November 2020

AllPrep® DNA/RNA/miRNA Universal Handbook

For simultaneous purification of genomic DNA and total RNA, including miRNA, from the same cell and tissue sample (including human blood cells and difficult-to-lyse tissues)

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep DNA/RNA/miRNA Universal Kit (50) **Catalog no.**	**(50)** **80224**
AllPrep DNA Mini Spin Columns (uncolored)	50
RNeasy® Mini Spin Columns (pink) (each in a 2 ml collection tube)	50
Collection Tubes (1.5 ml)	100
Collection tubes (2 ml)	150
Buffer RLT Plus*	45 ml
Proteinase K	4 x 1.4 ml
Buffer FRN*† (concentrate)	14 ml
RNase-Free DNase 1 (lyophilized)	1500 units‡
Buffer RDD	2 x 2 ml
Buffer RPE§ (concentrate)	11 ml
Buffer AW1*§ (concentrate)	19 ml
Buffer AW2§ (concentrate)	13 ml
Buffer EB	15 ml
RNase-Free Water	10 ml
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Before using for the first time, add the appropriate volume of isopropanol as indicated on the bottle to obtain a working solution.

‡ Kunitz units, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. **33**, 349 and 363).

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

Storage

RNase-Free DNase I should be immediately stored at 2–8°C upon arrival. Buffers, RNeasy Mini Spin Columns and AllPrep DNA Mini Spin Columns can be stored at room temperature (15–25°C). Under these conditions, the kit components can be kept for at least 9 months without any reduction in performance, if not otherwise stated on the label.

Proteinase K is supplied in a specially formulated storage buffer and is stable for at least 1 year after delivery when stored at room temperature. If longer storage is required or if ambient temperatures often exceed 25°C, we recommend storage at 2–8°C. Upon arrival, label one vial of Proteinase K for DNA preparation and the remaining three vials for RNA preparation.

Intended Use

The AllPrep DNA/RNA/miRNA Universal Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

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

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

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

Buffer AW1 contains guanidine hydrochloride; Buffer RLT Plus and Buffer FRN contain guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The AllPrep DNA/RNA/miRNA Universal Kit is designed to purify genomic DNA and total RNA, including miRNA, simultaneously from a single biological sample. Since there is no need to divide the sample into two for separate purification procedures, maximum yields of DNA and RNA/miRNA can be achieved. The kit is optimized for isolation of nucleic acids from challenging samples, such as difficult-to-lyse fibrous and lipid-rich tissues, without the need for toxic substances, such as phenol. The lysate is first passed through an AllPrep DNA Mini spin column to selectively retain DNA and then through an RNeasy Mini spin column to isolate RNA, including miRNA. Additional enzymatic digestions for RNA and DNA ensure high yields of nucleic acids from all sample types. In contrast to other procedures where either the biological sample or the purified total nucleic acids are divided into two before being processed separately, with the AllPrep DNA/RNA/miRNA Universal Kit, pure DNA and RNA, including miRNA, are purified from the entire sample. The AllPrep DNA/RNA/miRNA Universal Kit can be used with a broad range of tissues, cells and cellular components of human whole blood.

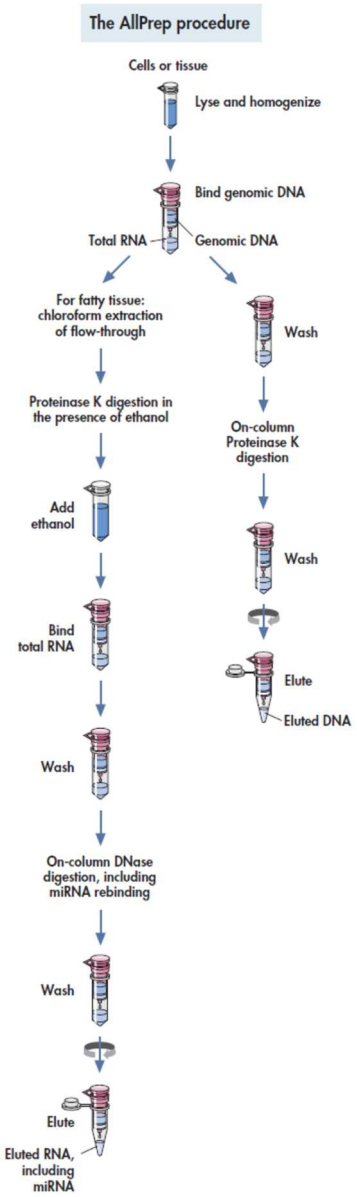
Genomic DNA purified with the AllPrep DNA/RNA/miRNA Universal Kit procedure has an average length of 15–30 kb, depending on homogenization conditions. All RNA molecules that are >18 nucleotides are easily isolated with the AllPrep DNA/RNA/miRNA Universal Kit.

Principle and procedure

The patent protected AllPrep DNA/RNA/miRNA Universal Kit integrates QIAGEN's technology for selective binding of double-stranded DNA with well-established RNeasy technology. Specially optimized enzymatic digestion steps enable the purification of RNA, miRNA and DNA — even from challenging samples.

Biological 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 lysate is then passed through an AllPrep DNA Mini spin column. This column, in combination with the high-salt buffer, allows selective and efficient binding of genomic DNA. On-column Proteinase K digestion in optimized buffer conditions allows purification of high DNA yields from all sample types. The column is then washed and pure, ready-to-use DNA is eluted.

Flow-through from the AllPrep DNA Mini spin column is digested by Proteinase K in the presence of ethanol. This optimized digestion, together with the subsequent addition of further ethanol, allows appropriate binding of total RNA, including miRNA, to the RNeasy Mini spin column. DNase I digestion ensures high-yields of DNA-free RNA. Following DNase I digestion, contaminants are efficiently washed away and high-quality RNA is eluted.


The AllPrep procedure
Cells or tissue
Lyse and homogenize
Bind genomic DNA
Total RNA
Genomic DNA
For fatty tissue: chloroform extraction of flow-through
Proteinase K digestion in the presence of ethanol
Add ethanol
Bind total RNA
Wash
On-column DNase digestion, including miRNA rebinding
Wash
Elute
Eluted RNA, including miRNA
Wash
On-column Proteinase K digestion
Wash
Elute
Eluted DNA

Description of protocols

Preoptimized, streamlined protocols for different starting materials are included in this handbook. Protocols for processing the following samples are included:

- Tissues (page 26)
- Cells (page 37)
- Human whole blood (page 48)

The protocols slightly differ in the lysis and homogenization of the sample.

Automated purification of DNA, RNA and miRNA on QIAcube Instruments

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

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

QIAcube Connect.

