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AllPrep® 96 DNA/RNA Handbook

For simultaneous purification of genomic DNA and total RNA from animal and human cells and tissues in 96-well format

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep 96 DNA/RNA Kit **Catalog no.** **Number of preps**	**(4)** **80311** **4 x 96**
AllPrep 96 DNA Plates	4
RNeasy® 96 Plates	4
Register Cards (96-well)	4
S-Blocks*	4
Elution Microtubes CL	8 x 96
Caps for Elution Microtubes CL	100 x 8
AirPore Tape Sheets	5 x 5
Buffer RLT†	220 ml
Buffer RW1†	2 x 220 ml
Buffer RPE‡ (concentrate)	4 x 55 ml
RNase-Free Water	2 x 50 ml
Buffer AW1†‡ (concentrate)	2 x 98 ml
Buffer AW2‡ (concentrate)	2 x 66 ml
Buffer EB	4 x 15 ml
Quick-Start Protocol	1

* Reusable; see page 26 for cleaning instructions.

† Contains a guanidine salt. Not compatible with disinfecting reagents containing bleach. See page 5 for safety information.

‡ Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

The following kit components are also available separately: S-Blocks, Elution Microtubes CL (including caps) and AirPore Tape Sheets. See page 90 for ordering information.

Storage

The AllPrep 96 DNA/RNA 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 AllPrep 96 DNA/RNA Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention or treatment of a disease.

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.

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

Buffer RLT contains guanidine thiocyanate, Buffer RW1 contains a small amount of guanidine thiocyanate and Buffer AW1 contains guanidine hydrochloride. 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AllPrep 96 DNA/RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AllPrep 96 DNA/RNA Kit is designed for high-throughput sample preparation from cells and easy-to-lyse tissues in 96-well format, providing simultaneous purification of genomic DNA and total RNA from each sample. Lysates are first passed through an AllPrep 96 DNA plate to selectively isolate DNA and then through an RNeasy 96 plate to selectively isolate RNA. Pure DNA and RNA are purified from the entire sample, in contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately. The kit is compatible with a wide range of cultured cells and tissues of animal and human origin, providing DNA and RNA purification from up to 2 x 10^6 cells or up to 10 mg tissue per sample.

The AllPrep 96 DNA/RNA Kit allows the parallel processing of 96 samples in less than 60 minutes. The AllPrep DNA/RNA procedure replaces methods involving the use of toxic substances, such as phenol and/or chloroform, or time-consuming and tedious methods, such as alcohol precipitation.

Genomic DNA purified with the AllPrep DNA/RNA procedure has an average length of 15–30 kb depending on homogenization conditions.* DNA of this length is particularly suitable for PCR, where complete denaturation of the template is important to achieve the highest amplification efficiency. The purified DNA is ready to use in any downstream application, including:

- Next-generation sequencing (NGS)
- PCR and real-time PCR
- Southern, dot and slot blot analyses
- Comparative genome hybridization (CGH)
- Genotyping, SNP analysis

* For purification of high-molecular-weight DNA (up to 150 kb), we recommend using either QIAGEN Genomic-tips or Blood & Cell Culture DNA Kits. For details, visit www.qiagen.com/DNA.

With the AllPrep DNA/RNA procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure provides an enrichment for mRNA, since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded.* The purified RNA is ready to use in any downstream application, including:

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

Optionally, the AllPrep DNA/RNA procedure can be modified to allow the purification of total RNA containing small RNAs, such as miRNA, from cultured cells (see Appendices E and F, pages 80 and 84).

* For purification of miRNA and other small RNAs from cells and tissues, we recommend using the miRNeasy 96 Kit. For details, visit www.qiagen.com/miRNA.

† Visit www.qiagen.com/geneXpression for information on standardized solutions for gene expression analysis from QIAGEN.

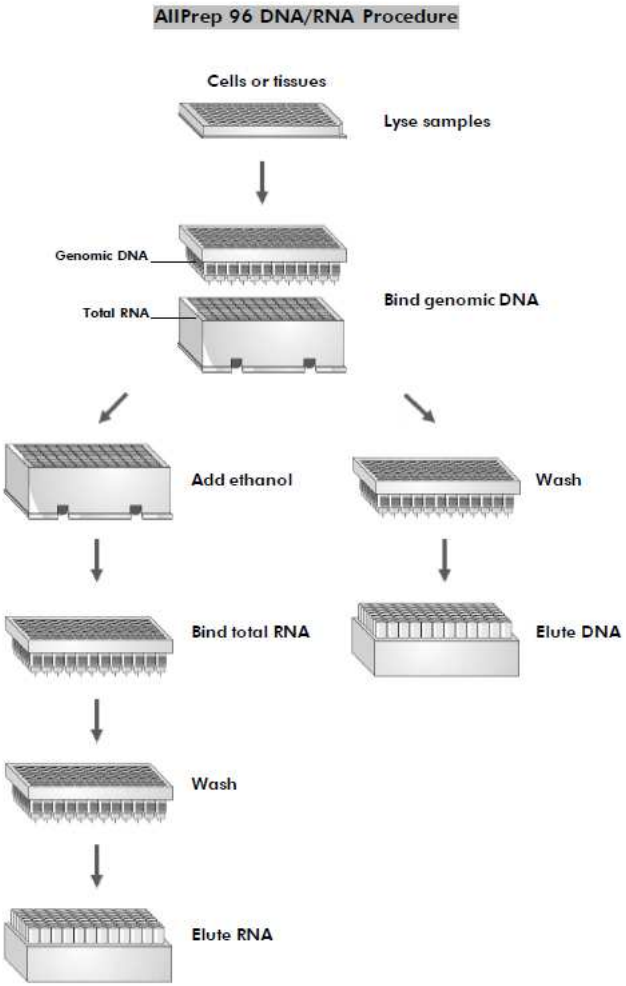
Principle and procedure

The AllPrep DNA/RNA procedure integrates QIAGEN's technology for selective binding of double-stranded DNA with well-established RNeasy technology. Efficient purification of high-quality DNA and RNA is guaranteed, without the need for additional RNase and DNase digestions.*

Cell or tissue samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate–containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysates are then passed through an AllPrep 96 DNA plate. This 96-well plate, in combination with the high-salt buffer, selectively and efficiently binds genomic DNA. The plate is washed and pure, ready-to-use DNA is then eluted.

Ethanol is added to the flow-throughs from the AllPrep 96 DNA plate to provide appropriate binding conditions for RNA, and the samples are then applied to an RNeasy 96 plate. Total RNA binds to the membranes of this 96-well plate and contaminants are efficiently washed away. High-quality RNA is then eluted in 45–70 µl water.

* Samples with particularly high DNA content may require additional DNase digestion if the purified RNA will be used in downstream applications sensitive to trace amounts of genomic DNA.


AllPrep 96 DNA/RNA Procedure
Cells or tissues
Lyse samples
Genomic DNA
Total RNA
Bind genomic DNA
Add ethanol
Wash
Bind total RNA
Elute DNA
Wash
Elute RNA

Description of protocols

For each sample type (cells or tissues), there is a vacuum/spin protocol and a spin protocol. With a vacuum/spin protocol, certain binding and washing steps are performed on the QIAvac 96 vacuum manifold for faster and easier sample processing; all other steps are performed in the Centrifuge 4-16KS. With a spin protocol, all steps are performed in the Centrifuge 4-16KS, and twice as much starting material can be used compared with the vacuum/spin protocol.

Simultaneous Purification of DNA and RNA from Cells Using Vacuum/Spin Technology

In this protocol, DNA and RNA are simultaneously purified from up to **1 x 10^6 cells**. The DNA binding step, the final wash steps including membrane drying and the elution steps, are performed in the Centrifuge 4-16KS (see page 18). All other steps are performed on the QIAvac 96 vacuum manifold (see page 15). Residual traces of salt are removed by centrifugation in the final wash steps. The Plate Rotor 2 x 96 holds 2 AllPrep 96 DNA plates or 2 RNeasy 96 plates, allowing up to 192 cultured-cell samples to be processed in parallel.

Simultaneous Purification of DNA and RNA from Cells Using Spin Technology

In this protocol, DNA and RNA are simultaneously purified from up to **2 x 10^6 cells**. All protocol steps are performed in the Centrifuge 4-16KS (see page 18). The Plate Rotor 2 x 96 holds 2 AllPrep 96 DNA plates or 2 RNeasy 96 plates, allowing up to 192 cultured-cell samples to be processed in parallel.

Simultaneous Purification of DNA and RNA from Tissues Using Vacuum/Spin Technology

In this protocol, DNA and RNA are simultaneously purified from up to **5 mg tissue**. The DNA binding step, the final wash steps, including membrane drying and the elution steps, are performed in the Centrifuge 4-16KS (see page 18). All other steps are performed on the QIAvac 96 vacuum manifold (see page 15). Residual traces of salt are removed by

centrifugation in the final wash steps. The Plate Rotor 2 x 96 holds 2 AllPrep 96 DNA plates or 2 RNeasy 96 plates, allowing up to 192 tissue samples to be processed in parallel.

