November 2020

AllPrep® DNA/RNA/Protein Mini Handbook

For simultaneous purification of genomic DNA, total RNA and total protein from the same cell or tissue sample

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep DNA/RNA/Protein Mini Kit	**(50)**
Catalog no.	**80004**
Number of preps	**50**
AllPrep DNA Mini Spin Columns (uncolored) (each in a 2 ml Collection Tube)	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*	45 ml
Buffer RW1*	45 ml
Buffer RPE† (concentrate)	11 ml
RNase-Free Water	10 ml
Buffer AW1*† (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer EB	22 ml
Buffer APP	55 ml
Buffer ALO‡	10 ml
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for safety information.

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

‡ DTT must be added before use. See "Things to do before starting" in the protocols.

Storage

The AllPrep DNA/RNA/Protein Mini Kit should be stored dry at room temperature (15–25°C) and is stable for at least 6 months under these conditions. All kit components are stable for at least 6 months under these conditions, if not otherwise stated on the label.

Intended Use

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

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

Safety Information

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

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

Buffer AW1 contains guanidine hydrochloride, Buffer RLT contains guanidine thiocyanate, and Buffer RW1 contains a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The AllPrep DNA/RNA/Protein Mini Kit is designed to purify genomic DNA, total RNA and total protein simultaneously from a single biological sample. In contrast to other procedures, the kit allows maximal recovery of DNA, RNA and protein. There is no need to divide the sample into 3 before purifying DNA, RNA and protein separately. There is also no need to purify total nucleic acids first and then to divide the purified nucleic acids into 2 before purifying DNA and RNA separately. The kit is compatible with small amounts of a wide range of cultured cells and harvested tissues of animal and human origin.

The AllPrep DNA/RNA/Protein Mini Kit allows the parallel processing of multiple samples. Methods involving the use of toxic substances, such as phenol and/or chloroform, or time-consuming and tedious methods, such as alcohol precipitation, are replaced by the AllPrep DNA/RNA/Protein procedure. Genomic DNA purified with the AllPrep DNA/RNA/Protein procedure has an average length of 15–30 kb, depending on homogenization conditions.* DNA of this length is particularly suitable for PCR, where complete denaturation of the template is important to achieve the highest amplification efficiency. The purified DNA is ready to use in any downstream application, including:

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

With the AllPrep DNA/RNA/Protein procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure provides an enrichment for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together comprise 15–

* For purification of high-molecular-weight DNA, we recommend using either QIAGEN Genomic-tips or Blood & Cell Culture DNA Kits. Both allow purification of DNA of up to 150 kb in size. See page 68 for ordering information.

20% of total RNA) are selectively excluded. The purified RNA is ready to use in any downstream application, including:

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

Proteins recovered with the AllPrep DNA/RNA/Protein procedure are suitable for downstream applications, such as:

- 1D and 2D gel electrophoresis
- Western blotting

Principle and procedure

The AllPrep DNA/RNA/Protein procedure integrates QIAGEN's patented technology for selective binding of double-stranded DNA with well-established RNeasy technology and combines this with a new protein precipitation chemistry. Efficient purification of high-quality DNA, RNA and proteins is guaranteed, without the need for additional RNase and DNase digestions.†