Equipment and Reagents to Be Supplied by User

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

For all protocols:

- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)
- 2 ml Safe-Lock microcentrifuge tubes (available from Brinkmann, cat. no. 022363352, or Eppendorf, cat. no. 0030 120.094), or 2 ml SafeSeal microcentrifuge tubes (Sarstedt, cat. no.72.695)
- Microcentrifuge (with rotor for 2 ml tubes)
- 96–100% ethanol*
- Isopropanol
- Chloroform (for lipid-rich tissues e.g., fat, brain, breast)
- Disposable gloves

For the tissue protocol:

- **Optional**: RNAprotect® Tissue Reagent (50 ml) (cat. no. 76104), Allprotect Tissue Reagent (100 ml) (cat. no. 76405), or liquid nitrogen
- For lipid-rich tissues: Refrigerated (4°C) microcentrifuge for chloroform extraction

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

For the blood protocol:

- Buffer EL (erythrocyte lysis buffer) (cat. no. 79217)
- Tubes for erythrocyte lysis (1.5 ml – 15 ml depending on sample size); use of sterile, disposable, polypropylene tubes is recommended
- Refrigerated (4°C) microcentrifuge (for blood samples ≤250 µl) or refrigerated laboratory centrifuge with a rotor for 12 ml or 15 ml centrifuge tubes (for blood samples >250 µl)

For sample disruption and homogenization, depending on the method chosen, one or more of the following are required:

- Trypsin and PBS
- QIAshredder® (50) homogenizer (cat. no. 79654)
- Blunt-ended needle and syringe
- Mortar and pestle
- TissueLyser LT (cat. no. 85600)
- TissueLyser II (cat. no. 85300)
- TissueRuptor® II (cat. no. 9001271)

Important Notes

Determining the amount of starting material

All protocols

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 limited by:

- The type of sample and its DNA and RNA content
- The DNA binding capacity of the AllPrep DNA Mini spin column
- The RNA binding capacity of the RNeasy Mini spin column

When processing samples containing high amounts of DNA or RNA, less than the maximum amount of starting material shown in Table 1 (page 15) should be used so that the binding capacity of the spin column is not exceeded.

When processing samples containing average or low amounts of DNA and RNA, the maximum amount of starting material shown in Table 1 (page 15) can be used. However, even though the spin column binding capacity is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of nucleic acids to the spin column membranes, resulting in lower yield and purity of DNA and RNA.

More information on using the correct amount of starting material is given in each protocol. Table 2. **Yields of genomic DNA and total RNA (including miRNA)**(page 16) shows expected DNA and RNA yields from various cells and tissues.

Note: If lysis of the starting material is incomplete, DNA and RNA yields will be lower than expected, even if the binding capacity of the spin columns is not exceeded.

Table 1. Spin column specifications

Specification	AllPrep DNA Mini spin column	RNeasy Mini spin column
Maximum binding capacity	100 µg DNA	100 µg RNA
Maximum loading volume	700 µl	700 µl
Nucleic acid size distribution	DNA size of 15–30 kb*	RNA >18 nt
Minimum elution volume	100 µl	30 µl
Maximum amount of starting material:		
Animal cells	1 x 10^7 cells	Entire flow-through from the AllPrep DNA Mini spin column
Animal tissue	30 mg†	Entire flow-through from the AllPrep DNA Mini spin column
Blood	1.5 ml (maximum of 1x10^7 leukocytes)	Entire flow-through from the AllPrep DNA Mini spin column

* Depending on homogenization conditions.

† Do not use more than 20 mg of tissue stabilized in RNAprotect Tissue Reagent or Allprotect Tissue Reagent.

Table 2. Yields of genomic DNA and total RNA (including miRNA)

Sample type	Average yield of genomic DNA (µg)	Average yield of total RNA* (µg)
Human blood from healthy subjects (1 ml)	10–20	2–5
Cell cultures (1 x 10^6 cells)		
Jurkat	6–10	10–15
K562	7–12	11–18
HeLa	15	18–20
Cos7	9–10	25–30
Cho-K1	7–9	12–15
HepG2	12–13	15–17
MCF-7	20–22	20–23
Rat tissues (10 mg)		
Brain	5–10	5–10
Heart	5–10	5–15
Muscle	4–7	5–10
Skin	2–5	5–8
Kidney	15–25	20–40
Liver	15–25	30–80
Spleen	50–70	30–80
Adipose tissue	0.5–2.5	0.5–2.5
Lung	10–35	15–30
Intestine	10–15	30–60

* Includes miRNA. Amounts can vary due to factors, such as species, developmental stage and growth conditions.

Counting cells or weighing tissue is the most accurate way to quantify the amount of starting material. However, the following may be used as a guide.

Cultured cells

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

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

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

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

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Cell numbers will vary for different kinds of cells.

Handling and storing starting material

Tissue

RNA in harvested tissue is not protected until the sample is treated with RNAprotect Tissue Reagent or Allprotect 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 or Allprotect Tissue Reagent.

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

Cells

RNA in harvested cells is not protected until the sample is treated with RNAprotect Cell Reagent, (cat. no. 76526) 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.

After disruption and homogenization in Buffer RLT Plus (lysis buffer), samples can be stored at –70°C for months.

Blood

The AllPrep DNA/RNA/miRNA Universal Kit also enables purification of total cellular genomic DNA and RNA/miRNA from fresh, human whole blood. Whole blood should be collected in the presence of an anticoagulant, such as EDTA or citrate.

Do not use heparin as it can interfere with downstream applications.

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

Note: The AllPrep DNA/RNA/miRNA Universal Kit cannot be used for frozen blood samples.

Lysis and homogenization

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 an absolute requirement 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. On the other hand, excessive homogenization results in shorter genomic DNA fragments.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step.

Cells and tissue

Disruption of cells is achieved by vortexing or mixing in Buffer RLT Plus. The method of homogenization depends on the cell count of the sample. If the cell count is 1 x 10^5 cells or fewer, sufficient homogenization is achieved by vortexing the sample for 1 minute. If the cell count is higher, homogenization must be performed using one of 4 methods:

- TissueLyser II, TissueLyser LT or other bead mill
- TissueRuptor II or other rotor–stator homogenizer
- Syringe and needle
- QIAshredder

Disruption and homogenization of tissue can be performed using one of 3 methods:

- Disruption and homogenization using the TissueLyser II, TissueLyser LT or other bead mill
- Disruption and homogenization using the TissueRuptor II or other rotor–stator homogenizer
- Disruption using a mortar and pestle and homogenization using a needle and syringe or a QIAshredder homogenizer

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

The different disruption and homogenization methods are described in more detail below.

Disruption and homogenization using the TissueLyser system

In bead-milling, tissues and cells 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 sample. Two bead mills are available from QIAGEN: the TissueLyser LT for low- to medium-throughput disruption, and the TissueLyser II for medium- to high-throughput disruption.

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

The TissueLyser II disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24, which holds 48 x 2 ml microcentrifuge tubes containing stainless steel beads of 5 mm or 7 mm mean diameter. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. If using other bead mills for sample disruption and homogenization, refer to suppliers' guidelines.

The TissueLyser II can also disrupt and homogenize up to 192 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 96, which holds 192 x 1.2 ml microtubes containing stainless steel beads of 5 mm mean diameter.

Stainless steel beads with a diameter of 5 mm are optimal for use with animal tissues in combination with the AllPrep DNA/RNA/miRNA Universal Kit. All other disruption parameters should be determined empirically for each application.

