

QIAamp[®] RNA Blood Mini Handbook

For total RNA purification from
human whole blood



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Kit Contents

QIAamp RNA Blood Mini Kit	
Catalog no.	52304
Preparations per Kit	50
QIAamp Spin Columns (clear)	50
QIAshredder Spin Columns (lilac)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	200
Buffer EL*	5 x 120 ml
Buffer RLT*†	45 ml
Buffer RW1†	45 ml
Buffer RPE‡	11 ml
RNase-free Water	10 ml
Handbook	1

* Also available separately (see ordering information, page 44).

† Not compatible with disinfecting reagents that contain bleach. Contains a chaotropic salt. See page 6 for safety information.

‡ Buffer RPE is supplied as a concentrate. Before use, add 4 volumes of ethanol (96–100%) to make a working solution.

Storage

QIAamp® RNA Blood Mini Kits should be stored dry, at room temperature (15–25°C) and are stable for at least 9 months under these conditions.

Product Use Limitations

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

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

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. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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 back 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 the QIAamp RNA Blood Mini Kit 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 back cover).

Quality Control

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

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.

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

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

The following risk and safety phrases apply to components of the QIAamp RNA Blood Mini Kit.

Buffer RLT

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

Buffer RW1

Contains ethanol: flammable. Risk and safety phrases: * R10

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R32: Contact with acids liberates very toxic gas; 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 container or label.

Introduction

QIAamp RNA Blood Mini Kits provide a fast, easy method for the preparation of total cellular RNA from up to 1.5 ml of human whole blood. Multiple blood samples can be processed simultaneously in less than 1 hour. Previous time-consuming and tedious methods, such as CsCl step-gradient ultracentrifugation and alcohol precipitation, or methods involving the use of toxic substances such as phenol and/or chloroform, are replaced by the QIAamp procedure. Contaminants and enzyme inhibitors such as hemoglobin and heparin are completely removed, leaving purified RNA ready for use in downstream applications such as:

- RT-PCR
- Real-time RT-PCR
- cDNA synthesis
- Northern, dot, and slot blotting
- RNase/S1 nuclease protection
- Differential display
- Poly A⁺ RNA selection
- Primer extension

In addition, QIAamp RNA Blood Mini Kits can be used to purify total RNA from tissues and cultured cells, and to separate RNA from proteins, salt and other reaction components after enzymatic reactions such as DNase digestions, proteinase digestions, RNA ligation, and labeling reactions.

Principle and procedure

QIAamp spin columns represent a technology for total RNA preparation that combines the selective binding properties of a silica-based membrane with the speed and convenience of microspin technology. A specialized high-salt buffering system allows RNA species longer than 200 bases to bind to the QIAamp membrane.

During the QIAamp procedure for purification of RNA from blood (see flowchart, page 9), erythrocytes are selectively lysed and leukocytes are recovered by centrifugation. The leukocytes are then lysed using highly denaturing conditions that immediately inactivate RNases, allowing the isolation of intact RNA. After homogenization of the lysate by a brief centrifugation through a QIAshredder spin column, ethanol is added to adjust binding conditions and the sample is applied to the QIAamp spin column. RNA is bound to the silica membrane during a brief centrifugation step. Contaminants are washed away and total RNA is eluted in 30 µl or more of RNase-free water for direct use in any downstream application. Since the procedure relies on intact leukocytes, frozen blood cannot be used.

QIAamp RNA Blood Kits enrich for RNA species larger than 200 nucleotides since small RNAs such as 5.8S RNA, 5S RNA, and tRNA (approximately 160, 120, and 70–90 nucleotides in length, respectively), which make up 15–20% of the total RNA, do not bind in quantity under the conditions used. Thus the size distribution of RNA isolated with the QIAamp procedure is comparable to that of RNA isolated by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently. Phenol-based procedures copurify RNAs <200 nt, accounting for the 20% higher yield of RNA purified using phenol-based procedures in comparison to QIAamp based procedures.

QIAamp RNA Blood Mini Procedure

Blood



Selectively lyse erythrocytes



Lysed erythrocytes
Intact leukocytes



Lyse leukocytes



Homogenize with QIAshredder



Add ethanol



Bind total RNA to QIAamp membrane



Wash 3x



Elute



Ready-to-use RNA

Equipment and Reagents to Be Supplied by User

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

For all protocols

- Pipets and sterile, RNase-free pipet tips
- Microcentrifuge with rotor for 2 ml tubes
- Ethanol (96–100%)*
- 70% ethanol in water*
- 14.3 M β -mercaptoethanol (β -ME) (commercially available solutions are usually 14.3 M)[†]

For blood protocol

- Tubes for erythrocyte lysis (1.5–15 ml depending on sample size); use of sterile, disposable, polypropylene tubes is recommended
- Refrigerated (4°C) microcentrifuge (for blood samples $\leq 250 \mu\text{l}$) or refrigerated laboratory centrifuge with rotor for 12 ml or 15 ml centrifuge tubes (for blood samples $>250 \mu\text{l}$)

For tissue protocol

- Equipment for sample disruption and homogenization (see pages 14–16); depending on the method chosen, one or more of the following are required:
 - QIAGEN TissueRuptor or equivalent rotor–stator homogenizer
 - QIAGEN TissueLyser
 - Mortar and pestle and liquid nitrogen

For cell protocol using trypsin

- Phosphate-buffered saline (PBS)
- Trypsin
- Medium, containing serum

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

[†] β -ME must be added to Buffer RLT before use. See “Things to do before starting”, pages 18, 22, 26, and 30.

Important Notes

Sample collection, storage, and handling

Blood

QIAamp RNA Blood Mini Kits are designed for isolation of total cellular RNA from fresh, human whole blood. Whole blood should be collected in the presence of an anti-coagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or ACD (acid citrate dextrose) can also be used.