Simultaneous Purification of DNA and RNA from Tissues Using Spin Technology

In this protocol, DNA and RNA are simultaneously purified from up to **10 mg tissue**. All protocol steps are performed in the Centrifuge 4-16KS (see page 18). The Plate Rotor 2 x 96 holds 2 AllPrep 96 DNA plates or 2 RNeasy 96 plates, allowing up to 192 tissue samples to be processed in parallel.

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

Multichannel pipet with tips. For efficient liquid handling, we recommend using an electric multichannel pipet with a minimum capacity of 650 µl per pipet tip. Matrix Technologies Corporation (**www.matrixtechcorp.com**) provides cordless electronic multichannel pipets with a unique expandable tip-spacing system, allowing transfer of liquid between different types of multiwell plate.*

- Reagent reservoirs for multichannel pipets
- Disposable gloves
- Centrifuge 4-16KS†
- Plate Rotor 2 x 96 (cat. no. 81031)
- 96–100% ethanol and 70% ethanol in water‡
- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M) or, alternatively, 2 M dithiothreitol (DTT) in water
- **Optional**: Tape Pads (cat. no. 19570) (for sealing unused wells)
- **Optional**: S-Blocks (cat. no. 19585) (if performing several 96-well preps per day, it may be convenient to have additional S-Blocks)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.
† For ordering information, please inquire (www.qiagen.com).
‡ Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.

For cell protocols

- Vortexer

For tissue protocols

- For stabilization of RNA in tissues: RNAprotect® Tissue Reagent (cat. no. 76104 [50 ml] or cat. no. 76106 [250 ml]), Allprotect Tissue Reagent (cat. no. 76405 [100 ml]) or liquid nitrogen
- For disruption and homogenization of tissues (low-throughput): TissueRuptor®† II and TissueRuptor Disposable Probes (cat. no. 990890)
- For disruption and homogenization of tissues (high-throughput): TissueLyser II (cat. no. 85300), TissueLyser Adapter Set 2 x 96 (cat. no. 69984), Stainless Steel Beads, 5 mm (cat. no. 69989), Collection Microtubes (cat. no. 19560) and Collection Microtube Caps (cat. no. 19566)

For protocols using vacuum/spin technology

- QIAvac 96 vacuum manifold (cat. no. 19504)
- QIAGEN Vacuum Pump* or other vacuum pump capable of generating a vacuum pressure of –800 to –900 mbar
- QIAGEN Vacuum Regulator (cat. no. 19530) or pressure gauge

* For ordering information, please inquire (www.qiagen.com).

QIAvac 96 vacuum manifold

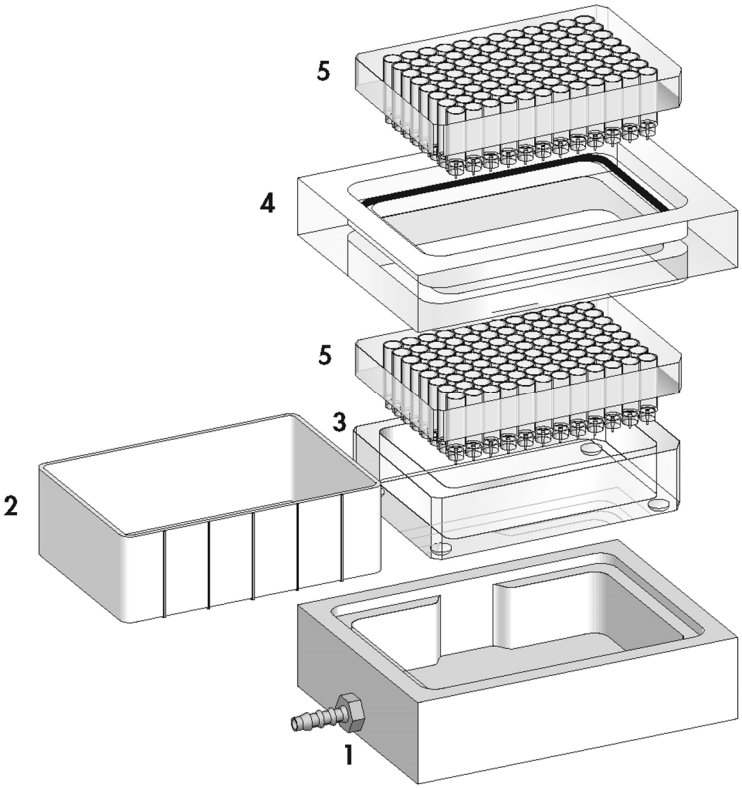


Figure 1. Components of the QIAvac 96 vacuum manifold.
1. QIAvac base, which holds a waste tray, a plate holder or a microtube rack.
2. Waste tray.
3. Plate holder (shown with 96-well plate) — not used in AllPrep 96 DNA/RNA protocol.
4. QIAvac 96 top plate with aperture for 96-well plate.
5. 96-well plate (i.e., AllPrep 96 DNA plate or RNeasy 96 plate).*

* Not included with QIAvac 96. Included in the AllPrep 96 DNA/RNA Kit.

QIAvac 96 handling guidelines

The QIAvac 96 vacuum manifold facilitates vacuum processing of AllPrep 96 DNA plates and RNeasy 96 plates through its convenient, modular design. The following recommendations should be followed when handling the manifold.

Operation of the manifold requires a house vacuum or a vacuum pump. If the house vacuum is weak or inconsistent, we recommend using a vacuum pump with a capacity of 18 liters per minute. Use of insufficient vacuum pressure may reduce nucleic acid yield and purity.

A vacuum pressure of –800 to –900 mbar should develop when an AllPrep 96 DNA plate or RNeasy 96 plate sealed with tape is used on the QIAvac 96 vacuum manifold. Vacuum pressures exceeding –900 mbar should be avoided. The vacuum pressure is the pressure difference between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 mbar or 760 mm Hg) and can be regulated and measured using a pressure gauge or vacuum regulator. Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere. Table 1 provides pressure conversions to other units.

Between loading steps, the vacuum must be switched off and the manifold ventilated to maintain uniform conditions for each sample. This can be done with a vacuum regulator inserted between the vacuum source and the QIAvac 96 vacuum manifold.

Wear safety glasses when working near a manifold under pressure.

For safety reasons, do not use 96-well plates that have been damaged in any way.

Always place the QIAvac 96 vacuum manifold on a secure benchtop or work area. If dropped, the manifold may crack.

Always store the QIAvac 96 vacuum manifold clean and dry. To clean, simply rinse all components with water, and dry with paper towels. Do not air dry, as the screws may rust and need to be replaced. Do not use abrasives. After rinsing and drying, wipe manifold components with paper towels wetted with 70% ethanol, and dry with fresh paper towels.

The QIAvac 96 vacuum manifold and components are not resistant to ethanol, methanol or other organic solvents when exposed for long periods. If solvents are spilled on the unit, rinse thoroughly with distilled water at the end of the AllPrep DNA/RNA procedure. Ensure that no residual buffers remain in the vacuum manifold.

To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 96 vacuum manifold. The spring lock on the top plate and the self-sealing gasket provide an airtight seal when vacuum is applied to the assembled unit. To maximize gasket life, rinse the gasket free of salts and buffers after each use, and dry with paper towels before storage.

Table 1. Pressure conversions

To convert from millibars (mbar) to:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per square inch (psi)	0.0145

Centrifuge 4-16KS

For optimal centrifugation of AllPrep 96 DNA plates and RNeasy 96 plates, QIAGEN, in cooperation with the centrifuge manufacturer Sigma Laborzentrifugen GmbH, has developed a centrifugation system consisting of the Plate Rotor 2 x 96 and the refrigerated table-top Centrifuge 4-16KS. A wide range of other rotors can be used with the Centrifuge 4-16KS in addition to the Plate Rotor 2 x 96.

Standard table-top centrifuges and 96-well–microplate rotors are not suitable for use with the AllPrep 96 DNA/RNA Kit. Usually, 96-well–microplate buckets are not deep enough to carry the complete plate assembly without interfering with how the buckets swing out. Furthermore, high *g*-forces (>5500 x *g*) are required for optimal performance of the AllPrep 96 DNA/RNA Kit.

For further information about the Centrifuge 4-16KS and the Plate Rotor 2 x 96, please contact QIAGEN or your local distributor.

Important: Do not centrifuge the Plate Rotor 2 x 96 metal holders without the complete plate assembly, which can be an AllPrep 96 DNA plate or RNeasy 96 plate on top of an S-Block or elution microtubes rack. If unsupported, the holders will collapse under high *g* force. Therefore, remove the holders during test runs. Standard 96-well microplates may be centrifuged in the holders if a *g*-force of 500 x *g* is not exceeded.