Disruption and homogenization using the TissueRuptor II

Using the TissueRuptor II, samples are simultaneously disrupted and homogenized by rapid rotation of the blade of the disposable probe. The transparent probe enables visual control of the sample disruption process. Cells and tissues are disrupted at room temperature (15–25°C) in lysis buffer.

Protocols for purification of nucleic acids from tissues or cells contain guidelines for disruption and homogenization using the TissueRuptor II and disposable probes. For other rotor–stator homogenizers, please refer to suppliers' guidelines for further details.

IMPORTANT: To prevent the rotor from becoming stuck in the stator tube, be sure to choose a suitably sized vessel for disruption. During homogenization and disruption, the tip of the disposable probe must be submerged in lysis buffer to prevent damage to the instrument and probe.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into an appropriately sized tube cooled with liquid nitrogen and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add the lysis buffer and continue as quickly as possible with homogenization using either the TissueLyser II or TissueLyser LT (or

similar bead mill), the TissueRuptor II (or similar rotor–stator homogenizer), the QIAshredder homogenizer, or a syringe and needle (see below).

Note: Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization must be performed separately before continuing with the AllPrep DNA/RNA/miRNA Universal procedure.

Homogenization using a syringe and needle

Cell and tissue lysates can be homogenized using a syringe and needle. Lysate is passed through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may be required to facilitate handling and minimize loss. However, for subsequent nucleic acid purification, do not use more than 600 µl lysate.

Homogenization using the QIAshredder homogenizer

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

Blood

Human erythrocytes (red blood cells) do not contain nuclei and are therefore not relevant for nucleic acid isolation since they neither synthesize nor contain nucleic acids. The target of nucleic acid isolation from whole blood is leukocytes (white blood cells), which are nucleated and therefore contain nucleic acids. Leukocytes consist of 3 main cell types: lymphocytes, monocytes and granulocytes.

Since healthy blood contains approximately 1000 times more erythrocytes than leukocytes, removing the erythrocytes simplifies nucleic acid isolation.

Erythrocyte removal

The QIAGEN erythrocyte lysis procedure can be used in combination with the AllPrep DNA/RNA/miRNA Universal Kit to achieve selective lysis of erythrocytes. Erythrocytes are more susceptible than leukocytes to hypotonic shock and burst rapidly in the presence of a hypotonic buffer. Intact leukocytes are then recovered by centrifugation. The conditions for selective lysis of erythrocytes have been optimized to allow fast removal of erythrocytes without affecting the stability of the leukocytes. Buffer EL for erythrocyte lysis is supplied separately; see ordering information on page 72.

A common alternative to erythrocyte lysis is Ficoll® density-gradient centrifugation. In contrast to erythrocyte-lysis procedures, Ficoll density-gradient centrifugation only recovers mononuclear cells (lymphocytes and monocytes), and removes granulocytes.

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

Leukocyte lysis and homogenization

Leukocytes are lysed and homogenized in the same way as cultured cells (see above).

Preparation of buffers

Preparing Buffer RLT Plus

Before starting the procedure, check whether a precipitate has formed in Buffer RLT Plus. If necessary, dissolve by warming with gentle agitation.

When processing samples lysed in Buffer RLT Plus, excessive foaming may occur. This foaming is substantially reduced by adding Reagent DX to Buffer RLT Plus at a final concentration of

0.5% (v/v) before lysing the samples. Reagent DX has been tested with the AllPrep DNA/RNA/miRNA Universal Kit and has no effect on RNA and DNA purity or on downstream applications, such as RT-PCR or real-time PCR. Buffer RLT Plus containing Reagent DX can be stored at room temperature (15–25°C) for at least 9 months. Reagent DX is supplied separately; see ordering information on page 72.

Preparing Buffer FRN

Add 42 ml isopropanol to the bottle containing 14 ml Buffer FRN concentrate. Tick the check box on the bottle label to indicate that isopropanol has been added.

Before using Buffer FRN for the first time, check whether a precipitate has formed. If necessary, dissolve by warming with gentle agitation. After equilibration to room temperature (15–25°C), add the isopropanol as indicated above. Tick the check box on the bottle label to indicate that isopropanol has been added.

Note: Before starting the procedure, mix reconstituted Buffer FRN by shaking.

Preparing DNase I stock solution

Prepare DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µl RNase-free water. In some cases, the vial of DNase may appear to be empty due to lyophilized enzyme sticking to the septum. To avoid loss of DNase I, do not open the vial. Instead, inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex. Due to the production process, insoluble material may be present in the lyophilized DNase. Insoluble material may remain when dissolving DNase. This does not affect DNase performance. Rigorous QC tests are carried out to ensure that DNase activity remains consistent from lot to lot.

Note: Do not vortex reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial. For long-term storage of DNase I, remove the stock solution from the vial, divide it into single-use aliquots,

and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Preparing Buffer RPE

Add 4 volumes (44 ml) ethanol (96–100%) to the bottle containing 11 ml Buffer RPE concentrate. Tick the check box on the bottle label to indicate that ethanol has been added.

Note: Before starting the procedure, mix reconstituted Buffer RPE by shaking.

Preparing Buffer AW1

Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added.

Note: Before starting the procedure, mix reconstituted Buffer AW1 by shaking.

Preparing Buffer AW2

Add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added.

Note: Before starting the procedure, mix reconstituted Buffer AW2 by shaking.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA, including miRNA, from Tissues

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal nucleic acid yield and purity. A maximum amount of 30 mg fresh or frozen tissue or 20 mg of tissue that has been stabilized with RNAprotect Tissue Reagent or Allprotect Tissue Reagent can generally be processed. For most tissues, the DNA binding capacity of the AllPrep DNA Mini spin column, the RNA binding capacity of the RNeasy Mini spin column and the lysing capacity of Buffer RLT Plus will not be exceeded by these amounts. Average DNA and RNA yields from various tissues are given in Table 2. **Yields of genomic DNA and total RNA (including miRNA)** (page 16).

Some tissues, such as thymus, contain very high amounts of DNA, which will overload the AllPrep DNA Mini spin column (unless less than 5 mg tissue is used as starting material).

If there is no information about the nature of your starting material, we recommend starting with no more than 10 mg tissue.

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

Important points before starting

- If using the AllPrep DNA/RNA/miRNA Universal Kit for the first time, read "Important Notes" (page 14).
- If preparing RNA for the first time, read Appendix A (page 57).

- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Handbook*) or in Allprotect Tissue Reagent (see Allprotect *Tissue Reagent Handbook*). Tissues can be stored in RNAprotect Tissue Reagent for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or archived at –30°C to –15°C or –90°C to –65°C , or tissues can be stored in Allprotect Tissue Reagent for up to 1 day 37°C, 7 days at 15–25°C, 6 months at 2–8°C, or archived at –30°C to –15°C or –90°C to –65°C.
- Fresh, frozen or RNAprotect- or Allprotect-stabilized tissues can be used.
- Flash-freeze fresh tissues in liquid nitrogen, and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT Plus. Homogenized tissue lysates from step 3 can also be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- If desired, more than 30 mg tissue can be disrupted and homogenized at the start of the procedure (increase the volume of Buffer RLT Plus proportionately). Use a portion of the homogenate corresponding to no more than 30 mg tissue for nucleic acid purification, and store the rest at –80°C.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25°C) for 1 month after addition of β-ME. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- Buffer RLT Plus, Buffer FRN 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. During the procedure, work quickly.