For optimal results, blood samples should be processed within a few hours of collection. mRNAs from blood cells have different stabilities. mRNAs of regulatory genes have shorter half-lives than mRNAs of housekeeping genes. To ensure that the isolated RNA contains a representative distribution of mRNAs, blood samples should not be stored for long periods before isolating RNA.

Note: The QIAamp RNA Blood Mini Kit cannot be used for frozen blood samples.

If blood samples must be stored after collection, we recommend using the PAXgene™ Blood RNA System (see page 45 for ordering information). The system consists of a blood collection tube (PAXgene Blood RNA Tube) and nucleic acid purification kit (PAXgene Blood RNA Kit). It is intended for the collection, storage, and transport of blood and stabilization of intracellular RNA in a closed tube and subsequent isolation and purification of intracellular RNA from whole blood for RT-PCR used in molecular diagnostic testing.

Tissues and cells

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. See the *RNA^{later} Handbook* for information about RNA^{later} RNA Stabilization Reagent and about stabilizing RNA in tissues.

After harvesting or excision, samples can be immediately flash frozen in liquid nitrogen* and stored at -70°C . Frozen 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.

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

Starting amounts of samples

Blood

A maximum amount of 1.5 ml of whole blood from healthy adults (typically 4000–7000 leukocytes per microliter) can be processed on a single QIAamp Mini spin column. For blood with elevated numbers of leukocytes, less than 1.5 ml must be used. The maximum number of leukocytes that can be processed is 1×10^7 per spin column. If more leukocytes are processed, they will not be fully lysed and contaminants will not be completely removed, even if the volume of Buffer RLT is increased. To process larger amounts of blood, please call QIAGEN Technical Services or your local distributor for guidelines to scale up the procedure.

Maximum RNA yields using QIAamp RNA MinieKits are generally determined by two criteria: lysis volume and binding capacity of the spin column. Using the maximum amount of leukocytes that can be processed in the procedure (1×10^7), however, the binding capacity of the QIAamp spin column is not usually attained due to the low RNA content of leukocytes.

Tissues and cells

It is essential to use the correct amount of starting material in order to obtain optimal results. The two main factors used to determine the amount of material are:

- the volume of Buffer RLT required for efficient lysis
- the RNA-binding capacity of the QIAamp Mini spin column (100 µg)

For samples containing very high amounts of RNA, this means that smaller amounts of starting material than listed in Table 1 should be used in order to avoid exceeding the RNA-binding capacity of the spin column. Average RNA yields from various sources are provided in Table 2 and can be used as a guide for calculating amounts of starting material.

Table 1. QIAamp Spin-Column Specifications

Maximum binding capacity	100 µg nucleic acid
Maximum loading volume	700 µl
RNA size distribution	>200 nt
Minimum elution volume	30 µl
Maximum amount of starting material	
Blood	1.5 ml*
Cells	1×10^7 *
Tissue	30 mg*

* To process larger amounts, please call your local Technical Services or distributor for details.

Note: If the RNA-binding capacity of the QIAamp spin column is exceeded, yields of total RNA will not be consistent and less than the maximum possible total RNA may be recovered. If the starting material is incompletely lysed, the yield of total RNA will be lower than expected even if the binding capacity of the QIAamp spin column is not exceeded.

Table 2. Yields of Total RNA with QIAamp RNA Blood Mini Kits

Source	Average yield of total RNA* (µg)
Human blood from healthy subjects (1 ml)	3
Mammalian tissues (10 mg)	
Brain	8
Heart	10
Kidney	35
Liver	40
Spleen	35
Lung	10
Cell cultures (1 x 10⁶ cells)	
NIH-3T3	10
HeLa	15
COS-7	35
Huh7	15
Jurkat	14

* Yields can vary due to developmental stage, growth conditions used, etc. Since QIAamp RNA Blood Mini Kits enrich for RNAs >200 nt long, RNA yield does not include 5S RNA, tRNA, and other low-molecular-weight RNAs.

Note: If your starting material is not shown in Table 2 and you have no information regarding RNA content, we recommend beginning with half the maximum amount of starting material indicated in Table 1. Depending on the yield obtained, the sample size may be increased in subsequent preparations.

Examples of how to determine the correct amount of starting material

Lung: Low RNA content (approximately 10 µg RNA per 10 mg tissue)
 — no more than 30 mg tissue can be used (the maximum amount that can be efficiently lysed) even though the RNA-binding capacity of the QIAamp Mini spin column will not be reached.

COS cells: High RNA content (approximately 35 µg RNA per 1 x 10⁶ cells)
 — no more than 3 x 10⁶ cells can be used, otherwise the RNA-binding capacity of the QIAamp Mini spin column will be exceeded.

Lysis and homogenization

Efficient disruption and homogenization of the starting material is essential for all intracellular RNA isolation procedures. Disruption and homogenization are two distinct steps.

Disruption/lysis: Complete disruption and lysis of cell and organelle plasma membranes 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 high-molecular-weight cellular components, creating a homogeneous lysate. Incomplete homogenization results in significantly reduced yields.

Blood

Blood cells are lysed in two separate procedures, erythrocyte lysis and leukocyte lysis. Erythrocytes (red blood cells) of human blood do not contain nuclei and are therefore not important for RNA isolation since they neither synthesize nor contain RNA. The target of RNA isolation from whole blood is leukocytes (white blood cells), which are nucleated and therefore do contain RNA. Leukocytes consist of 3 main cell types: lymphocytes, monocytes, and granulocytes.

