations, RNA yields may be reduced. |
|-------------------------|--|

RNA does not perform well in downstream experiments

- | | |
|----------------------------------|---|
| a) Salt carryover during elution | Ensure that Buffer RPE is at 20–30°C.
When reusing Waste Tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels. |
| b) Ethanol carryover | During the second wash with Buffer RPE, be sure to centrifuge at $\geq 8000 \times g$ ($\geq 10,000$ rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. After centrifugation, carefully remove the column from the Waste Tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
Perform the optional centrifugation to dry the RNeasy spin column membrane if any flow-through is present on the outside of the column (step 10 of the protocols). |

References

1. 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.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and 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 purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA*, followed by RNase-free water (see "Solutions", page 44), or rinse with chloroform* if the plasticware is chloroform-resistant.

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

To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS)*, rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant) and allow to dry.

Disposable plasticware

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

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 mL DEPC to 100 mL of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO₂. When preparing Tris buffers, treat water with DEPC first and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine

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

residues has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

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

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

Storage of RNA

Purified RNA may be stored at -70°C to -15°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see “Spectrophotometric quantification of RNA” below). For small amounts of RNA, however, it may be difficult to determine amounts photometrically. Small amounts of RNA can be quantified using quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

Using a standard spectrophotometer

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 μg of RNA per mL ($A_{260} = 1 \rightarrow 4 \mu\text{g}/\text{mL}$). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH*. As discussed below (see “Purity of RNA”, page 47), 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

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

cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 44). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

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

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

A_{260} = 0.2
Concentration of RNA sample = 44 μ g/mL $\times A_{260}$ \times dilution factor
= 44 μ g/mL \times 0.2 \times 50
= 440 μ g/mL

Total amount = concentration \times volume in milliliters
= 440 μ g/mL \times 0.1 mL
= 44 μ g of RNA

Purity of RNA

For standard photometric measurements, the ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Because water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination (1). For accurate values, we recommend measuring absorbance in 10 mM Tris-Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1* in 10 mM Tris-Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution. For determination of RNA concentration, however, we

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

recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 $\mu\text{g}/\text{mL}$ RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see “Spectrophotometric quantification of RNA”, page 46).

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

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiNova Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see www.qiagen.com/GeneGlobe). For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, we recommend using the QuantiNova Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see Ordering Information, page 61).

Integrity of RNA

The integrity and size distribution of total RNA purified with QIAwave RNA Plus Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by

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

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

Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

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

FA gel preparation

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

1.2 g agarose*

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

Add RNase-free water to 100 mL

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

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

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

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x loading buffer (see composition below) to 4 volumes of RNA sample (e.g., 10 μL of loading buffer and 40 μL of RNA) and mix.

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

Gel running conditions

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

Composition of FA gel buffers

10x FA gel buffer

200 mM 3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)*
50 mM sodium acetate*
10 mM EDTA*
pH to 7.0 with NaOH*

1x FA gel running buffer

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

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

5x RNA loading buffer

16 μ L saturated aqueous bromophenol blue solution *†

80 μ L 500 mM EDTA, pH 8.0

720 μ L 37% (12.3 M) formaldehyde

2 mL 100% glycerol*

3.084 mL formamide*

4 mL 10 x FA gel buffer

RNase-free water to 10 mL

Stability: approximately 3 months at 4°C

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

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

Appendix D: Purification of Total RNA Containing Small RNAs from Cells

The following procedure allows the purification of total RNA containing small RNAs, such as miRNA from cultured animal and human cells.

This protocol is not suitable for miRNA purification from animal or human tissues. If you need to purify miRNA from animal or human tissues, we recommend using the miRNeasy Mini Kit (cat. no. 217004).

Reagents to be supplied by user

- Ethanol (100%)*

Important points before starting

- This protocol is for use with up to 5×10^6 cells.
- Perform all centrifugation steps at room temperature.

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β -ME to Buffer RLT Plus before use. Add 10 μ L β -ME per 1 mL Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β -ME can be stored at room temperature for up to 1 month. Alternatively, add 20 μ L of 2 M DTT per 1 mL Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in

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

single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.

- **Preparation of Buffer RPE (cat. no. 74634):** Transfer the entire volume of Buffer RPE/C from the 2 mL tube into a glass bottle larger than 65 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 52 mL ethanol (96–100%) to obtain a final volume of 65 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer RPE (cat. no. 74636):** Transfer the entire volume of Buffer RPE/C from the 15 mL bottle into a glass bottle larger than 325 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 260 mL ethanol (96–100%) to obtain a final volume of 325 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

Procedure

1. Add 350 μ L Buffer RLT Plus to the sample, and disrupt and homogenize immediately (see “Disrupting and homogenizing starting material”, page 20, for more information).
2. Transfer the homogenate to a gDNA Eliminator spin column placed in a 2 mL collection tube. Centrifuge for 30 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the column, and save the flow-through.