- Unless otherwise indicated perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- In the procedure below, ▲ indicates protocol procedures when processing 350 µl lysate and ■ indicates protocol procedures when processing 600 µl lysate.

Things to do before starting

- If using Buffer FRN, Buffer RPE, Buffer AW1, Buffer AW2 and RNase-Free DNase I for the first time, reconstitute and dilute them as described in "Preparation of buffers" (page 23).
- If necessary, warm and gently agitate Buffer RLT Plus and Buffer FRN to redissolve any precipitates that may have formed.
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer FRN, Buffer RPE, Buffer AW1 and Buffer AW2 by shaking.

Procedure

1. Excise the tissue sample from the animal or remove it from storage. Remove RNAprotect- or Allprotect-stabilized tissues from the reagent using forceps. Determine the amount of tissue. Do not use more than 30 mg of fresh or frozen tissue or 20 mg of stabilized tissue.

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

2. Follow step 2a for RNAprotect- or Allprotect-stabilized tissues or step 2b for unstabilized fresh or frozen tissues.

   2a. For stabilized tissues:

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

The RNA in RNAprotect- or Allprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (18–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect Tissue Reagent or Allprotect Tissue Reagent. Previously stabilized tissues can be stored at –90°C to –65°C without additional reagent.

2b. For unstabilized fresh or frozen tissues:

- If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately to step 3.
- If using only a portion of the tissue, weigh the piece to be used, and place it into a suitably sized vessel for disruption and homogenization. To protect the RNA, tissues should be cut on dry ice. Proceed immediately to step 3.

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

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

3. Disrupt the tissue and homogenize the lysate in Buffer RLT Plus according to step 3a if using the TissueRuptor II or other rotor–stator, 3b if using a TissueLyser instrument, 3c if disrupting tissue using a mortar and pestle, followed by homogenization using a needle and syringe, or 3d if disrupting tissue using a mortar and pestle, followed by homogenization using a QIAshredder homogenizer.

   See “Lysis and Homogenization”, page 19, for more details on disruption and homogenization.

   Note: Ensure that β-ME is added to Buffer RLT Plus before use (see “Important points before starting”, page 26).

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

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

3a. Disruption using the TissueRuptor II or other rotor–stator:

- Place the weighed (fresh, frozen or RNAprotect- or Allprotect-stabilized) tissue in a suitably sized vessel.
- Add the appropriate volume of Buffer RLT Plus (see Table 4, page 31). Immediately disrupt and homogenize the tissue using a conventional rotor–stator homogenizer until it is uniformly homogeneous (usually 20–40 s).
- Proceed to step 4.

3b. Disruption using the TissueLyser II:

- Place the weighed (fresh, frozen or RNAprotect- or Allprotect-stabilized) tissue in a suitably sized vessel.
- Add the appropriate volume of Buffer RLT Plus (see Table 4, page 31) and add one stainless steel bead (5 mm diameter).
- If using the TissueLyser II, homogenize for 2–5 min at 20–25 Hz. Rotate the TissueLyser rack and homogenize for another 2–5 min at 20–25 Hz. Continue the protocol with step 4.
- If using the TissueLyser LT, place the tubes in the insert of the TissueLyser LT Adapter and operate the TissueLyser LT for 2–5 min at 30–50 Hz. Continue the protocol with step 4.

For detailed information, refer to the *TissueLyser Handbook* or the *TissueLyser LT Handbook*.

Note: The instructions in step 3b are only guidelines. They may need to be changed depending on the sample being processed and on the bead mill being used.

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

- Immediately place the weighed (fresh, frozen or RNAprotect- or Allprotect-stabilized) tissue in liquid nitrogen and grind thoroughly with a mortar and pestle.
- Decant tissue powder and liquid nitrogen into an RNase-free, liquid nitrogen-cooled 2 ml microcentrifuge tube (not supplied).
- Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.
- Add the appropriate volume of Buffer RLT Plus (see Table 4) and homogenize by passing lysate at least 5 times through a 20-gauge needle fitted to an RNase-free syringe.
- Proceed to step 4.

3d. Disruption using a mortar and pestle, followed by homogenization using the QIAshredder:

- Immediately place the weighed (fresh, frozen or RNAprotect- or Allprotect-stabilized) tissue in liquid nitrogen and grind thoroughly with a mortar and pestle.
- Decant tissue powder and liquid nitrogen into an RNase-free, liquid nitrogen-cooled 2 ml microcentrifuge tube (not supplied).
- Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.
- Add the appropriate volume of Buffer RLT Plus (see Table 4). Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube and centrifuge for 2 min at maximum speed.
- Proceed to step 4.

Table 4. Volumes of Buffer RLT Plus for tissue disruption and homogenization

Amount of starting material	Volume of Buffer RLT Plus
<10 mg	350 µl or 600 µl*
10–30 mg	600 µl

* Use 600 µl Buffer RLT Plus for tissues stabilized in RNAprotect Tissue Reagent or Allprotect Tissue Reagent.

4. **Optional**: Briefly centrifuge the tube to reduce foam.
5. Transfer the homogenized lysate to an AllPrep DNA Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 30 s at full speed (maximum speed of 20,000 x *g*).

   Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.
6. Place the AllPrep DNA Mini spin column in a new 2 ml collection tube (supplied) and store at room temperature (15–25°C) or at 2–8°C for DNA purification later in steps 24–30. Transfer the flow-through into a new 2 ml microcentrifuge tube (not supplied) for RNA purification in steps 7–23.

   Note: Do not store the DNA-containing AllPrep DNA Mini spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA (including miRNA) purification

7. Only for lipid-rich, fatty tissues (e.g., fat, brain, breast): Add ▲ 90 µl or ■ 150 µl chloroform to the flow-through from step 6 and vortex thoroughly. Centrifuge at 4°C for 3 min at full speed (maximum speed of 20,000 x *g*) to separate the phases. Carefully transfer the aqueous phase to a new 2 ml microcentrifuge tube (not supplied).
8. Add ▲ 50 µl or ■ 80 µl Proteinase K to the flow-through from step 6 or the aqueous phase from step 7 and mix by pipetting.
9. Add ▲ 200 µl or ■ 350 µl of 96–100% ethanol and mix well. Do not centrifuge.
10. Incubate for 10 min at room temperature.
11. Add ▲ 400 µl or ■ 750 µl of 96–100% ethanol and mix well. Do not centrifuge.

12. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 *x g*). Discard the flow-through.*

    Reuse the collection tube in step 13.

13. Repeat step 12 until the entire sample has passed through the RNeasy Mini spin column.

    Reuse the collection tube in step 14.

14. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Preparing Buffer RPE", page 25).

    Reuse the collection tube in step 15.

15. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    Note: DNase I is supplied lyophilized and should be reconstituted as described in "Preparing DNase I stock solution" (page 24).

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

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

17. Add 500 µl Buffer FRN to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Save the flow-through for use in step 18.

    IMPORTANT: Do not discard the flow-through, as it contains small RNAs.

    Note: Buffer FRN is supplied as a concentrate. Ensure that isopropanol is added before use as described in "Preparing Buffer FRN" (page 24).

* Flow-through contains Buffer RLT Plus and is therefore not compatible with bleach. See page 5 for safety information.

18. Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied). Apply the flow-through from step 17 to the spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.*

    Reuse the collection tube in step 19.

19. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*).

    Discard the flow-through.

    Reuse the collection tube in step 20.

20. Add 500 µl of 96–100% ethanol to the RNeasy Mini spin column. Close the lid gently and centrifuge for 2 min at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane.

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

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

21. **Optional**: Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied) and discard the old collection tube with the flow-through. Centrifuge at full speed for 2 min.

    Perform this step to eliminate any possible carryover of ethanol or to collect the residual flow-through that remains on the outside of the RNeasy Mini spin column after step 20.

22. Place the RNeasy Mini spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

* Flow-through contains Buffer RLT Plus and is therefore not compatible with bleach. See page 5 for safety information.

23. If the expected RNA yield is >30 µg, repeat step 22 using another 30–50 µl RNase-free water, or using the eluate from step 22 (if high RNA concentration is required). Reuse the collection tube from step 22.

    If using the eluate from step 22, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Genomic DNA purification

24. Add 350 µl Buffer AW1 to the AllPrep DNA Mini spin column from step 6. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane. Discard the flow-through.*

    Reuse the collection tube in step 25.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Preparing Buffer AW1", page 25).

25. Add 20 µl Proteinase K to 60 µl Buffer AW1, mix gently, and apply the mixture to the AllPrep DNA Mini spin column membrane.

    Note: Be sure to add the Proteinase K incubation mix directly to the membrane. Digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

26. Incubate for 5 min at room temperature.

27. Add 350 µl Buffer AW1 to the AllPrep DNA Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane. Discard the flow-through.*

    Reuse 7the collection tube in step 28.

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 5 for safety information.

28. Add 500 µl Buffer AW2 to the AllPrep DNA Mini spin column. Close the lid gently and centrifuge for 2 min at full speed to wash the spin column membrane. Discard the flow-through.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Preparing Buffer AW2", page 25).

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

    Note: After centrifugation, carefully remove the AllPrep DNA Mini spin column from the collection tube. If the column is in contact with the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

29. Place the AllPrep DNA Mini spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature for 1 min and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

30. Repeat step 29 to elute further DNA.

    To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 29.

    Note: To achieve a higher DNA concentration, elute with 2 x 50 µl Buffer EB. The final DNA yield, however, may be reduced.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA, including miRNA, from Cells

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal nucleic acid yield and purity. See Table 2 (page 16) for details. The minimum amount that can be processed is generally 100 cells, while the maximum amount depends on:

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

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

- HeLa cells have average RNA content (approximately 18 µg RNA per 10^6 cells). Do not use more than 6 x 10^6 cells; otherwise the RNA binding capacity of the RNeasy Mini spin column will be exceeded.

If processing a cell type not listed in Table 2 (page 16), and if there is no information about its RNA content, we recommend starting with no more than 3–4 x 10^6 cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

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

As a guide, Table 3 (page 17) shows the expected numbers of HeLa cells in different cell-culture vessels.

Important points before starting

- If using the AllPrep DNA/RNA/miRNA Universal Kit for the first time, read "Important Notes" (page 14).
- If preparing RNA for the first time, read Appendix A (page 57).
- Cell pellets can be stored at –70°C or in RNAprotect Cell Reagent, either for later use or for use directly in this procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2. Cells in RNAprotect Cell Reagent should be handled according to the *RNAprotect Cell Reagent Handbook* before starting the purification procedure.
- Homogenized cell lysates from step 3 can be stored at –70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25°C) for 1 month after addition of β-ME. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.

- Buffer RLT Plus, Buffer FRN 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. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- In the procedure below, ▲ indicates protocol procedures when processing 350 µl lysate and ■ indicates protocol procedures when processing 600 µl lysate.

Things to do before starting

- If using Buffer FRN, Buffer RPE, Buffer AW1, Buffer AW2, and RNase-Free DNase I for the first time, reconstitute and dilute them as described in "Preparation of buffers" (page 23).
- If necessary, warm and gently agitate Buffer RLT Plus and Buffer FRN to redissolve any precipitates that may have formed.
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer FRN, Buffer RPE, Buffer AW1 and Buffer AW2 by shaking.

Procedure

1. Harvest cells according to step 1a if cells are grown in suspension or according to step 1b if cells are grown in a monolayer. Do not use more than 1 x 10^7 cells.

   1a. For cells grown in suspension:
   - Determine the number of cells.
   - Pellet the appropriate number by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied).
   - Carefully remove all supernatant by aspiration.
   - Proceed to step 2.

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.

1b. For cells grown in a monolayer in cell-culture vessels:

- Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.
- To lyse cells directly: Determine the number of cells according to Table 3 (page 17). Completely aspirate the cell-culture medium, and proceed immediately to step 2b.

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.

- To trypsinize and collect cells: Determine the number of cells according to Table 3 (page 17). Aspirate the medium and wash the cells with PBS. Aspirate the PBS and add 0.10–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min. Completely aspirate the supernatant and proceed to step 2a.

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.

2. Disrupt the cells by adding Buffer RLT Plus according to step 2a for pelleted cells or step 2b for direct lysis of cells grown in a monolayer.

2a. For pelleted cells:

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

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced nucleic acid yields. Ensure that β-ME is added to Buffer RLT Plus before use (see "Important points before starting", page 38).

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

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

Note: Ensure that β-ME is added to Buffer RLT Plus before use (see "Important points before starting", page 38).

Table 5. Growth area HeLa cell number in various culture vessels

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

Table 6. Volume of Buffer RLT Plus for direct cell lysis Table 5

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

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

3. Homogenize the lysate according to step 3a if using a QIAshredder, step 3b if using a TissueRuptor II or other rotor–stator, step 3c if using a TissueLyser system, or step 3d if using a syringe and needle.

   See "Lysis and Homogenization", page 19, for more details on homogenization. If processing ≤1 x 10^5 cells, homogenize by vortexing for 1 min. After homogenization, proceed to step 4.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep DNA Mini and RNeasy Mini spin columns.

Homogenization with a TissueRuptor II or a rotor–stator, TissueLyser II, or QIAshredder homogenizer generally results in higher nucleic acid yields than with a syringe and needle.

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

3b. Homogenize the lysate for 30 s using a TissueRuptor II or other rotor–stator. Proceed to step 4.