Erythrocyte lysis

Since healthy blood contains approximately 1000 times more erythrocytes than leukocytes, removing the erythrocytes simplifies RNA isolation. The QIAamp procedure uses selective lysis of erythrocytes to achieve this. Erythrocytes are more susceptible than leukocytes to hypotonic shock and burst rapidly in the presence of a hypotonic buffer. Intact leukocytes are then recovered by centrifugation. The conditions for selective lysis of erythrocytes in the QIAamp procedure have been optimized to allow fast removal of erythrocytes without affecting the stability of the leukocytes. The erythrocyte-lysis procedure can be scaled up for volumes of whole blood >50 µl.

A common alternative to erythrocyte lysis is Ficoll® density-gradient centrifugation. In contrast to erythrocyte-lysis procedures, Ficoll density-gradient centrifugation only recovers mononuclear cells (lymphocytes and monocytes) and removes granulocytes. Mononuclear cells isolated by Ficoll density-gradient centrifugation can be processed with the QIAamp RNA Blood Mini Kit.

The erythrocyte-lysis and Ficoll density-centrifugation procedures both rely upon intact blood cells, so fresh blood must be used.

Leukocyte lysis

During the QIAamp procedure, leukocytes are efficiently lysed under highly denaturing conditions that immediately inactivate RNases, allowing isolation of intact RNA.

Homogenization of lysate with QIAshredder spin columns

Isolation of total cellular RNA from leukocytes requires efficient disruption of cells and homogenization of lysate for optimal yield and purity. Traditional homogenization methods usually require a rotor–stator homogenizer or a syringe and needle. However, all QIAamp RNA Blood Mini Kits include QIAshredder spin columns, unique tools which supplement the QIAamp procedure. QIAshredder spin columns allow fast and simple homogenization of cell lysates without risk of cross-contamination. Cell lysates are loaded onto the QIAshredder spin column, briefly microcentrifuged, and the homogenized lysate collected in a tube.

Cells

Cultured cells are lysed and homogenized in the same way as leukocytes (see above).

Tissues

Tissues can be disrupted using a rotor–stator homogenizer, such as the QIAGEN TissueRuptor, a mortar and pestle, or a bead mill, such as the QIAGEN TissueLyser. Rotor–stator homogenizers and the TissueLyser system simultaneously disrupt and homogenize the tissue sample, whereas tissues are only disrupted using a mortar and pestle, and a separate homogenization step using a QIAshredder spin column must be performed. Thus, use of a rotor–stator homogenizer or the TissueLyser are the preferred methods.

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

In the presence of lysis buffer, rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize tissues in 5–90 s, depending on the toughness of the sample. The TissueRuptor uses transparent disposable probes, which helps to minimize the risk of cross-contamination and enables visual control of the sample disruption process. See page 45 for ordering information.

Disruption and homogenization using the Tissuelyser system

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

- size and composition of beads
- ratio of buffer to beads
- amount of starting material
- speed and configuration of the Tissuelyser
- disintegration time

Stainless steel beads with a diameter of 5 mm are optimal to use for tissues in combination with the QIAamp RNA Blood Mini Kit. All other disruption parameters should be determined empirically for each application.

The protocol for purification of total RNA from tissues (page 22) contains guidelines for disruption and homogenization of tissues using the Tissuelyser and stainless steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

Homogenization using QIAshredder spin columns

See page 15 for a detailed description.

Disruption using a mortar and pestle

To disrupt tissue 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 an RNase-free, liquid-nitrogen-cooled polypropylene or Corex® tube. Allow the liquid nitrogen to evaporate, but do not allow the sample to thaw. Add lysis buffer, and continue as quickly as possible with homogenization using a QIAshredder spin column, as described above.

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

DNase treatment

Generally, DNase digestion is not required with the QIAamp RNA Blood Mini Kit since the 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., real-time RT-PCR analysis with a low-abundance target). In these cases, residual DNA can be removed by optional on-column DNase digestion using the RNase-Free DNase Set (see Appendix D, page 41). The DNase is efficiently removed in subsequent wash steps. Alternatively, residual DNA can be removed by a DNase digestion after RNA purification (see Appendix E, page 43). The DNase digestion can then be cleaned up, if desired, using “Protocol: RNA Cleanup” (page 30).

QIAamp RNA Blood Mini Kit for RNA cleanup

QIAamp RNA Blood Mini Kits can also be used to purify RNA from enzymatic reactions (e.g., DNase digestion, RNA labeling) or for desalting RNA samples (maximum 100 µg RNA). The QIAamp RNA Mini Protocol for RNA Cleanup (page 30) omits the erythrocyte-lysis step. Buffer RLT and ethanol are added to the sample, which is then loaded onto the QIAamp spin column. Homogenization with a QIAshredder spin column is not required. RNA binds to the QIAamp membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in water.

Protocol: Purification of Total Cellular RNA from Human Whole Blood

Important points before starting

- If using the QIAamp RNA Blood Mini Kit for the first time, please read “Important Notes” (page 11).
- If preparing RNA for the first time, please read “General Remarks for Handling RNA” (Appendix A, page 35).
- Blood and body fluids of all human subjects are considered potentially infectious. All necessary precautions recommended by the Food and Drug Administration (in the USA), the Bundesseuchengesetz (in Germany), or the appropriate regulatory authorities in the country of use, should be taken when working with whole blood.
- The maximum amount of human whole blood that can be processed (1.5 ml) has been determined for blood from healthy adults (approximately 4000–7000 leukocytes per microliter). Reduce amount appropriately if using blood with elevated numbers of leukocytes. A maximum of 1×10^7 leukocytes can be processed on a QIAamp spin column.
- After erythrocyte lysis, all steps of this protocol should be performed at room temperature (15–25°C), as quickly as possible.
- Homogenized cell lysates from step 6 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. Continue with step 7. The use of frozen leukocyte pellets (without addition of Buffer RLT) is not recommended.
- Frozen whole blood cannot be used.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.
- β -Mercaptoethanol (β -ME) must be added to Buffer RLT before use. Add 10 μ l of β -ME per 1 ml of Buffer RLT. Buffer RLT is stable for 1 month at room temperature (15–25°C) after addition of β -ME.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 41).