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

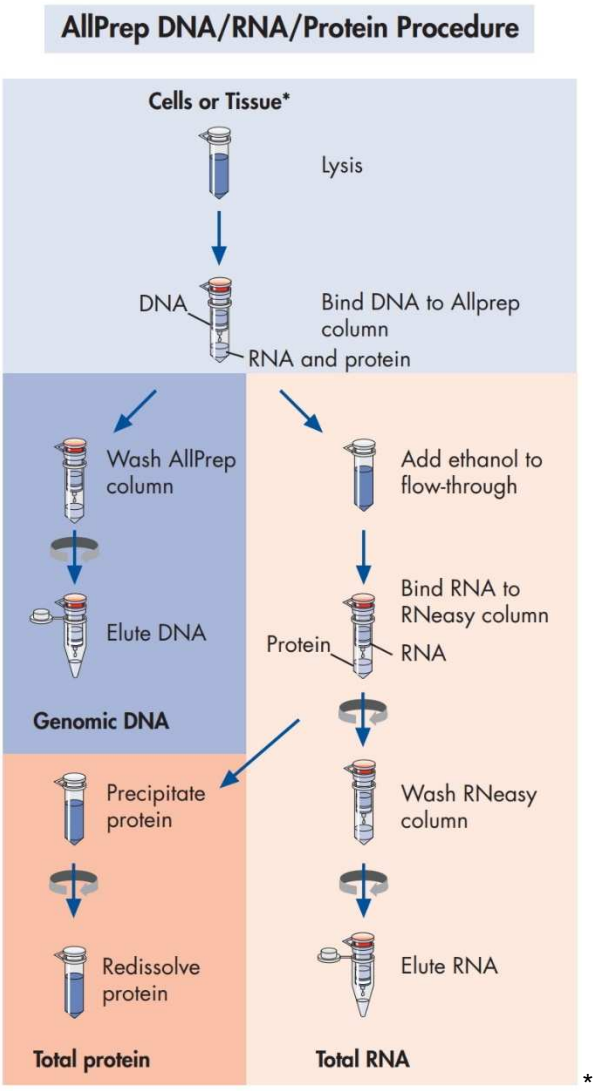
† Samples with particularly high DNA content may require additional DNase digestion.

Biological samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate–containing buffer, which immediately inactivates DNases and RNases as well as proteases to ensure isolation of intact DNA, RNA and proteins. The lysate is then passed through an AllPrep DNA spin column. This column, in combination with the high-salt buffer, allows selective and efficient binding of genomic DNA. The column is washed and pure, ready-to-use DNA is then eluted.

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

Buffer APP, a novel aqueous protein precipitation solution, is added to the flow-through of the RNeasy spin column, and the precipitated proteins are pelleted by centrifugation. Intact total proteins are redissolved in an appropriate buffer and then ready to use in downstream applications. The kit includes Buffer ALO, which is compatible with SDSPAGE, for dissolving the protein pellet.

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



* We recommend stabilizing harvested tissues immediately in Allprotect Tissue Reagent to protect DNA, RNA and proteins (see page 14).

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)
- Dithiothreitol (DTT)
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- 96–100% ethanol*
- 70% ethanol* in water
- Disposable gloves
- For tissue samples: Allprotect Tissue Reagent (see ordering information, page 62) or liquid nitrogen
- Equipment for sample disruption and homogenization (see "Disrupting and homogenizing starting material", page 15). Depending on the method chosen, one or more of the following are required:
  - Trypsin and PBS
  - QIAshredder homogenizer (see ordering information, page 62)
  - Blunt-ended needle and syringe
  - Mortar and pestle
  - TissueLyser II (see ordering information, page 62)
  - TissueRuptor® II (see ordering information, page 62)
- **Optional**: 5% (w/v) sodium dodecyl sulfate (SDS) or 8 M urea (for details, see the protocols)

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

Important Notes

Determining the amount of starting material

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

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

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

When processing samples containing average or low amounts of DNA and RNA, the maximum amount of starting material shown in Table 1 can be used. However, even though the binding capacity of the spin columns are 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 shows expected DNA and RNA yields from various cells and tissues.

Note: Although the AllPrep DNA spin column can bind a maximum of 100 µg DNA, the use of starting materials containing more than 20 µg DNA may lead to the purification of RNA containing small amounts of DNA. If the binding capacity of the RNeasy spin column is exceeded, RNA yields will not be consistent and less than expected. 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. Specifications of the spin columns in the AllPrep DNA/RNA/Protein Mini Kit

Specification	AllPrep DNA spin-column	RNeasy spin column
Maximum binding capacity	100 µg DNA*	100 µg RNA
Maximum loading volume	700 µ	700 µl
Nucleic acid size distribution	DNA of 15–30 kb†	RNA >200 nucleotides
Minimum elution volume	100 µl	30 µl
Maximum amount of starting material:		
Animal and human cells	1 x 10^7 cells	Entire flow-through from AllPrep DNA spin column
Animal and human tissues	30 mg‡	Entire flow-through from AllPrep DNA spin column

* Loading more than 20 µg DNA may lead to DNA contamination of the RNA eluate.
† Depending on homogenization conditions.
‡ If using Allprotect stabilized tissues, 15–20 mg tissue should be used.