3c. If using the TissueLyser II, transfer the lysate to a 2 ml microcentrifuge tube, add one stainless steel bead (5 mm diameter), and homogenize the lysate for 1 min at 20 Hz. Rotate the TissueLyser rack and homogenize for another 1 min at 20 Hz. Continue the protocol with step 4. If using the TissueLyser LT, place the tubes, including one stainless steel bead (5 mm diameter) in the insert of the TissueLyser LT Adapter and operate the TissueLyser LT for 1–2 min at 30–50 Hz. Continue the protocol with step 4.
For more information, refer to the *TissueLyser Handbook* or the *TissueLyser LT Handbook*.

Note: The instructions in step 3c are guidelines that may need to be changed depending on the bead mill being used.

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

4. **Optional**: Briefly centrifuge the tube to reduce foam.

5. Transfer the homogenized lysate to an AllPrep DNA Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 30 s at full speed (maximum 20,000 x *g*).

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

6. Place the AllPrep DNA Mini spin column in a new 2 ml collection tube (supplied) and store at room temperature (15–25°C) or at 2–8°C for DNA purification later in steps 23–29. Transfer the flow-through to a new 2 ml microcentrifuge tube (not supplied) for RNA purification (steps 7–22).

   Note: Do not store the DNA-containing AllPrep DNA Mini spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA (including miRNA) purification

7. Add ▲50 µl or ■ 80 µl Proteinase K to the flow-through from step 6, and mix by pipetting.
8. Add ▲200 µl or ■ 350 µl of 96–100% ethanol and mix well. Do not centrifuge.
9. Incubate for 10 min at room temperature.
10. Add ▲400 µl or ■ 750 µl of 96–100% ethanol and mix well. Do not centrifuge.
11. Transfer up to 700 µl of sample, including any precipitate that may have formed, to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 15 s at full speed (maximum 20,000 x *g*). Discard the flow-through.*

    Reuse the collection tube in step 12.
12. Repeat step 11 until the entire sample has passed through the RNeasy Mini spin column.

    Reuse the collection tube in step 13.
13. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Preparing Buffer RPE", page 25).

    Reuse the collection tube in step 14.

* Flow-through contains Buffer RLT Plus and is therefore not compatible with bleach. See page 5 for safety information.

14. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    Note: DNase I is supplied lyophilized and should be reconstituted as described in "Preparing DNase I stock solution" (page 24).

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

15. Add the DNase I incubation mix (80 µl) directly onto the RNeasy Mini spin column membrane and incubate on the benchtop (20–30°C) for 15 min.

16. Add 500 µl Buffer FRN to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Save the flow-through for use in step 17.

    IMPORTANT: Do not discard the flow-through, as it contains small RNAs.

    Note: Buffer FRN is supplied as a concentrate. Ensure that isopropanol is added before use as described in "Preparing Buffer FRN" (page 24).

17. Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied). Apply the flow-through from step 16 to the spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.*

    Reuse the collection tube in step 18.

18. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.

    Reuse the collection tube in step 19.

* Flow-through contains Buffer RLT Plus or Buffer FRN and is therefore not compatible with bleach. See page 5 for safety information.

19. Add 500 µl of 96–100% ethanol to the RNeasy Mini spin column. Close the lid gently and centrifuge for 2 min at full speed (maximum speed of 20000 x *g*) to wash the spin column membrane.

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

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

20. **Optional**: Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied) and discard the old collection tube with the flow-through. Centrifuge at full speed for 2 min.

    Perform this step to eliminate any possible carryover of ethanol or to collect residual flow-through that remains on the outside of the RNeasy Mini spin column after step 19.

21. Place the RNeasy Mini spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

22. If the expected RNA yield is >30 µg, repeat step 21 using another 30–50 µl RNase-free water, or using the eluate from step 21 (if high RNA concentration is required). Reuse the collection tube from step 21.

    If using the eluate from step 21, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Genomic DNA purification

23. Add 350 µl Buffer AW1 to the AllPrep DNA Mini spin column from step 6. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane. Discard the flow-through.*

    Reuse the collection tube in step 24.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see Preparing Buffer AW1", page 25).

24. Add 20 µl Proteinase K to 60 µl Buffer AW1, mix gently, and apply the mixture to the AllPrep DNA Mini spin column membrane.

    Note: Be sure to add the Proteinase K incubation mix directly to the membrane. Digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

25. Incubate for 5 min at room temperature.

26. Add 350 µl Buffer AW1 to the AllPrep DNA Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane. Discard the flow-through.*

    Reuse the collection tube in step 27.

27. Add 500 µl Buffer AW2 to the AllPrep DNA Mini spin column. Close the lid gently and centrifuge for 2 min at full speed to wash the spin column membrane.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Preparing Buffer AW2", page 25). The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.

    Note: After centrifugation, carefully remove the AllPrep DNA Mini spin column from the collection tube. If the column is in contact with the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 5 for safety information.

28. Place the AllPrep DNA Mini spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature for 1 min then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

29. Repeat step 28 to further elute DNA.

    To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 28.

    Note: To achieve a higher DNA concentration, elute with 2 x 50 µl Buffer EB. The final DNA yield, however, may be reduced.

Protocol: Simultaneous Purification of Cellular Genomic DNA and Total RNA, including miRNA, from Human Whole Blood

Determining the correct amount of starting material

A maximum amount of 1.5 ml of whole blood from healthy adults (typically 4000–7000 leukocytes per microliter) can be processed. For blood with elevated numbers of leukocytes, less than 1.5 ml must be used. The maximum number of leukocytes that can be processed is 1×10^7 per spin column. If more leukocytes are processed, they will not be fully lysed and contaminants will not be completely removed, even if the volume of Buffer RLT Plus is increased.

Important points before starting

- If using the AllPrep DNA/RNA/miRNA Universal Kit for the first time, read "Important Notes" (page 14).
- If preparing RNA for the first time, read Appendix A (page 57).
- Homogenized cell lysates in Buffer RLT Plus 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.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25°C) for 1 month after addition of β-ME. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- If using Ficoll density-gradient centrifugation for erythrocyte removal, start with step 6 for nucleic acid purification.

- Buffer RLT Plus, Buffer FRN and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Unless otherwise indicated, perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Unless otherwise indicated, perform all centrifugation steps at 20–25°C in a standard microcentrifuge. For blood samples >250 µl use a refrigerated laboratory centrifuge with rotor for 12 ml or 15 ml centrifuge tubes when performing erythrocyte lysis.
- In the procedure below, ▲ indicates protocol procedures when processing 350 µl lysate and ■ indicates protocol procedures when processing 600 µl lysate.

Things to do before starting

- If using Buffer FRN, Buffer RPE, Buffer AW1, Buffer AW2 and RNase-Free DNase I for the first time, reconstitute and dilute them as described in "Preparation of buffers" (page 23).
- If necessary, warm and gently agitate Buffer RLT Plus and Buffer FRN to redissolve any precipitates that may have formed.
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer FRN, Buffer RPE, Buffer AW1 and Buffer AW2 by shaking.

Procedure

Erythrocyte lysis

1. Mix 1 volume of human whole blood with 5 volumes of Buffer EL (not provided; see ordering information, page 72) in an appropriately sized tube (not provided).