Procedure

- Mix 1 volume of human whole blood with 5 volumes of Buffer EL in an appropriately sized tube (not provided).**

For optimal results, the volume of the mixture (blood + Buffer EL) should not exceed 3/4 of the volume of the tube to allow efficient mixing. For example, add 5 ml of Buffer EL to 1 ml of whole blood, and mix in a tube which has a total volume of ≥ 8 ml.

Note: Use an appropriate amount of whole blood. Up to 1.5 ml of healthy blood (typically 4000–7000 leukocytes per microliter) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used. (In this case, also adjust amount of Buffer RLT in step 6.)

- Incubate for 10–15 min on ice. Mix by vortexing briefly 2 times during incubation.**

The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 min.

- Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant.**

Leukocytes will form a pellet after centrifugation. Ensure supernatant is completely removed. Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in the following wash step. See page 32 if larger amounts of erythrocytes remain.

- Add Buffer EL to the cell pellet (use 2 volumes of Buffer EL per volume of whole blood used in step 1). Resuspend cells by vortexing briefly.**

For example, add 2 ml of Buffer EL per 1 ml of whole blood used in step 1.

- Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant.**

Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the QIAamp spin column, resulting in lower yield.

- Add Buffer RLT to pelleted leukocytes according to the table below. Vortex or pipet to mix.**

When not using healthy blood, refer to number of leukocytes to determine the volume of Buffer RLT required. Buffer RLT disrupts the cells. No cell clumps should be visible before you proceed to the homogenization step. Vortex or pipet further to remove any clumps.

Buffer RLT* (μ l)	Healthy whole blood (ml)	No. of leukocytes
350	Up to 0.5	Up to 2×10^6
600	0.5 to 1.5	2×10^6 to 1×10^7

* Ensure β -ME is added to Buffer RLT (see "Things to do before starting").

- 7. Pipet lysate directly into a QIAshredder spin column in a 2 ml collection tube (provided) and centrifuge for 2 min at maximum speed to homogenize. Discard QIAshredder spin column and save homogenized lysate.**

To avoid aerosol formation, adjust pipet to $\geq 750 \mu\text{l}$ to ensure that the lysate can be added to the QIAshredder spin column in a single step.

If too many cells have been used, after homogenization the lysate will be too viscous to pipet. If this is the case, please refer to page 32.

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

A precipitate may form after the addition of ethanol. This will not affect the QIAamp procedure.

- 9. Carefully pipet sample, including any precipitate which may have formed, into a new QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Maximum loading volume is 700 μl . If the volume of the sample exceeds 700 μl , successively load aliquots onto the QIAamp spin column and centrifuge as above.**

Discard flow-through* and collection tube.

Optional: If performing optional on-column DNase digestion (see “DNase treatment”, page 41), follow steps D1–D4 (page 42) after performing this step.

- 10. Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Apply 700 μl Buffer RW1 to the QIAamp spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash.**

Discard flow-through* and collection tube.

- 11. Place QIAamp spin column in a new 2 ml collection tube (provided). Pipet 500 μl of Buffer RPE into the QIAamp spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm).**

Discard flow-through* and collection tube.

Note: Ensure ethanol is added to Buffer RPE (see “Things to do before starting”).

- 12. Carefully open the QIAamp spin column and add 500 μl of Buffer RPE. Close the cap and centrifuge at full speed (20,000 $\times g$, 14,000 rpm) for 3 min.**

Note: Some centrifuge rotors may distort slightly upon deceleration, resulting in flow-through, containing Buffer RPE, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

* Flow-through contains Buffer RW1 or RLT and is therefore incompatible with bleach. See page 6 for safety information.

- 13. Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

This step helps to eliminate the chance of possible Buffer RPE carryover.

- 14. Transfer QIAamp spin column into a 1.5 ml microcentrifuge tube (provided) and pipet 30–50 μ l of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute. Repeat if >0.5 ml whole blood (or $>2 \times 10^6$ leukocytes) has been processed.**

Protocol: Purification of Total RNA from Tissues

Important points before starting

- Use an appropriate amount of tissue (see page 13).
- When using QIAamp RNA Blood Mini Kits for the first time, please read “Important Notes”, page 11.
- When preparing RNA for the first time, please read “General Remarks for Handling RNA” (Appendix A, page 35).
- Some tissues, including heart, spleen, and brain, are difficult to homogenize. The volume of lysis buffer may have to be increased to facilitate complete homogenization and to avoid reduced yields. See protocol for amounts.
- For best results, stabilize tissues immediately in *RNAlater* RNA Stabilization Reagent. Tissues can be stored in *RNAlater* TissueProtect Tubes 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. See the *RNAlater Handbook* for more information about *RNAlater* RNA Stabilization Reagent and about stabilizing RNA in tissues.
- Fresh, frozen, or *RNAlater* 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 before disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 1) can also be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature (15–25°C) or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 2.
- All steps of this protocol (including centrifugation) should be performed at room temperature (15–25°C). During the procedure, work quickly.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.
- β -Mercaptoethanol (β -ME) must be added to Buffer RLT before use. Add 10 μ l β -ME per 1 ml of Buffer RLT. This solution is stable for 1 month at room temperature (15–25°C).
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 41).

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

Procedure

1. Disrupt tissue and homogenize lysate according to step 1a, 1b, or 1c.

See “Lysis and homogenization”, pages 14–16, for more details on disruption and homogenization.