Table 2. Typical yields of genomic DNA, total RNA and total protein with the AllPrep DNA/RNA/Protein Mini Kit

	Typical yield (µg) of		
Sample type	**Genomic DNA§ n**	**Total RNA¶**	**Total protein**
Cell cultures (1 x 10^6 cells)			
NIH/3T3	8	10	50
HeLa, Jurkat	6	15	60
COS-7	7	35	150
Mouse/rat tissues (10 mg)			
Kidney	15–25	20–30	1300
Liver	15–25	40–60	1000
Spleen	50–70	30–80	1000
Thymus	50–100	40–80	800
Lung	15–20	10–20	800

§ Amounts can vary, depending on the disruption and homogenization method (see page 18).

¶ Amounts can vary due to factors, such as species, developmental stage and growth conditions. Since the AllPrep DNA/RNA/Protein procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storing starting material

RNA and protein in harvested tissue are not protected until the sample is treated with 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 at both the mRNA and protein levels will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C or immediately immersed in Allprotect Tissue Reagent.*

The procedures for tissue harvesting and RNA and protein protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or

* RNAprotect Tissue Reagent, which stabilizes RNA only, can be used instead if proteins will not be purified.

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

Disrupting and homogenizing starting material

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

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

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 3 gives an overview of various disruption and homogenization methods and is followed by a detailed description of each method.

Table 3. Disruption and homogenization methods

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

* Simultaneously disrupts and homogenizes individual samples.

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

Disruption and homogenization using the TissueRuptor System

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

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

Note: Rotor–stator homogenization using a metal probe must be avoided, as the probe can warm the homogenate, resulting in protein degradation. To preserve intact proteins, rotor–stator homogenization should be carried out with the TissueRuptor II, which uses a plastic disposable probe.

Disruption and homogenization using the TissueLyser System

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. 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 mean diameter. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.

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

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.

Disruption using a mortar and pestle

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

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

Homogenization using QIAshredder homogenizers

Using QIAshredder homogenizers is a fast and efficient way to homogenize 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 spun for 2 minutes at maximum

speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column. QIAshredder homogenizers typically result in less DNA fragmentation compared with rotor–stator homogenizers.

Homogenization using a syringe and needle

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.

Effect of homogenization on DNA yield and integrity

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

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

Protocol: Simultaneous Purification of Genomic DNA, Total RNA and Total Protein from Animal and Human 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. The minimum amount is generally 100 cells, while the maximum amount depends on:

- The RNA content of the cell type
- The DNA binding capacity of the AllPrep DNA spin column
- The RNA binding capacity of the RNeasy spin column (100 µg RNA)
- The volume of Buffer RLT required for efficient lysis (the maximum volume of Buffer RLT 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 examples illustrate how to determine the maximum amount of starting material:

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

If processing a cell type not listed in Table 2 (page 14) 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.

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

Counting cells is the most accurate way to quantitate the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is given in Table 4.

Table 4. Growth area and number of HeLa cells in various culture vessels

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

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

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Cell numbers will vary for different kinds of animal and human cells, which vary in length from 10 to 30 µm.

Important points before starting

- If using the AllPrep DNA/RNA/Protein Mini Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 46).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- Cell pellets can be stored at –70°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2. Homogenized cell lysates from step 3 can be stored at –70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.
- Buffer RLT, Buffer RW1 and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C. Things to do before starting.
- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.
- Dithiothreitol (DTT) must be added to Buffer ALO before use. Add 8 mg DTT per 1 ml Buffer ALO.

- Buffer RPE, Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. Harvest cells according to step 1a or 1b.
   1a. Cells grown in suspension (do not use more than 1 x 10^7 cells): Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and proceed to step 2.
   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.
   1b. Cells grown in a monolayer (do not use more than 1 x 10^7 cells): Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.
   To lyse cells directly: Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.
   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.
   To trypsinize and collect cells:

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

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

2. Disrupt the cells by adding Buffer RLT.

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

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

Table 5. Volumes of Buffer RLT for lysing pelleted cells

Number of pelleted cells	Volume of Buffer RLT
$<5 \times 10^6$	350 µl
$5 \times 10^6 – 1 \times 10^7$	600 µl

   For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RLT (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 before use (see "Things to do before starting").