Note: If necessary, repeat the centrifugation until all liquid has passed through the membrane.

3. Add 1.5 volumes (usually 525 μ L) of 100% ethanol to the flow-through, and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 4.
4. Transfer 700 μ L of the sample, including any precipitate that may have formed, to an RNeasy Mini spin column placed in a 2 mL Waste Tube. Close the lid gently, and

centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through. Reuse the Waste Tube.

Repeat step 4 until the whole sample has passed through the membrane. Discard the flow-through each time.

5. Add 500 μL Buffer RPE to the spin column. Close the lid gently, and centrifuge for 2 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through. Reuse the Waste Tube.

Note: Buffer RPE is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see “Things to do before starting”, page 53).

6. Add 500 μL Buffer RPE to the RNeasy Mini spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane.
7. Discard the flow-through and reuse the Waste Tube. Close the lid, and centrifuge at full speed for 1 min.
8. Place the RNeasy Mini spin column in a 1.5 mL microcentrifuge tube (not supplied). Add 30–50 μL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute the RNA (total RNA containing miRNA).

If the expected RNA yield is $>30 \mu\text{g}$, repeat step 8 with a second volume of RNase-free water. Elute into the same collection tube.

For real-time RT-PCR with the purified RNA, QIAGEN offers the miRCURY® LNA® PCR System, which allows detection of hundreds of miRNAs from a single cDNA synthesis reaction. For details, visit www.qiagen.com/miRNA

Appendix E: Separate Purification of Small RNA (Containing miRNA) and Larger RNA from Animal Cells using the QIAwave RNA Plus Mini Kit and RNeasy MinElute® Cleanup Kit

The following procedure allows the purification of total RNA containing small RNAs, such as miRNA from cultured animal and human cells.

This protocol is not suitable for miRNA purification from animal or human tissues. If you need to purify miRNA from animal or human tissues, we recommend using the miRNeasy Mini Kit (cat. no. 217004).

Reagents to be supplied by user

- RNeasy MinElute Cleanup Kit (50), cat. no. 74204
- Ethanol (70%, 80%, and 100%)*
- Either Collection Tubes or Waste Tubes (2 mL) are supplied with RNeasy® MinElute® Cleanup Kit or QIAwave RNA Plus Mini Kit; if necessary, extra 2 mL collection tubes can be purchased separately (cat. no. 19201)

Important points before starting

- This protocol is for use with up to 5×10^6 cells.
- Perform all centrifugation steps at room temperature.
- Read the handbook supplied with the RNeasy MinElute Cleanup Kit.

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

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β -ME to Buffer RLT Plus before use. Add 10 μ L β -ME per 1 mL Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β -ME can be stored at room temperature for up to 1 month. Alternatively, add 20 μ L of 2 M DTT per 1 mL Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- **Preparation of Buffer RPE (cat. no. 74634):** Transfer the entire volume of Buffer RPE/C from the 2 mL tube into a glass bottle larger than 65 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 52 mL ethanol (96–100%) to obtain a final volume of 65 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer RPE (cat. no. 74636):** Transfer the entire volume of Buffer RPE/C from the 15 mL bottle into a glass bottle larger than 325 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 260 mL ethanol (96–100%) to obtain a final volume of 325 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

Procedure

Separating small RNA from total RNA using the QIAwave RNA Plus Mini Kit

1. Add 350 μ L Buffer RLT Plus to the sample, and disrupt and homogenize immediately (see “Disrupting and homogenizing starting material”, page 20, for more information).

2. Transfer the homogenate to a gDNA Eliminator spin column placed in a 2 mL collection tube. Centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the column, and save the flow-through.

Note: If necessary, repeat the centrifugation until all liquid has passed through the membrane.

3. Add 1 volume (usually 350 μL) of 70% ethanol, and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 4.
4. Transfer the sample, including any precipitate that may have formed, to an RNeasy Mini spin column placed in a 2 mL collection tube (not supplied). Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm).
5. If purifying small RNA (containing miRNA) only, discard the RNeasy Mini spin column and follow steps 6–12 only.

If purifying both small RNA (containing miRNA) and larger RNA (>200 nt), save the RNeasy Mini spin column for use in step 13 (the spin column can be stored at 4°C or at room temperature, but not for long periods). Follow steps 6–12 to purify small RNA, and then steps 13–17 to purify larger RNA.