   For optimal results, the volume of the mixture (blood + Buffer EL) should not exceed ¾ of the volume of the tube to allow efficient mixing. For example, add 5 ml of Buffer EL to 1 ml of whole blood, and mix in a tube which has a total volume of ≥8 ml.

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

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

3. Centrifuge at 400 x *g* for 10 min at 4°C. Completely remove and discard supernatant.

   Leukocytes will form a pellet after centrifugation. Ensure that the supernatant is completely removed. Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in the following wash step. See page 56 if larger amounts of erythrocytes remain.

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

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

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

   Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of nucleic acids to the spin columns, resulting in lower yield.

Nucleic acid purification

6. Add Buffer RLT Plus to pelleted leukocytes/cellular components according to Table 7. Vortex or pipet to mix.

   Buffer RLT disrupts the cells. No cell clumps should be visible before you proceed to the homogenization step. Vortex or pipet further to remove any clumps.

   Note: If using Ficoll density-gradient centrifugation for erythrocyte removal, start the nucleic acid purification procedure at this step.

Table 7. Volume of Buffer RLT Plus

Buffer RLT Plus (µl)*	Healthy whole blood (ml)	No. of leukocytes
350	Up to 0.5	Up to 2 x 10^6
600	0.5 to 1.5	2 x 10^6–1 x 10^7

* Ensure β-ME is added to Buffer RLT Plus (see "Important points before starting", page 48).

7. Homogenize the lysate according to step 7a if using a QIAshredder column, step 7b if using a TissueRuptor II or other rotor-stator, step 7c if using a TissueLyser instrument, or step 7d if using a needle and syringe.

   See "Lysis and Homogenization", page 19, for more details on homogenization.

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

   7a. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube and centrifuge for 2 min at maximum speed. Proceed to step 8.

   7b. Homogenize the lysate for 30 s using the TissueRuptor II or other rotor–stator. Proceed to step 8.

   7c. If using the TissueLyser II, transfer the lysate to a 2 ml microcentrifuge tube, add one stainless steel bead (5 mm diameter), and homogenize the lysate for 1 min at 20 Hz. Rotate the TissueLyser rack, and homogenize for another 1 min at 20 Hz. Continue the protocol with step 8. If using the TissueLyser LT, place the tubes in the insert of the TissueLyser LT Adapter and operate the TissueLyser LT for 1–2 min at 30–50 Hz. Continue the protocol with step 8.
   For detailed information, refer to the *TissueLyser Handbook* or the *TissueLyser LT Handbook*.

   Note: The instructions in step 7c are only guidelines. They may need to be changed depending on the bead mill being used.

   7d. Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 8.

8. **Optional**: Briefly centrifuge the tube to reduce foam.
9. Transfer the homogenized lysate to an AllPrep DNA Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 30 s at full speed (maximum speed of 20,000 x *g*).

   Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.
10. Place the AllPrep DNA Mini spin column in a new 2 ml collection tube (supplied) and store at room temperature (15–25°C) or at 2–8°C for later DNA purification in steps 27–33. Transfer the flow-through to a new 2 ml microcentrifuge tube (not supplied) for RNA purification in steps 11–26.

    Note: Do not store the DNA-containing AllPrep DNA Mini spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA (including miRNA) purification

11. Add ▲50 µl or ■ 80 µl Proteinase K to the flow-through from step 10 and mix by pipetting.
12. Add ▲200 µl or ■ 350 µl of 96–100% ethanol and mix well. Do not centrifuge.
13. Incubate for 10 min at room temperature.
14. Add ▲400 µl or ■ 750 µl of 96–100% ethanol and mix well. Do not centrifuge.
15. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.*

    Reuse the collection tube in step 16.
16. Repeat step 15 until the entire sample has passed through the RNeasy Mini spin column.

    Reuse the collection tube in step 17.

* Flow-through contains Buffer RLT Plus and is therefore not compatible with bleach. See page 5 for safety information.

17. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Preparing Buffer RPE", page 25).

    Reuse the collection tube in step 18.

18. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    Note: DNase I is supplied lyophilized and should be reconstituted as described in "Preparing DNase I stock solution" (page 24).

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

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

20. Add 500 µl Buffer FRN to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Save the flow-through for use in step 21.

    IMPORTANT: Do not discard the flow-through, as it contains small RNAs.

    Note: Buffer FRN is supplied as a concentrate. Ensure that isopropanol is added before use as described in "Preparing Buffer FRN" (page 24).

21. Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied). Apply the flow-through from step 20 to the spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*). Discard the flow-through.*

    Reuse the collection tube in step 22.

* Flow-through contains Buffer FRN and is therefore not compatible with bleach. See page 5 for safety information.

22. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane. Discard the flow-through.

    Reuse the collection tube in step 23.

23. Add 500 µl of 96–100% ethanol to the RNeasy Mini spin column. Close the lid gently and centrifuge for 2 min at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane.

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

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

24. **Optional**: Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied) and discard the old collection tube with the flow-through. Centrifuge at full speed for 2 min.

    Perform this step to eliminate any possible carryover of 96–100% ethanol, or to collect residual flow-through that remains on the outside of the RNeasy Mini spin column after step 23.

25. Place the RNeasy Mini spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

26. If the expected RNA yield is >30 µg, repeat step 25 using another 30–50 µl of RNase-free water, or using the eluate from step 25 (if high RNA concentration is required). Reuse the collection tube from step 25.

    If using the eluate from step 25, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

Genomic DNA purification

27. Add 350 µl Buffer AW1 to the AllPrep DNA Mini spin column from step 10. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20,000 x *g*) to wash the spin column membrane. Discard the flow-through.*

    Reuse the collection tube in step 28.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Preparing Buffer AW1", page 25).

28. Add 20 µl Proteinase K to 60 µl Buffer AW1, mix gently, and apply the mixture to the AllPrep DNA Mini spin column membrane.

    Note: Be sure to add the Proteinase K incubation mix directly to the membrane. Digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

29. Incubate for 5 min at room temperature.

30. Add 350 µl Buffer AW1 to the AllPrep DNA Mini spin column. Close the lid gently and centrifuge for 15 s at full speed (maximum speed of 20000 x *g*) to wash the spin column membrane. Discard the flow-through.*

    Reuse the spin column in step 31.

31. Add 500 µl Buffer AW2 to the AllPrep DNA Mini spin column. Close the lid gently and centrifuge for 2 min at full speed to wash the spin column membrane.

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Preparing Buffer AW2", page 25).

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

    Note: After centrifugation, carefully remove the AllPrep DNA Mini spin column from the collection tube. If the column contacts the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 5 for safety information.

32. Place the AllPrep DNA Mini spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

33. Repeat step 32 to elute further DNA.

    To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 32.

    Note: To achieve a higher DNA concentration, elute with 2 x 50 µl Buffer EB. The final DNA yield, however, may be reduced.

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 AllPrep DNA Mini or RNeasy Mini spin column	
a) Inefficient disruption and/or homogenization	See "Lysis and Homogenization" (page 19) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material 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 "Important Notes", page 14).
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 spin column. If this happens, set the centrifugation temperature to 25°C. Warm the lysate to 37°C before transferring it to the AllPrep DNA Mini spin column.
Incomplete erythrocyte lysis	
a) The cloudy suspension does not become translucent in step 2	Extend incubation on ice to 20 min.