Note: Incomplete disruption and homogenization will lead to significantly reduced yields, and can cause clogging of the QIAamp spin column. Homogenization with the TissueLyser, TissueRuptor, or equivalent rotor–stator homogenizers generally results in higher RNA yields than with other methods.

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

After storage in RNA/ater RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem. For easier disruption and homogenization, we recommend using 600 μ l Buffer RLT.

1a. Disruption and homogenization using the QIAGEN TissueRuptor or equivalent rotor–stator homogenizer:

Place the weighed (fresh, frozen, or RNA/ater stabilized) tissue in a suitably sized vessel for the homogenizer. Add the appropriate volume of Buffer RLT (see Table 3). Immediately disrupt and homogenize the tissue using the TissueRuptor or equivalent rotor–stator homogenizer until it is uniformly homogeneous (usually 20–40 s). Centrifuge the lysate for 3 min at maximum speed and use only the supernatant. Proceed to step 2.

Following centrifugation, for some samples, very small amounts of insoluble material may be present, making the pellet invisible.

Table 3. Volumes of Buffer RLT Used for Sample Lysis

Amount of starting material	Volume of Buffer RLT
Up to 20 mg	350 μ l
20 to 30 mg, if tissue is difficult to lyse	600 μ l

As a guide, 3 mm³ of most tissues weighs 30–35 mg.

1b. Disruption and homogenization using the TissueLyser:

Place the weighed (fresh, frozen, or RNA/ater stabilized) tissue in a 2 ml microcentrifuge tube. Add the appropriate volume of Buffer RLT (see Table 3), and add one stainless steel bead (5 mm diameter). Homogenize the lysate on the TissueLyser for 2 min at 20 Hz. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Centrifuge the lysate for 3 min at maximum speed and use only the supernatant. Proceed to step 2.

Note: The instructions in step 1b are only guidelines. They may need to be changed depending on the sample being processed or if a different bead mill is used. See the TissueLyser Handbook for more details.

1c. Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer:

Immediately place the weighed (fresh, frozen, or RNA^{later} stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle, keeping the sample immersed in liquid nitrogen. Transfer the tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen-cooled 2 ml microcentrifuge tube. Allow the liquid nitrogen to evaporate, but do not let allow the tissue to thaw.

Add 600 μ l Buffer RLT. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge at maximum speed for 2 min to homogenize the sample. Discard the QIAshredder spin column, and use only the supernatant. Proceed to step 2.

Note: This method may result in lower yields than those obtained when using the TissueLyser, TissueRuptor, or equivalent rotor-stator homogenizer (see above).

2. Add 1 volume (usually 350 μ l or 600 μ l) of 70% ethanol to the cleared lysate and mix well by pipetting. Do not centrifuge.

If some lysate is lost during homogenization, reduce volume of ethanol accordingly. A precipitate may form after the addition of ethanol but this will not affect the QIAamp procedure.

3. Carefully pipet 700 μ l of the sample, including any precipitate that may have formed, into a QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). If sample volume exceeds 700 μ l, load aliquots successively onto the QIAamp spin column and centrifuge as above.

Discard flow-through* and collection tube.

Optional: If performing optional on-column DNase digestion (see “DNase treatment”, page 41), follow steps D1–D4 (page 42) after performing this step.

4. Transfer QIAamp spin column to a new 2 ml collection tube (provided). Pipet 700 μ l Buffer RW1 onto the QIAamp spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash.

Discard flow-through* and collection tube.

5. Transfer QIAamp spin column to a new 2 ml collection tube (provided). Pipet 500 μ l of Buffer RPE onto the QIAamp spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash.

Discard flow-through* and collection tube.

* Flow-through contains Buffer RW1 or RLT and is therefore incompatible with bleach. See page 6 for safety information.

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

- 6. Carefully open the QIAamp spin column and add 500 μ l of Buffer RPE. Close the cap and centrifuge at full speed (20,000 x g, 14,000 rpm) for 3 min.**

Note: Some centrifuge rotors may distort slightly upon deceleration, resulting in flow-through, containing Buffer RPE, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

- 7. Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

This step helps to eliminate the chance of possible Buffer RPE carryover.

- 8. Transfer QIAamp spin column into a 1.5 ml microcentrifuge tube (provided) and pipet 30–50 μ l of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at ≥ 8000 x g ($\geq 10,000$ rpm) to elute. Repeat if the expected RNA yield is >30 μ g.**

If a second elution step is performed, elute into the same tube using another 30–50 μ l RNase-free water.

Protocol: Purification of Total RNA from Cultured Cells

Important notes before starting

- Use an appropriate number of cultured cells (see page 13).
- When using QIAamp RNA Blood Mini Kits for the first time, please read “Important Notes”, page 11.
- When preparing RNA for the first time, please read “General Remarks for Handling RNA” (Appendix A, page 35).
- All steps of this protocol (including centrifugation) should be performed at room temperature (15–25°C). During the procedure, work quickly.
- After harvesting cells, all centrifugation steps should be performed in a microcentrifuge at 15–25°C.
- 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 2) can be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature (15–25°C) or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 3.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.
- β -Mercaptoethanol (β -ME) must be added to Buffer RLT before use. Add 10 μ l β -ME per 1 ml of Buffer RLT. This solution is stable for 1 month at room temperature (15–25°C).
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 41).

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 x g in a centrifuge tube. Carefully remove all supernatant by aspiration, and proceed to step 2.

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

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

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

To lyse cells directly:

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

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

To trypsinize and collect cells:

Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube, and centrifuge at $300 \times g$ for 5 min. Completely aspirate the supernatant, and proceed to step 2.