Table 6. Volumes of Buffer RLT for lysing pelleted cells

Dish diameter	Volume of Buffer RLT*
<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.Homogenize the lysate according to step 3a, 3b or 3c. See "Disrupting and homogenizing starting material", page 15, for more details on homogenization. If processing ≤1 x 10^5 cells, they can be homogenized by vortexing for 1 min. After homogenization, proceed to step 4.

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

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

2b. Place the tip of the TissueRuptor II disposable probe into the lysate and operate the TissueRuptor II at full speed until the lysate is homogenous (usually 30 s). Proceed to step 4.

Note: To avoid damage to the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

2c. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.

3. Transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at 8000 x *g* (10,000 rpm).

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.

4. Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 21–24. Use the flow-through for RNA purification in steps 6–13.

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

Total RNA purification

5. To the flow-through from step 5, add 96–100% ethanol: either 250 µl (if 350 µl Buffer RLT was used) or 400 µl (if 600 µl Buffer RLT was used). Mix well by pipetting. Do not centrifuge. Proceed immediately to step 7. If some lysate was lost during homogenization and DNA binding to the AllPrep DNA spin column, adjust the volume of ethanol accordingly.

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

6. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm). Transfer the flow-through* to a 2 ml tube (supplied) for protein purification in steps 14–20. Reuse the collection tube in step 8. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Transfer the flow-through after each centrifugation to the 2 ml tube.

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

   Reuse the collection tube in step 9.

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

* Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 8 for safety info

8. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

   Reuse the collection tube in step 10.

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

9. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at 8000 x *g* (10,000 rpm) to wash the spin column membrane.

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

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

10. **Optional**: Place the RNeasy 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 1 min.

    Perform this step to eliminate any possible carryover of Buffer RPE or if residual flow-through remains on the outside of the RNeasy spin column after step 10.

11. Place the RNeasy 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.

12. If the expected RNA yield is >30 µg, repeat step 12 using another 30–50 µl of RNase-free water or using the eluate from step 12 (if high RNA concentration is required). Reuse the collection tube from step 12.

    If using the eluate from step 12, 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.

Total protein precipitation

13. Add 1 volume (usually 600 or 1000 µl) of Buffer APP to the flow-through from step 7. Mix vigorously and incubate at room temperature for 10 min to precipitate protein.
14. Centrifuge at full speed for 10 min, and carefully decant the supernatant.*
15. Add 500 µl of 70% ethanol to the protein pellet. Centrifuge at full speed for 1 min, and remove the supernatant by using a pipet or by decanting as much liquid as possible.

    It is not necessary to resuspend or incubate the pellet.
16. Dry the protein pellet for 5–10 min at room temperature.

    Note: Incomplete drying may cause problems when loading the protein onto a gel due to residual ethanol.
17. Add up to 100 µl Buffer ALO and mix vigorously to dissolve the protein pellet.

    Note: Ensure that DTT is added to Buffer ALO before use (see "Things to do before starting")

    The volume of Buffer ALO to add depends on the amount of starting material. See Table 2 (page 14) for typical protein yields from various starting materials.

    Buffer ALO is a Laemmli-related sample buffer for use in SDS-PAGE and contains bromophenol blue dye. If the proteins will not be analyzed by SDS-PAGE, dissolve the pellet in a buffer compatible with the intended downstream application.

    Due to the strong denaturing conditions with Buffer RLT, which is necessary to inactivate RNases and proteases, the precipitated proteins may show reduced solubility. Vortex for several minutes or disaggregate the pellet by pipetting up and down several times.

    Depending on the sample type, the pellet may contain proteins or other cellular components that are not soluble. For details on how to solubilize these samples, see "Troubleshooting Guide" on page 42.

* Supernatant contains Buffer RLT and is therefore not compatible with bleach. See page 8 for safety information

For easier dissolving or if the proteins need to be quantified prior to SDS PAGE, dissolve the pellet in 5% (w/v) SDS or 8 M urea. For details about protein quantification, see Appendix F (page 61).

Buffer ALO may turn yellow upon dissolving protein, but this has no effect on downstream applications. The buffer will turn blue again in SDS-PAGE sample buffer. If using a stock solution of your own sample buffer (e.g., 5x concentration), be sure to dilute it accordingly so that the protein sample to be loaded on a gel has a 1x concentration of sample buffer.