Important Notes

Determining the amount of starting material

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

- The type of sample and its DNA and RNA content
- The volume of Buffer RLT required for efficient lysis
- The DNA binding capacity of the AllPrep 96 DNA plate
- The RNA binding capacity of the RNeasy 96 plate

Table 2 shows the specifications for the plates supplied with the AllPrep 96 DNA/RNA Kit. Each well of an AllPrep 96 DNA plate or RNeasy 96 plate has a maximum binding capacity of 100 µg DNA or RNA, respectively, but actual yields depend on the sample. Table 3 shows typical DNA and RNA yields from various cultured cells and tissues.

Table 2. Specifications of the plates in the AllPrep 96 DNA/RNA Kit

Specification	AllPrep 96 DNA plate	RNeasy 96 plate
Preps per plate	96	96
Maximum binding capacity	100 µg DNA*	100 µg RNA
Maximum loading volume	1 ml	1 ml
Nucleic acid size distribution	DNA of 15–30 kb†	RNA >200 nucleotides‡
Minimum elution volume	45 µl	45 µl
Maximum amount of starting material using vacuum/spin protocol	Up to 1 x 10^6 cells or up to 5 mg tissue	Entire flow-through from AllPrep plate
Maximum amount of starting material using spin protocol	Up to 2 x 10^6 cells or up to 10 mg tissue	Entire flow-through from AllPrep plate

* Loading more than 20 µg DNA may lead to DNA contamination of the RNA eluate.

† Depending on homogenization conditions (see page 25).

‡ Purification of total RNA containing small RNAs from cultured cells is possible through a modification of the AllPrep DNA/RNA procedure. For details, see Appendices E and F, pages 80 and 84.

Note: Although the AllPrep 96 DNA plate can bind a maximum of 100 µg DNA, the use of starting materials containing more than 20 µg DNA may lead to the purification of RNA containing small amounts of DNA. If the binding capacity of the RNeasy 96 plate is exceeded, RNA yields will not be consistent and will be less than expected.

Note: Do not overload the AllPrep 96 DNA plate, as this may lead to clogging of the wells and to copurification of DNA with RNA. Do not overload the RNeasy 96 plate, as this will significantly reduce RNA yield and purity.

Table 3. Typical yields of genomic DNA and total RNA with the AllPrep 96 DNA/RNA Kit

Sample type	Typical yield of genomic DNA (µg)	Typical yield of total RNA* (µg)
Cell cultures (5 x 10^5 cells)		
• NIH/3T3	4	5
• HeLa, Jurkat	3	8
• COS-7	4	15
Mouse/rat tissues (5 mg)		
• Brain	2–5	2–5
• Heart	2–5	2–4
• Kidney	7–15	10–15
• Liver	7–15	20–30
• Spleen	25–35	15–40
• Thymus	25–50	20–40
• Lung	5–10	5–10

* Amounts can vary due to factors, such as species, developmental stage and growth conditions. Since the AllPrep DNA/RNA procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5.8S rRNA, tRNA and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Cells

The AllPrep DNA/RNA procedure is optimized for use with 100 to 1 x 10^6 cells (vacuum/spin protocol) or 100 to 2 x 10^6 cells (spin protocol). Direct counting is the most accurate way to quantify the number of cells. As a guide, the number of HeLa cells obtained in various cell-culture plates after confluent growth is given in Table 4.

Table 4. Growth area and number of HeLa cells in various multiwell cell-culture plates

Cell-culture plate	Growth area per well (cm^2)*	Number of cells per well†
96-well	0.32–0.60	4–5 x 10^4
48-well	1.0	1.3 x 10^5
24-well	2.0	2.5 x 10^5
12-well	4.0	5.0 x 10^5
6-well	9.5	1.2 x 10^6

* Growth area varies slightly depending on the supplier.

† Confluent growth is assumed.

Tissues

If using the vacuum/spin protocol, a maximum amount of 5 mg fresh or frozen tissue or 2–3 mg RNAprotect- or Allprotect-stabilized tissue (which is partially dehydrated) can generally be processed. If using the spin protocol, a maximum amount of 10 mg fresh or frozen tissue or 4–6 mg RNAprotect- or Allprotect-stabilized tissue can usually be processed. For most tissues, the DNA binding capacity of the AllPrep 96 DNA plate, the RNA binding capacity of the RNeasy 96 plate, and the lysing capacity of Buffer RLT will not be exceeded by these amounts.

Weighing tissue is the most accurate way to quantify the amount of starting material. As a guide, a 1.5 mm cube (3.4 mm^3) of most animal tissues weighs 3.5–4.5 mg.

Handling and storing starting material

Cells

After harvesting, cells should be immediately lysed in Buffer RLT to prevent unwanted changes in the gene expression profile. This highly denaturing lysis buffer inactivates RNases and other proteins to prevent RNA degradation as well as downregulation or upregulation of transcripts.

If the cells are to be shipped to another lab for nucleic acid purification, they should be pelleted, frozen in liquid nitrogen and transported on dry ice. Alternatively, the cells can be mixed with RNAprotect Cell Reagent (see ordering information, page 90) at room temperature and then shipped at ambient temperature.

Tissues

RNA in harvested tissue is not protected until the sample is treated with RNAprotect Tissue Reagent, flash-frozen or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C or immediately immersed in RNAprotect Tissue Reagent. An alternative to RNAprotect Tissue Reagent is Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA and protein in tissue samples at room temperature.

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

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the starting material is an absolute requirement for all nucleic acid purification procedures. Disruption and homogenization are 2 distinct steps:

- **Disruption**: Complete disruption of plasma membranes of cells and organelles is absolutely required to release all the nucleic acids contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced nucleic acid yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of DNA and RNA and therefore significantly reduced yield and purity of nucleic acids. Excessive homogenization, on the other hand, results in shorter genomic DNA fragments.

Some disruption methods simultaneously disrupt and homogenize the sample, while others require an additional homogenization step. Table 5 gives an overview of various disruption and homogenization methods and is followed by a detailed description of each method.

Table 5. Disruption and homogenization methods

Sample	Disruption method	Homogenization method
Cells	Addition of lysis buffer	Mechanical shaking
Tissues	TissueRuptor II*	TissueRuptor II *
	TissueLyser II†	TissueLyser II †
	Mortar and pestle	QIAshredder homogenizer or syringe and needle
Sample	Disruption method	Homogenization method

* Simultaneously disrupts and homogenizes individual samples.

† Simultaneously disrupts and homogenizes up to 192 samples in parallel. Results are comparable to those obtained using the TissueRuptor II or other rotor–stator homogenizer.

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.

Note: Longer homogenization times with the TissueRuptor II result in greater DNA fragmentation. Therefore, the homogenization time should be kept as short as possible if the DNA will be used in downstream applications that require long DNA fragments.

Disruption and homogenization using the TissueLyser II

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. The TissueLyser II disrupts and homogenizes 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. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.

Note: Tungsten carbide beads react with Buffer RLT and must not be used to disrupt and homogenize tissues.

Disruption using a mortar and pestle

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

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

Homogenization using QIAshredder homogenizers

Using QIAshredder homogenizers is a fast and efficient way to homogenize tissue lysates without cross-contamination of samples. Up to 700 µl of lysate is loaded onto a QIAshredder spin column placed in a 2 ml collection tube and spun for 2 minutes at maximum speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column. QIAshredder homogenizers typically result in less DNA fragmentation compared with rotor–stator homogenizers.

Homogenization using a syringe and needle

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

Effect of homogenization on DNA yield and integrity

The yield and integrity of genomic DNA purified using the AllPrep 96 DNA/RNA Kit depends on the method used for disruption and homogenization.

Homogenization with the TissueRuptor II (or other rotor–stator homogenizer) or the TissueLyser II (or other bead mill) results in higher DNA yields, but also in greater DNA fragmentation, depending on the homogenization time and intensity. In contrast, gentler homogenization with the QIAshredder or a syringe and needle allows purification of longer DNA fragments. However, as longer DNA fragments are more difficult to elute, DNA yields may be significantly lower.

S-Blocks

The kit contains 4 S-Blocks. If carrying out several 96-well preps per day, it may be convenient to have extra S-Blocks available (cat. no. 19585; case of 24). Fresh S-Blocks must be used to collect the first flow-through from the AllPrep 96 DNA plate (i.e., the flow-through containing RNA). After use, S-Blocks may be cleaned and reused to collect waste liquid from AllPrep 96 DNA plates and RNeasy 96 plates. Do not reuse cleaned S-Blocks to collect the first flow-through from AllPrep 96 DNA plates. To clean S-Blocks, rinse them thoroughly with tap water, incubate for 2 hours or overnight in 0.1 M NaOH, 1 mM EDTA,* rinse in distilled water and dry at 50°C.