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

2. Disrupt cells by adding Buffer RLT.

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

For pelleted cells, loosen cell pellet by flicking the tube and add Buffer RLT (according to the table below). Vortex or pipet to mix. No cell clumps should be visible before proceeding to step 3.

Number of pelleted cells	Volume of Buffer RLT (μ l)
Up to 5×10^6	350
5×10^6 to 1×10^7	600

For monolayer cells, add Buffer RLT (according to the table on the next page) to monolayer cells. Collect cell lysate with a rubber cell scraper. Vortex or pipet to mix. No cell clumps should be visible before proceeding to step 3.

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

* Add the volumes indicated to completely cover the surface of the dish, regardless of the cell number.

- Pipet lysate directly into a QIAshredder spin column in a 2 ml collection tube (provided) and centrifuge for 2 min at maximum speed to homogenize. Discard QIAshredder spin column and save homogenized lysate.**

To avoid aerosol formation, adjust pipet to ≥ 750 µl to ensure that the lysate can be added to the QIAshredder spin column in a single step.

If too many cells have been used, after homogenization the lysate will be too viscous to pipet. If this is the case, please refer to page 32.

- 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 was lost during homogenization, adjust volume of ethanol accordingly. A precipitate may form after the addition of ethanol but this will not affect the QIAamp procedure.

- Carefully pipet sample, including any precipitate which may have formed, into a new QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Maximum loading volume is 700 µl. If the volume of the sample exceeds 700 µl, successively load aliquots onto the QIAamp spin column and centrifuge as above.**

Discard flow-through[†] and collection tube.

Optional: If performing optional on-column DNase digestion (see “DNase treatment”, page 41), follow steps D1–D4 (page 42) after performing this step.

- Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Pipet 700 µl Buffer RW1 into the QIAamp spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash.**

Discard flow-through[†] and collection tube.

- Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Pipet 500 µl Buffer RPE into the spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash.**

Discard flow-through.[†]

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

[†] Flow-through contains Buffer RW1 and is therefore incompatible with bleach. See page 6 for safety information.

- 8. Carefully open the QIAamp spin column and add 500 μ l Buffer RPE. Close the cap and centrifuge at full speed (20,000 \times g, 14,000 rpm) for 3 min.**

Note: Some centrifuge rotors may distort slightly upon deceleration, resulting in flow-through, containing Buffer RPE, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

- 9. Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

This step helps to eliminate the chance of possible Buffer RPE carryover.

- 10. Transfer the QIAamp spin column into a 1.5 ml collection tube (provided) and pipet 30–50 μ l of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute. Repeat if the expected RNA yield is $>30 \mu$ g.**

If a second elution step is performed, elute into the same collection tube using another 30–50 μ l RNase-free water.

Protocol: RNA Cleanup

Important point before starting

- Do not exceed the binding capacity (100 µg) of the QIAamp spin column.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl of β-ME per 1 ml of 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%) to obtain a working solution.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix D (page 41).

Procedure

1. **Adjust sample volume to 100 µl with RNase-free water (provided), add 350 µl Buffer RLT to the sample, and mix thoroughly.**

Note: β-ME must be added to Buffer RLT before use (see “Things to do before starting”).

2. **Add 250 µl ethanol (96–100%) to the lysate and mix by pipetting. Do not centrifuge.**
3. **Pipet sample (700 µl) into a QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim, and centrifuge for 15 s at $\geq 8,000 \times g$ ($\geq 10,000$ rpm).**

Discard flow-through* and collection tube.

Optional: If performing optional on-column DNase digestion (see “DNase treatment”, page 41), follow steps D1–D4 (page 42) after performing this step.

4. **Place the QIAamp spin column into a new 2 ml collection tube (provided), add 500 µl Buffer RPE, and centrifuge for 15 s at $\geq 8,000 \times g$ ($\geq 10,000$ rpm).**

Discard flow-through and collection tube.

Note: Ensure ethanol is added to Buffer RPE (see “Things to do before starting”).

5. **Carefully open the QIAamp spin column and add 500 µl of Buffer RPE. Close the cap and centrifuge at full speed ($20,000 \times g$, 14,000 rpm) for 3 min.**

* Flow-through contains Buffer RW1 and is therefore incompatible with bleach. See page 6 for safety information.

Note: Some centrifuge rotors may distort slightly upon deceleration, resulting in flow-through, containing Buffer RPE, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

- 6. Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

This step helps to eliminate the chance of possible Buffer RPE carryover.

- 7. Transfer the QIAamp spin column to a new 1.5 ml collection tube (provided) and pipet 30–50 μ l of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute. Repeat if the expected RNA yield is $>30 \mu$ g.**

A second elution step into the same collection tube with a further 30–50 μ l RNase-free water is recommended when the expected RNA yield is $>30 \mu$ g.

Troubleshooting Guide

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

Comments and suggestions

Incomplete erythrocyte lysis

- a) The cloudy suspension does not become translucent in step 2
- b) Pellet in step 3 is red

Extend incubation on ice to 20 min.

The leukocyte pellet should be white and may contain residual traces of erythrocytes. However, if erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5–10 min on ice after addition of Buffer EL at step 4.

Very viscous lysate after homogenization with QIAshredder

If too much material has been used, the lysate will be too viscous to pipet after homogenization with QIAshredder. In this case, divide the sample into two aliquots and adjust the volumes of each aliquot to 600 μ l with Buffer RLT. Continue with the procedure from step 7 (blood), step 1b (tissues), or step 2 (cultured cells) using two QIAshredder and two QIAamp spin columns.

Clogged QIAamp spin column

Too much starting material

Inefficient lysis and homogenization. Increase *g*-force and centrifugation time. In subsequent preparations, reduce the amount of starting material (see page 13) and/or increase volume of Buffer RLT.