18. Incubate for 5 min at 95°C to completely dissolve and denature the protein. Then cool the sample to room temperature.

19. Centrifuge for 1 min at full speed to pellet any residual insoluble material. Use the supernatant in downstream applications, such as SDS-PAGE and western blotting.

    The dissolved protein can be stored at –20°C for several months or at 4°C for several days.

Genomic DNA purification

20. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 5. Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through.*

    Reuse the spin column in step 22.

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

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 8 for safety information

21. Add 500 µl Buffer AW2 to the AllPrep DNA 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 "Things to do before starting", page 32). 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 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.

22. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at 8000 x *g* (10,000 rpm) to elute the DNA.

23. Repeat step 23 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 23.

    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, Total RNA and Total Protein from Animal and Human 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 15–20 mg Allprotect stabilized tissue* can generally be processed. For most tissues, the DNA binding capacity of the AllPrep DNA spin column, the RNA binding capacity of the RNeasy spin column, and the lysing capacity of Buffer RLT will not be exceeded by these amounts. However, smaller amounts may allow more efficient separation of DNA and RNA. Average DNA and RNA yields from various tissues are given in Table 2 (page 13).

Some tissues, such as spleen and thymus, contain very high amounts of DNA, which will overload the AllPrep DNA spin column (unless less than 5 mg tissue is used as starting material). For these tissues, we recommend performing DNase digestion on the RNeasy spin column membrane if the eluted RNA will be used in downstream applications sensitive to very small amounts of DNA (for further details, see Appendix E, page 58).

RNA yields from skeletal muscle, heart and skin tissue may be low due to the abundance of contractile proteins, connective tissue and collagen. For purification of genomic DNA and total RNA from these tissues, we recommend using the DNeasy® Blood & Tissue Kit and the RNeasy Fibrous Tissue Mini Kit, respectively (see Ordering Information, starting on page 62).

* RNAprotect Tissue Reagent, which stabilizes RNA only, can be used instead of Allprotect Tissue Reagent if protein will not be purified.

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

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

Weighing tissue is the most accurate way to quantitate the amount of starting material. As a guide, a 3 mm cube (27 mm^3) of most animal tissues weighs 30–35 mg.

Important points before starting

- If using the AllPrep DNA/RNA/Protein Mini Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 46).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- If using the TissueLyser II, ensure that you are familiar with operating it by referring to the operating instructions and *TissueLyser Handbook*.
- For optimal results, stabilize harvested tissues immediately in Allprotect Tissue Reagent (see the *Allprotect Tissue Reagent Handbook*). Tissues can be stored in the reagent for up to 1 day at 37°C, 7 days at 15–25°C or 6 months at 2–8°C or archived at –20°C or –80°C.

- Fresh, frozen or Allprotect stabilized tissues can be used. Tissues can be stored at –70°C for several months. Flash-freeze tissues in liquid nitrogen, and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized tissue lysates from step 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 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.
- Buffer RLT, Buffer RW1 and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.
- Dithiothreitol (DTT) must be added to Buffer ALO before use. Add 8 mg DTT per 1 ml Buffer ALO.
- Buffer RPE, Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. If using Allprotect Tissue Reagent, follow the protocols for tissue stabilization and for tissue disruption and homogenization in the *Allprotect Tissue Reagent Handbook* before proceeding to step 4 of this protocol. Otherwise, start at step 2 below.
2. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 30 mg. Proceed immediately to step 3. Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used. RNA and proteins in harvested tissues are not protected until the tissues are treated with 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 tissues can be placed into Allprotect Tissue Reagent to stabilize DNA, RNA and proteins (see the *Allprotect Tissue Handbook*). However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA and protein degradation.

3. Disrupt the tissue and homogenize the lysate in Buffer RLT (do not use more than 30 mg tissue) according to step 3a, 3b, 3c or 3d.

   See "Disrupting and homogenizing starting material", page 15, for more details on disruption and homogenization.

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

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

Table 7. Volumes of Buffer RLT for tissue disruption and homogenization

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

* Use 600 µl Buffer RLT for stabilized tissues or for difficult-to-lyse tissues.