Note: Used S-Blocks contain residual amounts of Buffer RLT, Buffer RW1 or Buffer AW1 and should therefore not be cleaned with bleach. See page 5 for safety information.

* 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.

Protocol: Simultaneous Purification of DNA and RNA from Cells using Vacuum/Spin Technology

Important points before starting

- If using the AllPrep 96 DNA/RNA Kit for the first time, read "Important Notes" (page 19).
- If preparing RNA for the first time, read Appendix A (page 67).
- All vacuum steps are performed on the QIAvac 96 vacuum manifold. If using the manifold for the first time, read "QIAvac 96 vacuum manifold" (page 15).
- All centrifugation steps are performed in the Centrifuge 4-16KS with the Plate Rotor 2 x 96. If using the centrifuge for the first time, read "Centrifuge 4-16KS" (page 18).
- Use of a multichannel pipet is recommended (see page 13). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for S-Blocks (see page 26).
- Cell pellets can be stored at –70°C for later use or used directly in the procedure. Frozen cell pellets should be thawed slightly before starting the procedure.
- Cell lysates in Buffer RLT from step 2 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. Mix by pipetting up and down 3 times before continuing with step 3.
- Cells stored in RNAprotect Cell Reagent can also be used in the procedure. Be sure to pellet the cells and remove the supernatant as described in steps 1–3 of the RNA purification protocol in the *RNAprotect Cell Reagent Handbook*. Then proceed immediately to step 1b of the procedure below.

- Buffer RLT, Buffer RW1 and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RPE, Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample lysis and homogenization

1. Harvest cells (up to 1 x 10^6 cells) according to step 1a or 1b.

   1a. Cells grown in a monolayer:
   Completely remove the cell-culture medium by pipetting, and add 300 µl Buffer RLT to each well. Transfer the lysates to a rack of collection microtubes (cat. no. 19560), and seal the tubes with collection microtube caps (cat. no. 19566).

   As an alternative to collection microtubes, a 96-well plate with caps can be used.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

   Note: The volume of Buffer RLT may be reduced to 200 µl if the well volume is too low and there is a risk of cross-contamination during handling.

   1b. Cells grown in suspension:
   Transfer up to 1 x 10^6 cells from each sample to a rack of collection microtubes (cat. no. 19560). Pellet the cells by centrifuging for 5 min at 300 x *g*. Completely remove all supernatant by pipetting, and add 300 µl Buffer RLT to each tube. Seal the tubes with collection microtube caps (cat. no. 19566).

   As an alternative to collection microtubes, a 96-well plate with caps can be used.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

   Note: The volume of Buffer RLT may be reduced to 200 µl if the well volume is too low and there is a risk of cross-contamination during handling.

2. Homogenize the lysates by vortexing the rack of collection microtubes at full speed for at least 30 s.

   Optional: For optimal DNA yields, the lysates can be homogenized by using the TissueLyser II instead of by vortexing:

   - Add 1 stainless steel bead (5 mm mean diameter) to each collection microtube, and seal with collection microtube caps.
   - Place the tubes in the TissueLyser Adapter Set 2 x 96.
   - Operate the TissueLyser II for 1 min at 20 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 1 min at 20 Hz. Rearranging the tubes allows even homogenization.
   - Proceed to step 3.

3. Place an AllPrep 96 DNA plate on top of a new S-Block. Mark the plate for later identification.

4. Transfer the lysates from step 2 to the wells of the AllPrep 96 DNA plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

5. Seal the AllPrep 96 DNA plate with an AirPore tape sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

   Centrifugation with sealed plates prevents cross-contamination.

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

6. Place the AllPrep 96 DNA plate on top of another S-Block (either new or reused), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 17–22. Keep the S-Block containing the flow-through for RNA purification in steps 7–16.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

   Note: Do not store the AllPrep 96 DNA plate for long periods, as this may lead to the membranes drying out. Do not freeze the plate.

Total RNA purification

7. Assemble the QIAvac 96 vacuum manifold: first place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place an RNeasy 96 plate in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.

   Note: Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to your right-hand side.

8. Add 1 volume (300 µl) of 70% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.

   Note: Add 200 µl of 70% ethanol if 200 µl Buffer RLT was used in step 1.

9. Transfer the samples (600 µl) to the wells of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the samples have completely passed through the membranes (15–60 s). Switch off the vacuum, and ventilate the manifold.

   Make sure the QIAvac 96 vacuum manifold is assembled correctly before loading the samples. The flow-through is collected in the waste tray.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

   Note: Tape unused wells with adhesive tape or Tape Pads (cat. no. 19570). Do not use the AirPore tape sheets supplied with the AllPrep 96 DNA/RNA Kit.

   Note: The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

10. Add 800 µl Buffer RW1 to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

    The flow-through is collected in the same waste tray from step 9.

11. Lift the QIAvac 96 top plate carrying the RNeasy 96 plate from the QIAvac base, and empty the waste tray.* Reassemble the QIAvac 96 vacuum manifold.

12. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

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

* The waste liquid contains Buffer RLT and Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

13. Place the RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

    If reusing an S-Block, make sure it is cleaned as described on page 26.

14. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the RNeasy membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during RNA elution.

15. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the RNA.

    Note: Be sure to pipet the RNase-free water directly onto the RNeasy membranes. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

16. Remove the AirPore tape sheet. Repeat step 15 with a second volume of 45–70 µl RNase-free water.

    Note: Repeating step 15 is required for complete recovery of RNA. The eluate volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane (the 15 µl corresponds to the membrane dead volume).

    Use the caps provided with the kit to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Genomic DNA purification

17. Assemble the QIAvac 96 vacuum manifold: first place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place the AllPrep 96 DNA plate (from step 6) in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.

    Note: Always place the AllPrep 96 DNA plate into the vacuum manifold with the beveled edges pointing to your right-hand side.

18. Add 800 µl Buffer AW1 to each well of the AllPrep 96 DNA plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

    The flow-through is collected in the same waste tray from step 12.*

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

19. Place the AllPrep 96 DNA plate on top of an S-Block (either new or reused).

    If reusing an S-Block, make sure it is cleaned as described on page 26.

* The waste liquid contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

20. Add 800 µl Buffer AW2 to each well of the AllPrep 96 DNA plate, and seal the plate with an AirPore tape sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the AllPrep DNA membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during DNA elution.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

21. Remove the AirPore tape sheet. Place the AllPrep 96 DNA plate on top of a rack of Elution Microtubes CL. Add 50–100 µl Buffer EB (prewarmed to 70°C) to each well, and seal the plate with a new AirPore tape sheet. Incubate for 5 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the DNA.

    Note: Be sure to pipet Buffer EB directly onto the AllPrep DNA membranes. Elution will be incomplete if some of the buffer sticks to the walls or the O-rings of the AllPrep 96 DNA plate.

22. Remove the AirPore tape sheet. Repeat step 21 with a second volume of 50–100 µl Buffer EB.

    Note: Repeating step 21 is required for complete recovery of DNA.

    Use the caps provided with the kit to seal the microtubes for storage.

Protocol: Simultaneous Purification of DNA and RNA from Cells using Spin Technology

Important points before starting

- If using the AllPrep 96 DNA/RNA Kit for the first time, read "Important Notes" (page 19).
- If preparing RNA for the first time, read Appendix A (page 67).
- All centrifugation steps are performed in the Centrifuge 4-16KS with the Plate Rotor 2 x 96. If using the centrifuge for the first time, read "Centrifuge 4-16KS" (page 18).
- Use of a multichannel pipet is recommended (see page 13). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for S-Blocks (see page 26).
- Cell pellets can be stored at –70°C for later use or used directly in the procedure. Frozen cell pellets should be thawed slightly before starting the procedure.
- Cell lysates in Buffer RLT from step 2 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. Mix by pipetting up and down 3 times before continuing with step 3.
- Cells stored in RNAprotect Cell Reagent can also be used in the procedure. Be sure to pellet the cells and remove the supernatant as described in steps 1–3 of the RNA purification protocol in the *RNAprotect Cell Reagent Handbook*. Then proceed immediately to step 1b of the procedure below.
- Buffer RLT, Buffer RW1 and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.

- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RPE, Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample lysis and homogenization

1. Harvest cells (up to 2 x 10^6 cells) according to step 1a or 1b.

1a. Cells grown in a monolayer:
Completely remove the cell-culture medium by pipetting, and add 300 µl Buffer RLT to each well. Transfer the lysates to a rack of collection microtubes (cat. no. 19560), and seal the tubes with collection microtube caps (cat. no. 19566).

As an alternative to collection microtubes, a 96-well plate with caps can be used.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

Note: The volume of Buffer RLT may be reduced to 200 µl if the well volume is too low and there is a risk of cross-contamination during handling.