Little or no RNA eluted

- a) **Blood:** Carryover of erythrocytes See "Incomplete erythrocyte lysis" above.
- b) **All:** Too much starting material used Insufficient lysis and homogenization. In subsequent preparations, reduce the amount of starting material (see page 13) and/or increase the volume of Buffer RLT.
- c) **All:** RNA still bound to the membrane Repeat elution. Incubate QIAamp spin column at room temperature (15–25°C) for 10 min with RNase-free water prior to centrifugation.

RNA degraded

- a) **Blood:** Age of blood Blood sample stored for too long prior to RNA isolation. See "Important Notes" (page 11).
- b) **Tissue:** Insufficient disruption/homogenization Use a rotor–stator homogenizer or the TissueLyser for disruption and homogenization of starting material.
- c) **All:** Quality of starting material RNA in sample degraded during handling prior to purification procedure (e.g., tissue excision, etc). See (d) below.
- d) **All:** Handling Ensure that the protocol, especially during the first few steps, has been performed quickly. See Appendix A, "General Remarks for Handling RNA" (page 35).
- e) **All:** Lysis buffer does not contain β -ME Ensure that β -ME has been added to the lysis buffer (Buffer RLT).
- f) **All:** RNase contamination Check for RNase contamination of buffers. Be certain not to introduce any RNase throughout the procedure or during further handling for analysis. See Appendix A, "General Remarks for Handling RNA" (page 35).

Residual DNA

DNase-digest the eluate containing the RNA (see page 43). Use RNA directly in the downstream experiment after DNase inactivation by heat treatment or repurify RNA using the QIAamp RNA Mini Protocol for RNA Cleanup (see "DNase treatment", page 17).

Alternatively, the QIAGEN RNase-Free DNase Digest Set can be used to completely remove copurified DNA during QIAamp procedures (see "DNase treatment", page 17).

Low A_{260}/A_{280} ratio

Use 10 mM Tris-Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see "Purity of RNA", Appendix B, page 38).

RNA does not perform well in downstream experiments

a) Salt carryover during elution

Ensure that Buffer RPE is at room temperature (15–25°C).

b) Ethanol carryover

Perform the recommended additional centrifugation step in the protocol to remove all traces of ethanol before eluting.

Appendix A: General Remarks for 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 only 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. During the procedure, work quickly and keep purified RNA on ice.

Disposable plasticware

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

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 36). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

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

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with detergent, thoroughly rinsed and oven baked at $\geq 180^{\circ}\text{C}$ for >4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases, but oven-baking will. 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 min to denature residual DEPC.

Note: Corex tubes should be rendered RNase-free by treatment with DEPC and not by baking. This will reduce the failure rate of this type of tube during centrifugation.

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 others) 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 per 100 ml of the solution to be treated, shake vigorously to thoroughly mix the DEPC with the solution and incubate for 12 hours at 37°C . Autoclave for 15 min to remove any trace of DEPC. It may be desirable to test water sources for the presence of contaminating RNases since many sources of distilled water are not RNase-free. DEPC will react with primary amines and so cannot be used to directly treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2 . When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues has been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C for 15 min.

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

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

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

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

Storage of total RNA

Total RNA may be stored at -20°C or -70°C in RNase-free water. Under these conditions at QIAGEN, no degradation of RNA has been detected even after 1 year.

Quantification of total RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a 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 milliliter ($A_{260} = 1 \rightarrow 44 \mu\text{g}/\text{ml}$). 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.* The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity (see "Purity of RNA", page 38).

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA* followed by a brief wash with RNase-free water (see "Solutions", page 36). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

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

Volume of RNA sample = 100 μl

Dilution = 20 μl of RNA sample + 80 μl 10 mM Tris-Cl,* pH 7.0 (1/5 dilution)

Measure dilution in a 1 ml cuvette (RNase-free): $A_{260} = 0.15$

Concentration of RNA stock = 40 $\mu\text{g}/\text{ml}$ x A_{260} x dilution factor

$$= 40 \mu\text{g}/\text{ml} \times 0.15 \times 5$$

$$= 30 \mu\text{g}/\text{ml}$$

Total yield = concentration x volume of stock in milliliters

$$= 30 \mu\text{g}/\text{ml} \times 0.1 \text{ ml}$$

$$= 3 \mu\text{g}$$

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

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 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 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 “Quantification of total RNA”, page 37).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While QIAamp RNA Blood Mini Kits will remove the vast majority of cellular DNA, trace amounts may still remain, depending on the amount and nature of the sample.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with ABI PRISM[®] and LightCycler[®] instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified.

For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, we recommend using the QuantiTect[®] Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination.

* 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.

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

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

For other sensitive applications, DNase digestion of the purified RNA with RNase-free DNase is recommended. A protocol for optional on-column DNase digestion using the RNase-Free DNase Set is provided in Appendix D (page 41). The DNase is efficiently washed away in subsequent wash steps. Alternatively, after the QIAamp RNA procedure, the RNA eluate can be treated with DNase (see page 43). The RNA can then be repurified according to the RNA cleanup protocol (page 30), or after heat inactivation of the DNase, the RNA can be used directly in downstream applications.

Integrity of RNA

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

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, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd 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. Before 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 material safety data sheets (MSDSs), available from the product supplier.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x RNA loading buffer (see composition below) per 4 volumes of RNA sample (for example: 10 μ l 5x RNA loading buffer and 40 μ l RNA).

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 per cm with 1x FA gel running buffer.

Composition of FA gel buffers

10x FA gel buffer

200 mM 3-[N-morpholino]propanesulfonic acid, free acid (MOPS)*

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

Note: Equilibrate gel in 1x FA gel running buffer for at least 30 min before starting electrophoresis.