Purifying small RNA (containing miRNA) using the RNeasy MinElute Cleanup Kit

6. Transfer the flow-through from step 4 (which contains miRNA) to a 2 mL reaction tube (not supplied).
7. Add 0.65 volumes (usually 455 μL) of 100% ethanol, and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 8.
8. Transfer 700 μL of the sample to an RNeasy MinElute spin column placed in a 2 mL collection tube. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through.
Repeat step 8 until the whole sample has passed through the membrane. Discard the flow-through each time.

9. Add 500 μL Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through.

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

10. Add 500 μL of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through and the collection tube.

11. Place the RNeasy MinElute spin column in a new 2 mL collection tube. Open the lid, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm).

12. Place the RNeasy MinElute spin column in a 1.5 mL microcentrifuge tube. Add 14 μL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute the RNA (small RNA containing miRNA).

This RNA eluate is enriched in various RNAs of < 200 nucleotides, including miRNA, 5S rRNA and tRNA. For this reason, the miRNA yield cannot be quantified by OD measurement or fluorogenic assays. To determine yield, we recommend using quantitative, real-time RT-PCR assays specific for the type of small RNA under study. For example, to estimate miRNA yield, an assay directed against any miRNA known to be adequately expressed in the samples being processed may be used.

Purifying larger RNA (> 200 nt) using the QIAwave RNA Plus Mini Kit

13. Add 700 μL Buffer RW1 to the RNeasy Mini spin column from step 4 placed in a Waste Tube. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through. Reuse the Waste Tube.

14. 500 μL Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through.

Note: Buffer RPE is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see “Things to do before starting”, page 57).

15. Add 500 μL Buffer RPE to the RNeasy Mini spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through. Reuse the Waste Tube.
16. Discard the flow-through and reuse the Waste Tube. Open the lid, and centrifuge at full speed for 1 min.
To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).
It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.
17. Place the RNeasy Mini spin column in a 1.5 mL microcentrifuge tube (not supplied). Add 30–50 μL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute the RNA (>200 nt).
If the expected RNA yield is $>30 \mu\text{g}$, repeat step 17 with a second volume of RNase-free water. Elute into the same collection tube.
For real-time RT-PCR with the purified RNA, QIAGEN offers the miRCURY LNA PCR System, which allows detection of hundreds of miRNAs from a single cDNA synthesis reaction. For details, visit www.qiagen.com/miRNA

Ordering Information

Product	Contents	Cat. no.
QIAwave RNA Plus Mini (50)	50 gDNA Eliminator Mini Spin Columns (uncolored), 50 RNeasy Mini Spin Columns, 50 Waste Tubes, 45 mL Buffer RLT Plus, 45 mL Buffer RW1, 1 mL Buffer RPE/C, 10 mL RNase-Free Water	74634
QIAwave RNA Plus Mini (250)	250 gDNA Eliminator Mini Spin Columns (uncolored), 250 RNeasy Mini Spin Columns, 250 Waste Tubes, 220 mL Buffer RLT Plus, 220 mL Buffer RW1, 5 mL Buffer RPE/C, 30 mL RNase-Free Water	74636
Accessories		
Waste Tubes	1000 Waste Tubes (2 mL)	19211
Nuclease-Free Water	5 liters nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); Provided in five 1 liter bottles, delivered in a cardboard box.	129117
Buffer RLT Plus	220 ml Lysepuffer zur Verwendung mit RNeasy Plus Micro Kit, RNeasy Plus Mini Kit, RNeasy Plus 96 Kit, AllPrep DNA/RNA Micro Kit und AllPrep DNA/RNA Mini Kit	1053393
Reagent DX	1 mL Antifoaming Reagent for QIAGEN Lysis Buffers	19088

Product	Contents	Cat. no.
RNAprotect Tissue Reagent (50 mL)*	50 mL RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Tubes (50 x 1.5 mL)*	For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 mL RNAprotect Tissue Reagent each	76154
QIAshredder (50)*	50 disposable cell-lysate homogenizers	79654
TissueRuptor II	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II (and the old TissueRuptor)	990890
TissueLyser LT	Compact bead mill, 100–240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately)	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 mL microcentrifuge tubes on the TissueLyser LT	69980
TissueLyser III	Bead mill; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)	9003240
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 mL microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser II	69984

Product	Contents	Cat. no.
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
Stainless Steel Beads, 5 mm (200)*	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989
QIAcube Connect – for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect†	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µL (1024), 1000 µL filter-tips (1024), 30 mL reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
QIAxcel Connect System – For effortless DNA and RNA analysis		
QIAxcel Connect	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel® Software, and 1-year warranty on parts and labor	9003110
RNeasy Midi Kit (50)*‡	50 RNeasy Midi Spin Columns, Collection Tubes (15 mL), RNase-free Reagents and Buffers	75144
RNeasy Maxi Kit (12)‡	12 RNeasy Maxi Spin Columns, Collection Tubes, RNase-free Reagents and Buffers	75162
miRNeasy Tissue/Cells Advanced Mini Kit (50)	For 50 preps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-Free Water and Buffers	217604