1b. Cells grown in suspension:
Transfer up to 2 x 10^6 cells from each sample to a rack of collection microtubes (cat. no. 19560). Pellet the cells by centrifuging for 5 min at 300 x *g*. Completely remove all supernatant by pipetting, and add 300 µl Buffer RLT to each tube. Seal the tubes with collection microtube caps (cat. no. 19566).

As an alternative to collection microtubes, a 96-well plate with caps can be used.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

Note: The volume of Buffer RLT may be reduced to 200 µl if the well volume is too low and there is a risk of cross-contamination during handling.

2. Homogenize the lysates by vortexing the rack of collection microtubes at full speed for at least 30 s.

   Optional: For optimal DNA yields, the lysates can be homogenized by using the TissueLyser II instead of by vortexing:

   - Add 1 stainless steel bead (5 mm mean diameter) to each collection microtube, and seal with collection microtube caps.
   - Place the tubes in the TissueLyser Adapter Set 2 x 96.
   - Operate the TissueLyser II for 1 min at 20 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 1 min at 20 Hz. Rearranging the tubes allows even homogenization.
   - Proceed to step 3.

3. Place an AllPrep 96 DNA plate on top of a new S-Block. Mark the plate for later identification.

4. Transfer the lysates from step 2 to the wells of the AllPrep 96 DNA plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

5. Seal the AllPrep 96 DNA plate with an AirPore tape sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

   Centrifugation with sealed plates prevents cross-contamination.

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

6. Place the AllPrep 96 DNA plate on top of another S-Block (either new or reused), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 16–19. Keep the S-Block containing the flow-through for RNA purification in steps 7–15.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

   Note: Do not store the AllPrep 96 DNA plate for long periods, as this may lead to the membranes drying out. Do not freeze the plate.

Total RNA purification

7. Place an RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

8. Add 1 volume (300 µl) of 70% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.

   Note: Add 200 µl of 70% ethanol if 200 µl Buffer RLT was used in step 1.

9. Transfer the samples (600 µl) to the wells of the RNeasy 96 plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

10. Seal the RNeasy 96 plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

    Centrifugation with sealed plates prevents cross-contamination.

11. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer RW1 to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

12. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

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

13. Empty the S-Block and remove the AirPore tape sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    It is important to dry the RNeasy membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during RNA elution.

14. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the RNA.

    Note: Be sure to pipet the RNase-free water directly onto the RNeasy membranes. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

* The waste liquid contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

15. Remove the AirPore tape sheet. Repeat step 14 with a second volume of 45–70 µl RNase-free water.

    Note: Repeating step 14 is required for complete recovery of RNA. The eluate volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane (the 15 µl corresponds to the membrane dead volume).

    Use the caps provided with the kit to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Genomic DNA purification

16. Add 800 µl Buffer AW1 to each well of the AllPrep 96 DNA plate from step 6, and seal the plate with an AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

17. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer AW2 to each well of the AllPrep 96 DNA plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    It is important to dry the AllPrep DNA membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during DNA elution.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

* The waste liquid contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

18. Remove the AirPore tape sheet. Place the AllPrep 96 DNA plate on top of a rack of Elution Microtubes CL. Add 50–100 µl Buffer EB (prewarmed to 70°C) to each well, and seal the plate with a new AirPore tape sheet. Incubate for 5 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the DNA.

    Note: Be sure to pipet Buffer EB directly onto the AllPrep DNA membranes. Elution will be incomplete if some of the buffer sticks to the walls or the O-rings of the AllPrep 96 DNA plate.

19. Remove the AirPore tape sheet. Repeat step 18 with a second volume of 50–100 µl Buffer EB.

    Note: Repeating step 18 is required for complete recovery of DNA.

    Use the caps provided with the kit to seal the microtubes for storage.

Protocol: Simultaneous Purification of DNA and RNA from Tissues using Vacuum/Spin Technology

Important points before starting

- If using the AllPrep 96 DNA/RNA Kit for the first time, read "Important Notes" (page 19).
- If preparing RNA for the first time, read Appendix A (page 67).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- If using the TissueLyser II, ensure that you are familiar with operating it by referring to the operating instructions and *TissueLyser Handbook*.
- All vacuum steps are performed on the QIAvac 96 vacuum manifold. If using the manifold for the first time, read "QIAvac 96 vacuum manifold" (page 15).
- All centrifugation steps are performed in the Centrifuge 4-16KS with the Plate Rotor 2 x 96. If using the centrifuge for the first time, read "Centrifuge 4-16KS" (page 18).
- Use of a multichannel pipet is recommended (see page 13). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for S-Blocks (see page 26).
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Handbook*) or Allprotect Tissue Reagent (see the *Allprotect Tissue Reagent Handbook*). Tissues can be stored in the reagent at 37°C for up to 1 day, at 15–25°C for up to 7 days, or at 2–8°C for up to 4 weeks (RNAprotect) or 6 months (Allprotect). Alternatively, tissues can be archived at –20°C or –80°C.

- Fresh, frozen or RNAprotect- Allprotect-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. Homogenized tissue lysates from step 2 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 3. Avoid prolonged incubation, which may compromise RNA integrity.
- Buffer RLT, Buffer RW1 and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RPE, Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 5 mg. Proceed immediately to step 2.

   Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used.

   For RNAprotect- or Allprotect-stabilized tissues: Remove the tissue from the stabilization reagent using forceps and be sure to remove any crystals that may have formed. RNA in RNAprotect- or Allprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect Tissue Reagent or Allprotect Reagent. Previously stabilized tissues can be stored at –80°C without the reagent.

   For unstabilized fresh or frozen tissues: RNA in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent or Allprotect Reagent, flash-frozen or disrupted and homogenized in step 2. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible. Remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA or in Allprotect Tissue Reagent to stabilize DNA, RNA and protein. However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

2. Disrupt the tissue and homogenize the lysate in Buffer RLT (do not use more than 5 mg tissue) according to step 2a (TissueRuptor II) or step 2b (TissueLyser II). If preferred, tissues can be disrupted under cryogenic conditions: in this case, follow the guidelines in Appendix G (page 87).

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

   After storage in RNAprotect Tissue Reagent or Allprotect Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep and RNeasy plates. Homogenization with the TissueRuptor II or TissueLyser II generally results in higher nucleic acid yields than with other methods. However, prolonged homogenization with these homogenizers results in greater DNA fragmentation.

   2a. Disruption and homogenization using the TissueRuptor II:

   - Place the tissue in a suitably sized vessel. Add 350 µl Buffer RLT.
     Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.
     Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.
   - Place the tip of the disposable probe into the vessel and operate the TissueRuptor II at full speed until the lysate is homogeneous (usually 30 s). Proceed to step 3.
     Note: To avoid damage to the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer. Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

2b. Disruption and homogenization using the TissueLyser II:

- Place the tissues in collection microtubes (cat. no. 19560) containing 1 stainless steel bead (5 mm mean diameter).
  If handling fresh or frozen tissue samples, keep the tubes on dry ice.
- Place the tubes at room temperature. Immediately add 350 µl Buffer RLT per tube.
- Attach collection microtube caps (cat. no. 19566), and place the tubes in the TissueLyser Adapter Set 2 x 96.
- Operate the TissueLyser II for 2 min at 25 Hz.
  The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.
- Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 2 min at 25 Hz. Rearranging the tubes allows even homogenization.
- Proceed to step 3.
  Do not reuse the stainless-steel beads.

3. Place an AllPrep 96 DNA plate on top of a new S-Block. Mark the plate for later identification.

4. Centrifuge the lysates from step 2 at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C. Carefully remove the supernatants by pipetting, and transfer them to the wells of the AllPrep 96 DNA plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

5. Seal the AllPrep 96 DNA plate with an AirPore tape sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

   Centrifugation with sealed plates prevents cross-contamination.

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

6. Place the AllPrep 96 DNA plate on top of another S-Block (either new or reused), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 17–22. Keep the S-Block containing the flow-through for RNA purification in steps 7–16.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

   Note: Do not store the AllPrep 96 DNA plate for long periods, as this may lead to the membrane drying out. Do not freeze the plate.

Total RNA purification

7. Assemble the QIAvac 96 vacuum manifold: first place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place an RNeasy 96 plate in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.

   Note: Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to your right-hand side.

8. Add 1 volume (350 µl) of 70% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.

   For maximum RNA yields from liver, 50% ethanol should be used instead of 70% ethanol.

9. Transfer the samples (700 µl) to the wells of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the samples have completely passed through the membranes (15–60 s). Switch off the vacuum, and ventilate the manifold.

   Make sure the QIAvac 96 vacuum manifold is assembled correctly before loading the samples. The flow-through is collected in the waste tray.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

   Note: Tape unused wells with adhesive tape or Tape Pads (cat. no. 19570). Do not use the AirPore tape sheets supplied with the AllPrep 96 DNA/RNA Kit.