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*

3084 μ l formamide*

4 ml 10x FA gel buffer

RNase-free water to 10 ml

Stability: Approximately 3 months at 4°C

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

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

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

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

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

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

Important points before starting

- Generally, DNase digestion is not required with the QIAamp RNA Blood Mini Kit since the 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., real-time RT-PCR analysis with a low-abundance target). DNA can also be removed by a DNase digestion following RNA purification.
- **Do not vortex the reconstituted DNase I.** DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 μ l of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at -20°C for up to 9 months. Thawed aliquots can be stored at $2-8^{\circ}\text{C}$ for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Prepare and load samples onto the QIAamp spin column as indicated in the individual protocols. Instead of performing the first wash step, follow steps D1–D4 below.

- D1. Add 350 µl Buffer RW1 to the QIAamp 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 collection tube in step D4.

- D2. Add 10 µl DNase I stock solution (see page 41) to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.**

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

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

- D3. Add the DNase I incubation mix (80 µl) directly to the QIAamp spin column membrane, and place on the benchtop (20–30°C) for 15 min.**

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

- D4. Add 350 µl Buffer RW1 to the QIAamp spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). 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 Buffer RW1 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. See page 6 for safety information.

Appendix E: DNase Digestion of RNA before RNA Cleanup

This protocol describes how to use the RNase-Free DNase Set (cat. no. 79254) to digest contaminating DNA in RNA solutions prior to RNA cleanup. DNase digestion can alternatively be carried out during RNA cleanup (see Appendix D, page 41). For samples highly contaminated with DNA, we recommend DNase digestion in solution, as it is more efficient than on-column DNase digestion.

Important points before starting

- Generally, DNase digestion is not required with the QIAamp RNA Blood Mini Kit since the 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., real-time RT-PCR analysis with a low-abundance target).
- **Do not vortex the reconstituted DNase I.** DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 μ l of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at -20°C for up to 9 months. Thawed aliquots can be stored at $2-8^{\circ}\text{C}$ for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Mix the following in a microcentrifuge tube:

- ≤ 87.5 μ l RNA solution (contaminated with genomic DNA)
- 10 μ l Buffer RDD
- 2.5 μ l DNase I stock solution

Make the volume up to 100 μ l with RNase-free water.

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

2. Incubate on the benchtop ($20-25^{\circ}\text{C}$) for 10 min.

3. Clean up the RNA according to "Protocol: RNA Cleanup" on page 30.

Ordering Information

Product	Contents	Cat. no.
QIAamp RNA Blood Mini Kit — for purification of total RNA from up to 1.5 ml of whole blood		
QIAamp RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304
Buffers and reagents		
Buffer EL	1000 ml Erythrocyte Lysis Buffer	79217
Buffer RLT	220 ml Lysis Buffer	79216
QIAshredder — for convenient cell lysate homogenization in RNA minipreps		
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid preparation, caps	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers for use in nucleic acid preparation, caps	79656
RNase-Free DNase Set — for DNase treatment during RNA purification		
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free DNA Digest Buffer, and RNase-free water for 50 RNA minipreps	79254
RNAlater TissueProtect Tubes — for collecting harvested tissues and immediate stabilization of the gene expression profile, and subsequent transport and storage		
RNAlater TissueProtect Tubes (50 x 1.5 ml)*	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAlater RNA Stabilization Reagent each	76154
RNAlater TissueProtect Tubes (20 x 5 ml)*	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNAlater RNA Stabilization Reagent each	76163

* RNAlater RNA Stabilization Reagent also available separately; please inquire.

Ordering Information

Product	Contents	Cat. no.
TissueRuptor — For low-throughput disruption of a wide range of biological samples using disposable probes		
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Inquire
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890
TissueLyser system — For high-throughput disruption of a wide range of biological samples		
TissueLyser	Universal laboratory mixer-mill disruptor	Inquire
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2.0 ml microcentrifuge tubes on the TissueLyser	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser	69984
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
Related products		
PAXgene Blood RNA System — for the collection, storage, and transport of whole blood and stabilization of intracellular RNA in a closed tube, and subsequent isolation and purification of RNA		
PAXgene Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes	762164* 762174†
PAXgene Blood RNA Tubes (100)	100 Blood Collection Tubes. To be used in conjunction with the PAXgene Blood RNA Kit (50)	762165‡

* USA and Canada.

† Rest of world; not available in all countries.

‡ Distributed by BD and BD authorized distributors. See www.PreAnalytiX.com.

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Blood Mini Kit — for DNA isolation from blood and body fluids		
QIAamp DNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, QIAGEN Protease, Collection Tubes, Reagents and Buffers	51104
QIAamp DNA Blood Mini Kit (250)	250 QIAamp Mini Spin Columns, QIAGEN Protease, Collection Tubes, Reagents and Buffers	51106
QIAamp DNA Mini Kit — for DNA isolation from tissues		
QIAamp DNA Mini Kit (50)	50 QIAamp Mini Spin Columns, Proteinase K, Collection Tubes, Reagents and Buffers	51304
QIAamp DNA Mini Kit (250)	250 QIAamp Mini Spin Columns, Proteinase K, Collection Tubes, Reagents and Buffers	51306
QIAamp 96 DNA Blood Kit — for high-throughput DNA isolation from blood and body fluids		
QIAamp 96 DNA Blood Kit (4)	4 QIAamp 96 Plates, QIAGEN Protease, Reagents and Buffers, Round-Well Blocks, Square-Well Blocks, Tape Pads, Collection Tubes	51161
QIAamp 96 DNA Blood Kit (12)	12 QIAamp 96 Plates, QIAGEN Protease, Reagents and Buffers, Round-Well Blocks, Square-Well Blocks, Tape Pads, Collection Tubes	51162

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