	Comments and suggestions
b) The pellet in step 3 is red	The leukocyte pellet should be white, and may contain residual traces of erythrocytes. However, if erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5–10 min on ice after the addition of Buffer EL at step 4 of the erythrocyte lysis protocol (page 50).
Low nucleic acid yield	
a) Insufficient disruption and homogenization	See "Lysis and Homogenization" (page 19) for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material and/or increase the volume of lysis buffer and the homogenization time.
b) Too much starting material	Overloading the spin columns significantly reduces nucleic acid yields. Reduce the amount of starting material (see "Important Notes", page 14).
c) RNA still bound to RNeasy Mini spin column membrane	Repeat RNA elution, but incubate the RNeasy Mini spin column on the benchtop for 10 min with RNase-free water before centrifuging.
d) DNA still bound to AllPrep DNA Mini spin column membrane	Repeat DNA elution, but incubate the AllPrep DNA Mini spin column on the benchtop for 10 min with Buffer EB before centrifuging. Alternatively, heat Buffer EB to 70°C prior to DNA elution.
e) Ethanol carryover	During the wash with 96–100% ethanol, be sure to centrifuge at full speed (max. 20,000 x *g*) for 2 min at 20–25°C to dry the RNeasy Mini spin column membrane. Perform the optional centrifugation to dry the RNeasy Mini spin column membrane if any flow-through is present on the outside of the column.
f) Incomplete removal of cell-culture medium (cell samples)	When processing cultured cells, ensure complete removal of cell-culture medium after harvesting cells (see protocol on page 37).

	Comments and suggestions
DNA contaminated with RNA	
a) Lysate applied to the AllPrep DNA Mini spin column contains ethanol	Only add ethanol to the lysate after passing the lysate through the AllPrep DNA Mini spin column.
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.
Contamination of RNA with DNA affects downstream applications	
a) Too much sample material	For some samples, the efficiency of DNA binding to the AllPrep DNA Mini spin column may be reduced when processing very high amounts. If the eluted RNA contains substantial DNA contamination, try processing smaller amounts.
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 DNA Mini spin column will not bind DNA effectively if the lysis buffer is diluted.
c) Tissue has high DNA content	For certain tissues with extremely high DNA content (e.g., thymus), some DNA will pass through the AllPrep DNA Mini spin column. Try using smaller samples.
Low A_{260}/A_{280} value	
Water used to dilute nucleic acid 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 65).

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

	Comments and suggestions
RNA degraded	
a) Inappropriate handling of starting material	For stabilized tissues, ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent or Allprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the AllPrep DNA/RNA/miRNA Universal Kit procedure quickly, especially the first few steps. See Appendix A (page 57) and "Handling and storing starting material" (page 17).
b) RNase contamination	Although all AllPrep buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 57) for general remarks on handling RNA.
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).
Nucleic acid concentration too low	
Elution volume too high	Elute nucleic acids in a smaller volume. Do not use less than 50 µl Buffer EB for the AllPrep DNA Mini spin column or less than 1 x 30 µl of water for the RNeasy Mini spin column. Although eluting in smaller volumes results in increased nucleic acid concentrations, yields may be reduced.

	Comments and suggestions
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. When reusing collection tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels.
b) Ethanol carryover	During the wash with ethanol 96–100%, be sure to centrifuge at full speed (max. 20,000 x *g*) for 2 min at 20–25°C to dry the RNeasy Mini spin column membranes. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. Perform the optional centrifugation to dry the RNeasy Mini spin column membrane if any flow-through is present on the outside of the column.
c) Heparin used as anticoagulant for whole blood	Residual heparin can interfere with RT-PCR. Use EDTA or citrate as anticoagulant for blood collection.

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 of RNaseKiller (cat. no 2500080) from 5 PRIME (**www.5prime.com**) is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 63), or rinse with chloroform* if the plasticware is chloroform-resistant. To

* 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.

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

Disposable plasticware

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

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), 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

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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.

Note: AllPrep 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 –30°C to –15°C or –90°C to –65°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA can 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 not be possible to accurately determine amounts photometrically. Small amounts of RNA can be quantified using the QIAxcel® Advanced system (**www.qiagen.com/products/qiaxceladvanced.aspx**) or Agilent® 2100 Bioanalyzer, quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260}=1 → 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 66), 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

* 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.

"Solutions", page 63). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl
Dilution = 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,† pH 7.0 (1/50 dilution)
Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)
A_{260} = 0.2

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

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring 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 65).

* 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.

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While the AllPrep DNA/RNA/miRNA Universal procedure will remove the vast majority of cellular DNA from the RNA fraction, trace amounts may still remain, depending on the amount and nature of the sample.

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

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiNova® Primer Assays from QIAGEN are designed for 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, the QuantiNova Reverse Transcription Kit (cat. no. 205311) provides fast cDNA synthesis with integrated removal of genomic DNA contamination.

Integrity of RNA

The integrity and size distribution of total RNA purified with AllPrep DNA/RNA/miRNA Universal Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel Advanced system or Agilent 2100 Bioanalyzer. The respective 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.

* 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.

The Agilent 2100 Bioanalyzer also provides an RNA Integrity Number (RIN) as a useful measure of RNA integrity. Ideally, the RIN should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

Appendix C: 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 –30°C to –15°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 safety data sheets (SDSs), 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. 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.

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

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

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

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 safety data sheets (SDSs), available from the product supplier.

Ordering Information

Product	Contents	Cat. no.
AllPrep DNA/RNA/miRNA Universal Kit (50)	For 50 preps: 50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes and Buffers	80224
Related products		
Stabilization		
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
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
Disruption and homogenization		
TissueRuptor II	Handheld rotor-stator homogenizer, 5 TissueRuptor Disposable Probes	Inquire
TissueLyser LT	Compact bead mill; requires the TissueLyser LT Adapter, 12-Tube (available separately)	Inquire
TissueLyser II	Bead mill; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)	Inquire

Product	Contents	Cat. no.
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79654
Reagent DX (1ml)	1 ml Antifoaming Reagent for QIAGEN Lysis Buffers	19088
Erythrocyte lysis		
Buffer EL	1000 ml Erythrocyte Lysis Buffer	79217
QuantiTect Reverse Transcription — for fast cDNA synthesis enabling sensitive real-time two-step RT-PCR for gene expression analysis cDNA		
QuantiTect Rev. Transcription Kit (50)	For 50 x 20 µl reactions: 100 µl 7x gDNA Wipeout Buffer, 50 µl Quantiscript Reverse Transcriptase, 200 µl 5x Quantiscript RT Buffer, 50 µl RT Primer Mix, 1.9 ml RNase-Free Water	205311
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395

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

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Document Revision History

Date	Changes
January 2020	Updated text, ordering information and intended use for QIAcube Connect.
November 2020	Updated branding of RNA protection products and information about DTT.

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