   Note: The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

10. Add 800 µl Buffer RW1 to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

    The flow-through is collected in the same waste tray from step 9.

11. Lift the QIAvac 96 top plate carrying the RNeasy 96 plate from the QIAvac base, and empty the waste tray.* Reassemble the QIAvac 96 vacuum manifold.

12. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

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

* The waste liquid contains Buffer RLT and Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

13. Place the RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

    If reusing an S-Block, make sure it is cleaned as described on page 26.

14. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the RNeasy membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during RNA elution.

15. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the RNA.

    Note: Be sure to pipet the RNase-free water directly onto the RNeasy membranes. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

16. Remove the AirPore tape sheet. Repeat step 15 with a second volume of 45–70 µl RNase-free water.

    Note: Repeating step 15 is required for complete recovery of RNA. The eluate volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane (the 15 µl corresponds to the membrane dead volume).

    Use the caps provided with the kit to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Genomic DNA purification

17. Assemble the QIAvac 96 vacuum manifold: first place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place the AllPrep 96 DNA plate (from step 6) in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.

    Note: Always place the AllPrep 96 DNA plate into the vacuum manifold with the beveled edges pointing to your right-hand side.

18. Add 800 µl Buffer AW1 to each well of the AllPrep 96 DNA plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

    The flow-through is collected in the same waste tray from step 12.*

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

19. Place the AllPrep 96 DNA plate on top of an S-Block (either new or reused).

    If reusing an S-Block, make sure it is cleaned as described on page 26.

* The waste liquid contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

20. Add 800 µl Buffer AW2 to each well of the AllPrep 96 DNA plate, and seal the plate with an AirPore tape sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the AllPrep DNA membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during DNA elution.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

21. Remove the AirPore tape sheet. Place the AllPrep 96 DNA plate on top of a rack of Elution Microtubes CL. Add 50–100 µl Buffer EB (prewarmed to 70°C) to each well, and seal the plate with a new AirPore tape sheet. Incubate for 5 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the DNA.

    Note: Be sure to pipet Buffer EB directly onto the AllPrep DNA membranes. Elution will be incomplete if some of the buffer sticks to the walls or the O-rings of the AllPrep 96 DNA plate.

22. Remove the AirPore tape sheet. Repeat step 21 with a second volume of 50–100 µl Buffer EB.

    Note: Repeating step 21 is required for complete recovery of DNA.

    Use the caps provided with the kit to seal the microtubes for storage.

Protocol: Simultaneous Purification of DNA and RNA from Tissues using Spin Technology

Important points before starting

- If using the AllPrep 96 DNA/RNA Kit for the first time, read "Important Notes" (page 19).
- If preparing RNA for the first time, read Appendix A (page 67).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- If using the TissueLyser II, ensure that you are familiar with operating it by referring to the operating instructions and *TissueLyser Handbook*.
- All centrifugation steps are performed in the Centrifuge 4-16KS with the Plate Rotor 2 x 96. If using the centrifuge for the first time, read "Centrifuge 4-16KS" (page 18).
- Use of a multichannel pipet is recommended (see page 13). Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for S-Blocks (see page 26).
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Handbook*) or Allprotect Tissue Reagent (see the *Allprotect Tissue Reagent Handbook*). Tissues can be stored in the reagent at 37°C for up to 1 day, at 15–25°C for up to 7 days, or at 2–8°C for up to 4 weeks (RNAprotect) or 6 months (Allprotect). Alternatively, tissues can be archived at –20°C or –80°C.

- Fresh, frozen or RNAprotect- or Allprotect-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. Homogenized tissue lysates from step 2 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 3. Avoid prolonged incubation, which may compromise RNA integrity.
- Buffer RLT, Buffer RW1 and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RPE, Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 10 mg. Proceed immediately to step 2.

   Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used.

   For RNAprotect- or Allprotect-stabilized tissues: Remove the tissue from the stabilization reagent using forceps and be sure to remove any crystals that may have formed. RNA in RNAprotect- or Allprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect or Allprotect Reagent. Previously stabilized tissues can be stored at –80°C without the reagent.

   For unstabilized fresh or frozen tissues: RNA in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent or Allprotect Reagent, flash-frozen or disrupted and homogenized in step 2. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible. Remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA or in Allprotect Tissue Reagent to stabilize DNA, RNA and protein. However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

2. Disrupt the tissue and homogenize the lysate in Buffer RLT (do not use more than 10 mg tissue) according to step 2a (TissueRuptor II) or step 2b (TissueLyser II). If preferred, tissues can be disrupted under cryogenic conditions: in this case, follow the guidelines in Appendix G (page 87).

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

   After storage in RNAprotect Tissue Reagent or Allprotect Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep and RNeasy plates. Homogenization with the TissueRuptor II or TissueLyser II generally results in higher nucleic acid yields than with other methods. However, prolonged homogenization with these homogenizers results in greater DNA fragmentation.

   2a. Disruption and homogenization using the TissueRuptor II:

   - Place the tissue in a suitably sized vessel. Add 350 µl Buffer RLT.
     Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.
     Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.
   - Place the tip of the disposable probe into the vessel and operate the TissueRuptor II at full speed until the lysate is homogeneous (usually 30 s). Proceed to step 3.
     Note: To avoid damage to the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer. Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

2b. Disruption and homogenization using the TissueLyser II:

- Place the tissues in collection microtubes (cat. no. 19560) containing 1 stainless steel bead (5 mm mean diameter).
  If handling fresh or frozen tissue samples, keep the tubes on dry ice.
- Place the tubes at room temperature. Immediately add 350 µl Buffer RLT per tube.
- Attach collection microtube caps (cat. no. 19566), and place the tubes in the TissueLyser Adapter Set 2 x 96.
- Operate the TissueLyser II for 2 min at 25 Hz.
  The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.
- Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 2 min at 25 Hz.
  Rearranging the tubes allows even homogenization.
- Proceed to step 3.
  Do not reuse the stainless-steel beads.

3. Place an AllPrep 96 DNA plate on top of a new S-Block. Mark the plate for later identification.

4. Centrifuge the lysates from step 2 at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C. Carefully remove the supernatants by pipetting, and transfer them to the wells of the AllPrep 96 DNA plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

5. Seal the AllPrep 96 DNA plate with an AirPore tape sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

   Centrifugation with sealed plates prevents cross-contamination.

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

6. Place the AllPrep 96 DNA plate on top of another S-Block (either new or reused), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 16–19. Keep the S-Block containing the flow-through for RNA purification in steps 7–15.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

   Note: Do not store the AllPrep 96 DNA plate for long periods, as this may lead to the membrane drying out. Do not freeze the plate.

Total RNA purification

7. Place an RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

8. Add 1 volume (350 µl) of 70% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.

   For maximum RNA yields from liver, 50% ethanol should be used instead of 70% ethanol.

9. Transfer the samples (700 µl) to the wells of the RNeasy 96 plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

10. Seal the RNeasy 96 plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

    Centrifugation with sealed plates prevents cross-contamination.

11. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer RW1 to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

12. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

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

13. Empty the S-Block and remove the AirPore tape sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    It is important to dry the RNeasy membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during RNA elution.

* The waste liquid contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

14. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the RNA.

    Note: Be sure to pipet the RNase-free water directly onto the RNeasy membranes. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

15. Remove the AirPore tape sheet. Repeat step 14 with a second volume of 45–70 µl RNase-free water.

    Note: Repeating step 14 is required for complete recovery of RNA. The eluate volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane (the 15 µl corresponds to the membrane dead volume).

    Use the caps provided with the kit to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Genomic DNA purification

16. Add 800 µl Buffer AW1 to each well of the AllPrep 96 DNA plate from step 6, and seal the plate with an AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

17. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer AW2 to each well of the AllPrep 96 DNA plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

    It is important to dry the AllPrep DNA membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during DNA elution.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

18. Remove the AirPore tape sheet. Place the AllPrep 96 DNA plate on top of a rack of Elution Microtubes CL. Add 50–100 µl Buffer EB (prewarmed to 70°C) to each well, and seal the plate with a new AirPore tape sheet. Incubate for 5 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the DNA.

    Note: Be sure to pipet Buffer EB directly onto the AllPrep DNA membranes. Elution will be incomplete if some of the buffer sticks to the walls or the O-rings of the AllPrep 96 DNA plate.

19. Remove the AirPore tape sheet. Repeat step 18 with a second volume of 50–100 µl Buffer EB.

    Note: Repeating step 18 is required for complete recovery of DNA.

    Use the caps provided with the kit to seal the microtubes for storage.

* The waste liquid contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

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 plate wells	
a) Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 22) for details on disruption and homogenization methods. Use the spin protocol instead of the vacuum/spin protocol. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see "Determining the amount of starting material", page 19) 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 19).
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 96-well plates. If this happens, set the centrifugation temperature to 25°C. Warm the lysates to 37°C before transferring them to the AllPrep 96 DNA plate.