3a. Disruption and homogenization using the TissueRuptor System:

- Place the tissue in a suitably sized vessel. Add the appropriate volume of Buffer RLT (see Table 7).

  Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization. Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.
- Place the tip of the disposable probe into the vessel and operate the TissueRuptor II at full speed until the lysate is homogeneous (usually 30 s). Proceed to step 4.

  Note: To avoid damage to the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

3b. Disruption and homogenization using the TissueLyser System:

- Place the tissues in 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter).
  If handling fresh or frozen tissue samples, keep the tubes on dry ice.
- Place the tubes at room temperature. Immediately add the appropriate volume of Buffer RLT (see Table 7) per tube.
- Place the tubes in the TissueLyser Adapter Set 2 x 24.
- Operate the TissueLyser II for 2 min at 20 Hz.
  The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.
- Rearrange the collection tubes so that the outermost tubes are innermost and the innermost tubes are outermost. Operate the TissueLyser II for another 2 min at 20 Hz.
  Rearranging the tubes allows even homogenization.
- Proceed to step 4.
  Do not reuse the stainless steel beads.

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

- Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle.
- Decant tissue powder and liquid nitrogen into an RNase-free, liquid nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.
- Add the appropriate volume of Buffer RLT (see Table 7).
- Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Proceed to step 4.

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

- Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle.
- Decant tissue powder and liquid nitrogen into an RNase-free, liquid nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.
- Add the appropriate volume of Buffer RLT (see Table 7), and homogenize by passing the lysate at least 5 times through a blunt 20-gauge (0.9 mm diameter) needle fitted to an RNase-free syringe. Proceed to step 4.

4. Centrifuge the lysate for 3 min at full speed. Carefully remove the supernatant by pipetting, and transfer it to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at 8000 x *g* (10,000 rpm).

   In some preparations, very small amounts of insoluble material will be present after the 3-min centrifugation, making the pellet invisible.

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

5. Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 21–24. Use the flow-through for RNA purification in steps 6–13.

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

Total RNA purification

6. To the flow-through from step 5, add 96–100% ethanol: either 250 µl (if 350 µl Buffer RLT was used) or 400 µl (if 600 µl Buffer RLT was used). Mix well by pipetting. Do not centrifuge. Proceed immediately to step

   If some lysate was lost during homogenization and DNA binding to the AllPrep DNA spin column, adjust the volume of ethanol accordingly.

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

7. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm). Transfer the flow-through* to a 2 ml tube (supplied) for protein purification in steps 14–20.

   Reuse the collection tube in step 8.

   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Transfer the flow-through after each centrifugation to the 2 ml tube.

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

   Reuse the collection tube in step 9.

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

   Optional: If purifying RNA from tissues with high DNA content and if the RNA will be used in sensitive downstream applications, we recommend performing DNase digestion by following steps E1–E4 (Appendix E, page 58) instead of step 8.

*. Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 8 for safety info

† Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 8 for safety info

9. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

   Reuse the collection tube in step 10.

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

10. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at 8000 x *g* (10,000 rpm) to wash the spin column membrane.

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

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

11. **Optional**: Place the RNeasy 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 1 min. Perform this step to eliminate any possible carryover of Buffer RPE or if residual flow-through remains on the outside of the RNeasy spin column after step 10.

12. Place the RNeasy 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.

13. If the expected RNA yield is >30 µg, repeat step 12 using another 30–50 µl of RNase-free water or using the eluate from step 12 (if high RNA concentration is required). Reuse the collection tube from step 12.

    If using the eluate from step 12, 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.

Total protein precipitation

14. Add 1 volume (usually 600 or 1000 µl) of Buffer APP to the flow-through from step 7. Mix vigorously and incubate at room temperature for 10 min to precipitate protein.
15. Centrifuge at full speed for 10 min, and carefully decant the supernatant.*
16. Add 500 µl of 70% ethanol to the protein pellet. Centrifuge at full speed for 1 min, and remove the supernatant by using a pipet or by decanting as much liquid as possible.

    It is not necessary to resuspend or incubate the pellet.
17. Dry the protein pellet for 5–10 min at room temperature.

    Note: Incomplete drying may cause problems when loading the protein onto a gel due to residual ethanol.
18. Add up to 100 µl Buffer ALO and mix vigorously to dissolve the protein pellet.