Product	Contents	Cat. no.
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74204
RNeasy Fibrous Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 mL and 2 mL), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	74704
RNeasy 96 Universal Tissue 8000 Kit	For 12 x 96 total RNA preps on the BioRobot 8000 or BioRobot Universal System: 12 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	967852
miRNeasy 96 Kit	For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
miRNeasy Micro Kit (50)	For 50 total RNA preps: 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 mL and 2 mL), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217084
miRNeasy Mini Kit (50)	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 mL and 2 mL), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217004

Product	Contents	Cat. no.
Related products for downstream applications		
Omniscript® RT Kit (50)*	For 50 reverse-transcription reactions: 200 units Omniscript Reverse Transcriptase, 150 µL 10x Buffer RT, 100 µL dNTP Mix (contains 5 mM each dNTP), 1.1 mL RNase-free water	205111
Sensiscript® RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 150 µL 10x Buffer RT, 100 µL dNTP Mix (contains 5 mM each dNTP), 1.1 mL RNase-free water	205211
QuantiNova Rev. Transcription Kit (10)*	For 10 x 20 µL reactions: 20 µL 8x gDNA Removal Mix, 10 µL Reverse Transcription Enzyme, 40 µL Reverse Transcription Mix (containing RT primers), 20 µL Internal Control RNA, 1.9 mL RNase-Free Water	205410
QuantiNova SYBR® Green PCR Kit (100)*	For 100 x 20 µL reactions: 1 mL 2x QuantiNova SYBR® Green PCR Master Mix, 500 µL QuantiNova Yellow Template Dilution Buffer, 250 µL QN ROX Reference Dye, 1.9 mL Water	208052
QuantiNova SYBR® Green RT-PCR Kit (100)*	For 100 x 20 µL reactions: 1 mL QuantiNova SYBR® Green RT-PCR Master Mix, 20 µL QuantiNova SYBR® Green RT Mix, 20 µL Internal Control RNA, 500 µL Yellow Template Dilution Buffer, 250 µL ROX Reference Dye, 1.9 µL RNase-Free Water	208152

Product	Contents	Cat. no.
QuantiNova Probe PCR Kit (100)*	For 100 x 20 µL reactions: 1 mL 2x QuantiNova Probe PCR Master Mix , 250 µL QN ROX Reference Dye, 500 µL QuantiNova Yellow Template Dilution Buffer, 1.9 mL Water	208252
QuantiNova Probe RT-PCR Kit (100)*	For 100 x 20 µL reactions: 1 mL QuantiNova Probe RT-PCR Master Mix, 20 µL QuantiNova Probe RT Mix, 20 µL Internal Control RNA, 500 µL Yellow Template Dilution Buffer, 250 µL ROX Reference Dye, 1.9 µL RNase-Free Water	208352
QuantiNova Multiplex PCR Kit (100)*	For 100 x 20 µL reactions: 500 µL QuantiNova Multiplex PCR Master mix, 500µL yellow template dilution buffer, 250µL ROX reference dye, 1.9µL RNase-free water	208452
QuantiNova Multiplex RT-PCR Kit (100)*	For 100 x 20 µL reactions: 0.5 mL 4x QuantiNova Multiplex RT-PCR Master Mix, 20 µL QuantiNova Multiplex RT-Mix, 20 µL QuantiNova IC RNA, 500 µL QuantiNova Yellow Template Dilution Buffer, 250 µL QN ROX Reference Dye, 1.9 mL RNase-Free Water	208552
miRCURY LNA SYBR® Green PCR Kit (200)	For 200 reactions: 2X miRCURY SYBR® Green Master Mix, RNase-Free Water	339345
miRCURY LNA SYBR® Green PCR Kit (600)	For 600 reactions: 2X miRCURY SYBR Green Master Mix, RNase-Free Water	339346

Product	Contents	Cat. no.
miRCURY LNA miRNA PCR Assay [§]	Contains forward and reverse primers for 200 reactions; for SYBR Green-based detection	339306

* Larger kit size available; see www.qiagen.com

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

‡ Requires use of a centrifuge capable of attaining 3000–5000 x g equipped with a swing-out rotor for 15 mL (Midi) or 50 mL (Maxi) centrifuge tubes.

§ Visit www.qiagen.com/GeneGlobe to search for and order this product.

Notes

Notes

Limited License Agreement for QIAwave RNA Plus Mini

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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Document Revision History

Date	Changes
06/2023	Initial release