		Comments and suggestions
Low nucleic acid yield		
a)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 22) for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material (see "Determining the amount of starting material", page 19) and/or increase the volume of lysis buffer and the homogenization time.
b)	Too much starting material	Overloading the AllPrep 96 DNA plate significantly reduces nucleic acid yields. Reduce the amount of starting material (see page 19).
c)	RNA still bound to RNeasy 96 plate membrane	Repeat RNA elution, but incubate the RNeasy 96 plate on the benchtop for 10 min with RNase-free water before centrifuging.
d)	DNA still bound to AllPrep 96 DNA plate membrane	Repeat DNA elution, but incubate the AllPrep 96 DNA plate on the benchtop for 10 min with Buffer EB (prewarmed to 70°C) before centrifuging. In subsequent preparations, homogenize samples more thoroughly (see "Effect of homogenization on DNA yield and integrity" (page 25).
e)	Ethanol carryover	During the second wash with Buffer RPE and during the wash with Buffer AW2, be sure to centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes of the RNeasy 96 plate and AllPrep 96 DNA plate.
f)	Incomplete removal of cell-culture medium	Ensure complete removal of cell-culture medium after harvesting cells (see protocols, pages 27 and 36).
Low A_{260}/A_{280} value in RNA eluate		
	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 70).

	Comments and suggestions
DNA contaminated with RNA	
a) Lysate applied to the AllPrep 96 DNA plate contains ethanol	Add ethanol to the lysates after passing the lysates through the AllPrep 96 DNA plate.
b) Sample is affecting pH of homogenate	The final homogenate should have a pH of 7. Make sure that the sample is not highly acidic or basic and remove any excess cell-culture medium or stabilization reagent before adding Buffer RLT.
Contamination of RNA with DNA affects downstream applications	
a) Too much starting material	For some cell or tissue types, the efficiency of DNA binding to the AllPrep 96 DNA plate may be reduced when processing larger amounts. If the eluted RNA contains substantial DNA contamination, try processing smaller samples.
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 AllPrep 96 DNA plate will not bind DNA effectively if the lysis buffer is diluted.
RNA degraded	
a) Inappropriate handling of starting material	Ensure that the samples have been properly handled and that the protocol has been performed without interruptions. For details, see Appendix A (page 67), "Handling and storing starting material" (page 21) and "Important points before starting" for each protocol.
b) RNase contamination	Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the AllPrep DNA/RNA procedure or later handling. See Appendix A (page 67) for general remarks on handling RNA.

	Comments and suggestions
DNA fragmented	
Homogenization too vigorous	The length of the purified DNA (usually 15–30 kb) depends strongly on the homogenization conditions. If longer DNA fragments are required, keep the homogenization time to a minimum or use a gentler homogenization method if possible (e.g., use a QIAshredder homogenizer instead of a rotor–stator homogenizer; however, see "Effect of homogenization on DNA yield and integrity" on page 25).
Nucleic acids do not perform well in downstream experiments	
a) Salt carryover during elution	Ensure that buffers are at 20–30°C. Ensure that the correct buffer is used for each step of the procedure. Ensure that all liquid has completely passed through the membranes in each step of the protocol. When reusing S-Blocks 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 and during the wash with Buffer AW2, be sure to centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes of the RNeasy 96 plate and AllPrep 96 DNA plate.

Appendix A: General Remarks on Handling RNA

Handling RNA

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

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 68), or rinse with chloroform* if the plasticware is chloroform-resistant. 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.

* 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.

Disposable plasticware

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

Solutions

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

* 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.

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

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

Storage of RNA

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

Quantification of RNA

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

Spectrophotometric quantification of RNA

Using the QIAxpert UV/VIS Spectrophotometer for microvolume analysis

To determine the concentration of your RNA sample purified with RNeasy QIAGEN kit, use the corresponding RNeasy App on the QIAxpert. For more information, see the QIAxpert product page (**www.qiagen.com/qiaxpert-system**).

Using a standard spectrophotometer

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260} = 1 → 4 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample,

this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 71), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 68). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

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

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

A_{260}	= 0.2
Concentration of RNA sample	= 44 µg/ml x A_{260} x dilution factor = 44 µg/ml x 0.2 x 50 = 440 µg/ml
Total amount	= concentration x volume in milliliters = 440 µg/ml x 0.1 ml = 44 µg of RNA

Purity of RNA

The assessment of RNA purity will be performed routinely, when using the QIAxpert with the corresponding RNeasy App. See the QIAxpert user manual for more information (**www.qiagen.com/qiaxpert-system/user manual**)

* 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 standard photometric measurements, the ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution. For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 70).

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. In AllPrep DNA/RNA Kits the vast majority of cellular DNA is removed by binding to the AllPrep DNA plate. 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. QuantiTect® 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

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

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

reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see ordering information, page 90).

Integrity of RNA

The integrity and size distribution of total RNA purified with the AllPrep 96 DNA/RNA Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel system or Agilent® 2100 Bioanalyzer. Ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification. As a useful measure of RNA integrity, the QIAxcel® Advanced system and the Agilent 2100 Bioanalyzer provide an RNA integrity score (RIS) and an RNA integrity number (RIN), respectively. Ideally, the value should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

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

Appendix C: 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 min.

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

RNA sample preparation for FA gel electrophoresis

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

Incubate for 3–5 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 µl	saturated aqueous bromophenol blue solution*
80 µl	500 mM EDTA, pH 8.0
720 µl	37% (12.3 M) formaldehyde
2 ml	100% glycerol*
3.084 ml	formamide*
4 ml	10 x FA gel buffer

RNase-free water to 10 ml

Stability: approximately 3 months at 4°C

* 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: Storage, Quantification and Determination of Quality of Genomic DNA

Storage of DNA

For long-term storage, purified DNA in Buffer EB can be stored at –20°C. Avoid any contamination, as this may lead to DNA degradation. We recommend storing samples in aliquots to avoid repeated freezing and thawing, which can cause formation of precipitates.

Quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer using a quartz cuvette. For greatest accuracy, readings should be between 0.1 and 1.0. Using a standard 1 cm path length, an absorbance of 1 unit at 260 nm corresponds to 50 µg genomic DNA per ml (A_{260} =1 → 50 µg/ml). This relation is valid only for measurements made at neutral pH. Therefore, samples should be diluted in a low-salt buffer with neutral pH (e.g., Tris·Cl, pH 7.0).* Use the buffer in which the DNA is diluted to zero the spectrophotometer. An example of the calculation involved in DNA quantification is shown below:

Volume of DNA sample	= 100 µl
Dilution	= 20 µl of DNA sample + 180 µl of buffer (1/10 dilution)

Measure absorbance of diluted sample in a 0.2 ml cuvette

A_{260}	= 0.2
Concentration of DNA sample	= 50 µg/ml x A_{260} x dilution factor
	= 50 µg/ml x 0.2 x 10
	= 100 µg/ml

* 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.

Total amount = concentration x volume of sample in milliliters
= 100 µg/ml x 0.1 ml
= 10 µg of DNA

RNA concentration can also be determined by measuring the absorbance at 260 nm. If the eluate contains both DNA and RNA, a fluorometer must be used to quantify the DNA.

Determination of DNA purity

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate A_{260}/A_{280} values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5). Make sure to zero the spectrophotometer with the appropriate buffer. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

* 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.

Determination of DNA length

The precise length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol* precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C), since over-dried genomic DNA is very difficult to redissolve. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows:

- 1% agarose* gel in 0.5x TBE electrophoresis buffer*
- Switch intervals = 5–40 s
- Run time = 17 h
- Voltage = 170 V

* 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.

Appendix E: Purification of Total RNA Containing Small RNAs from Cells using Vacuum/Spin Technology

The following procedure allows the purification of total RNA containing small RNAs, such as miRNA from animal and human cells, using vacuum/spin technology. This procedure should not be used with animal and human tissues.

Reagents to be supplied by user

- Ethanol (100%)*

Important points before starting

- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.

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%) as indicated on the bottle to obtain a working solution.

* 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.

Procedure

Carry out the protocol starting on page 27 up to and including step 6. Instead of performing steps 7–16 (purification of total RNA >200 nucleotides), follow steps 1–8 below (purification of total RNA containing small RNAs).

1. Assemble the QIAvac 96 vacuum manifold: first place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place an RNeasy 96 plate in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.

   Note: Always place the RNeasy 96 plate into the vacuum manifold with the beveled edges pointing to your right-hand side.

2. Add 1.5 volumes (450 µl) of 100% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.

   Note: Add 300 µl of 100% ethanol if 200 µl Buffer RLT was used in step 1.

3. Transfer the samples (750 µl) to the wells of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the samples have completely passed through the membranes (15–60 s). Switch off the vacuum, and ventilate the manifold.

   Make sure the QIAvac 96 vacuum manifold is assembled correctly before loading the samples. The flow-through is collected in the waste tray.*

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

   Note: Tape unused wells with adhesive tape or Tape Pads (cat. no. 19570). Do not use the AirPore tape sheets supplied with the AllPrep 96 DNA/RNA Kit.

   Note: The vacuum must be switched off and the manifold ventilated between pipetting steps to maintain uniform conditions for each sample.

* The waste liquid contains Buffer RLT and Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

4. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

   The flow-through is collected in the same waste tray from step 3.*

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

5. Place the RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

6. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–30°C to dry the membranes.

   Centrifugation with sealed plates prevents cross-contamination.

   It is important to dry the RNeasy membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during RNA elution.

7. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–30°C to elute the RNA.

   Note: Be sure to pipet the RNase-free water directly onto the RNeasy membranes. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

* The waste liquid contains Buffer RLT and is therefore not compatible with bleach. See page 7 for safety information.

8. Remove the AirPore tape sheet. Repeat step 7 with a second volume of 45–70 µl RNase-free water.

   Note: Repeating step 7 is required for complete recovery of RNA. The eluate volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane (the 15 µl corresponds to the membrane dead volume).

   Use the caps provided with the kit to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Appendix F: Purification of Total RNA Containing Small RNAs from Cells using Spin Technology

The following procedure allows the purification of total RNA containing small RNAs, such as miRNA from animal and human cells, using spin technology. This procedure should not be used with animal and human tissues.

Reagents to be supplied by user

- Ethanol (100%)*

Important points before starting

- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.

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%) as indicated on the bottle to obtain a working solution.

* 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.

Procedure

Carry out the protocol starting on page 36 up to and including step 6. Instead of performing steps 7–15 (purification of total RNA >200 nucleotides), follow steps 1–F8 below (purification of total RNA containing small RNAs).

1. Place an RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

   If reusing an S-Block, make sure it is cleaned as described on page 26.

2. Add 1.5 volumes (450 µl) of 100% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.

   Note: Add 300 µl of 100% ethanol if 200 µl Buffer RLT was used in step 1.

3. Transfer the samples (750 µl) to the wells of the RNeasy 96 plate.

   Note: Take care not to wet the rims of the wells, as this could lead to cross-contamination.

4. Seal the RNeasy 96 plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

   Centrifugation with sealed plates prevents cross-contamination.

5. Empty the S-Block* and remove the AirPore tape sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C.

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

* The waste liquid contains Buffer RLT and is therefore not compatible with bleach. See page 7 for safety information.

6. Empty the S-Block and remove the AirPore tape sheet. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with a new AirPore tape sheet. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.

   It is important to dry the RNeasy membranes, since residual ethanol may interfere with downstream reactions. The 10-min centrifugation ensures that residual traces of salt are removed and that no ethanol is carried over during RNA elution.

7. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the RNA.

   Note: Be sure to pipet the RNase-free water directly onto the RNeasy membranes. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.

8. Remove the AirPore tape sheet. Repeat step 7 with a second volume of 45–70 µl RNase-free water.

   Note: Repeating step 7 is required for complete recovery of RNA. The eluate volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane (the 15 µl corresponds to the membrane dead volume).

   Use the caps provided with the kit to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Appendix G: Cryogenic Disruption of Tissues

The tissue protocols in this handbook provide instructions for disrupting tissues in Buffer RLT. If preferred, tissues can be disrupted under cryogenic conditions without Buffer RLT. After disruption, the samples are mixed with Buffer RLT, and then homogenized to ensure optimal DNA yields. This appendix provides guidelines on cryogenic tissue disruption and subsequent homogenization. There are 2 alternative methods of disruption and 3 alternative methods of homogenization. Note that different homogenization methods have different effects on DNA yield and integrity (for details, see page 25).

Disruption using the TissueLyser II

Precool the TissueLyser Adapter Set 2 x 96 at –80°C for at least 2 h.

Place the collection microtubes (cat. no. 19560) on dry ice, and add 1 stainless steel bead (5 mm diameter) to each tube.

Place the frozen tissues in the collection microtubes, attach the collection microtube caps (cat. no. 19566), and place the tubes in the TissueLyser Adapter Set 2 x 96.

Operate the TissueLyser II for 1 min at 25 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 1 min at 25 Hz.

Immediately add 350 µl Buffer RLT to each tube.

Proceed with one of the 3 homogenization methods described in this appendix.

Important: Do not freeze the TissueLyser Adapter Set 2 x 96 and the collection microtubes in liquid nitrogen, as this may result in breakage of the tubes.

Disruption using a mortar and pestle

- Place the tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle.
- Decant 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 allow the tissue powder to thaw.
- Immediately add 350 µl Buffer RLT to the tissue powder.
- Proceed with one of the 3 homogenization methods described in this appendix.

Homogenization using the TissueLyser II

- If necessary, transfer the lysates to collection microtubes (cat. no. 19560), each containing 1 stainless steel bead (5 mm diameter).
- Attach collection microtube caps (cat. no. 19566) to the collection microtubes, and place the tubes in the TissueLyser Adapter Set 2 x 96.
- Operate the TissueLyser II for 1 min at 20 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 1 min at 20 Hz.
- Proceed to step 3 of the vacuum/spin protocol (page 44) or the spin protocol (page 54).

Homogenization using the QIAshredder

- Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed.
- Proceed to step 3 of the vacuum/spin protocol (page 44) or the spin protocol (page 54).

Homogenization using a needle and syringe

- Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-free syringe.
- Proceed to step 3 of the vacuum/spin protocol (page 44) or the spin protocol (page 54).

Ordering Information

Product	Contents	Cat. no.
AllPrep 96 DNA/RNA Kit (4)	For 4 x 96 preps: AllPrep 96 DNA Plates, RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, RNase-Free Water and Buffers	80311
Accessories		
S-Blocks (24)	96-well blocks with 2.2 ml wells; 24 per case	19585
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks; 50 sheets per pack	19571
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks; 25 sheets per pad, 5 pads per pack	19570
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates; includes QIAvac 96 top plate, base, waste tray, plate holder, rack of collection microtubes (1.2 ml)	19504
Vacuum Pump	Universal vacuum pump (capacity 34 liters per minute, 8 mbar vacuum abs.)	Inquire
Vacuum Regulator	For use with QIAvac manifolds	19530
Centrifuge 4-16KS	Universal refrigerated laboratory centrifuge with brushless motor	Inquire
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates; for use with QIAGEN centrifuges	81031
RNAprotect Cell Reagent (250 ml)	250 ml RNAprotect Cell Reagent	76526
RNAprotect Tissue Reagent (50 ml)	50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Reagent (250 ml)	250 ml RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106
RNAprotect Tissue Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAprotect Tissue Reagent each	76154
RNAprotect Tissue Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNAprotect Tissue Reagent each	76163
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
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	990890
TissueLyser II	Universal laboratory mixer-mill disruptor	inquire

Product	Contents	Cat. no.
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates and 2 racks for use with Collection Microtubes (racked) on the TissueLyser II	69984
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps	Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks, 960 in strips of 8	19566
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
QIAxcel system	Capillary electrophoresis device, including computer and BioCalculator Analysis software; 1-year warranty on parts and labor	9001421
QIAxcel Advanced Instrument	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9001941
Related products		
QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at www.qiagen.com/GeneGlobe)		
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions or 400 x 25 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies
QuantiNova® Reverse Transcription Kit for fast cDNA synthesis and reproducible real-time two-step RT-PCR		
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 Rev. Transcription Kit (50)	For 50 x 20 µl reactions: 100 µl 8x gDNA Removal Mix, 50 µl Reverse Transcription Enzyme, 200 µl Reverse Transcription Mix (containing RT primers), 100 µl Internal Control RNA, 1.9 ml RNase-Free Water	205411
QuantiNova Rev. Transcription Kit (200)	For 50 x 20 µl reactions: 100 µl 8x gDNA Removal Mix, 50 µl Reverse Transcription Enzyme, 200 µl Reverse Transcription Mix (containing RT primers), 100 µl Internal Control RNA, 1.9 ml RNase-Free Water	205413

* Visit www.qiagen.com/automation to find out more about the TissueRuptor II and TissueLyser II and to order.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Visit www.qiagen.com/geneXpression to find out more about standardized solutions for gene expression analysis — from RNA preparation to real-time RT-PCR.

Document Revision History

Date	Changes
February 2021	Updated branding of RNA protection products.

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the AllPrep 96 DNA/RNA Kit to the following terms:

1. The AllPrep 96 DNA/RNA Kit may be used solely in accordance with the AllPrep 96 DNA/RNA Handbook and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the AllPrep 96 DNA/RNA Handbook and additional protocols available at www.qiagen.com .
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
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5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

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The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges4-16S and 4-16KS from QIAGEN, and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.