    Note: Ensure that DTT is added to Buffer ALO before use (see "Things to do before starting").

    The volume of Buffer ALO to add depends on the amount of starting material. See Table 2 (page 14) for typical protein yields from various starting materials.

    Buffer ALO is a Laemmli-related sample buffer for use in SDS-PAGE and contains bromophenol blue dye. If the proteins will not be analyzed by SDS-PAGE, dissolve the pellet in a buffer compatible with the intended downstream application.

    Due to the strong denaturing conditions with Buffer RLT, which is necessary to inactivate RNases and proteases, the precipitated proteins may show reduced solubility. Vortex for several minutes or disaggregate the pellet by pipetting up and down several times.

    Depending on the sample type, the pellet may contain proteins or other cellular components that are not soluble. For details on how to solubilize these samples, see "Troubleshooting Guide" on page 42.

* Supernatant contains Buffer RLT and is therefore not compatible with bleach. See page 8 for safety Information.

For easier dissolving or if the proteins need to be quantified prior to SDS PAGE, dissolve the pellet in 5% (w/v) SDS or 8 M urea. For details about protein quantification, see Appendix F (page 61).

Buffer ALO may turn yellow upon dissolving protein, but this has no effect on downstream applications. The buffer will turn blue again in SDS-PAGE sample buffer. If using a stock solution of your own sample buffer (e.g., 5x concentration), be sure to dilute it accordingly so that the protein sample to be loaded on a gel has a 1x concentration of sample buffer.

19. Incubate for 5 min at 95°C to completely dissolve and denature the protein. Then cool the sample to room temperature.

20. Centrifuge for 1 min at full speed to pellet any residual insoluble material. Use the supernatant in downstream applications, such as SDS-PAGE and western blotting.

    The dissolved protein can be stored at –20°C for several months or at 4°C for several days.

Genomic DNA purification

21. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 5. Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through.*

    Reuse the spin column in step 22.

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

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 8 for safety information

22. Add 500 µl Buffer AW2 to the AllPrep DNA 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 "Things to do before starting").

    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 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.

23. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at 8000 x *g* (10,000 rpm) to elute the DNA.

24. Repeat step 23 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 23.

    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 or RNeasy spin column		
a)	Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting homogenization material" (page 15) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see protocols, pages 19 and 30) and/or increase the homogenization time.
b)	Too much starting material	Reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 12).
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 spin column.
Low nucleic acid yield		
a)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 15) for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material (see protocols, pages 19 and 30) 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 page 12).
c)	RNA still bound to RNeasy spin column membrane	Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifuging.

		Comments and suggestions
d)	DNA still bound to AllPrep DNA spin column membrane	Repeat DNA elution, but incubate the AllPrep DNA spin column on the benchtop for 10 min with Buffer EB before centrifuging.
e)	Ethanol carryover	During the second wash with Buffer RPE, be sure to centrifuge at 8000 x *g* (10,000 rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. Perform the optional centrifugation to dry the RNeasy spin column membrane if any flowthrough is present on the outside of the column (step 11 of the protocols
f)	Incomplete removal of cell-culture medium (cell samples)	e When processing cultured cells, ensure) complete removal of cell-culture medium after harvesting cells (see protocol, page 30).
DNA contaminated with RNA		
a)	Lysate applied to the AllPrep DNA spin column contains ethanol	Add ethanol to the lysate after passing the lysate through the AllPrep DNA spin column.
a)	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 application		
a)	Cell number too high	For some cell types, the efficiency of DNA binding to the AllPrep DNA spin column may be reduced when processing very high cell numbers. If the eluted RNA contains substantial DNA contamination, try processing smaller cell numbers.
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 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 spin column. Try using smaller samples. Alternatively, perform DNase digestion on the RNeasy spin column membrane (see Appendix E, page 58) or perform DNase digestion of the eluted RNA followed by RNA cleanup.

		Comments and suggestions
Low A_{260}/A_{280} value in RNA eluate		
	Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris·Cl,* pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 49).
RNA degraded		
a)	Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in Allprotect Tissue Reagent or RNAprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the AllPrep DNA/RNA/Protein procedure quickly, especially the first few steps. See Appendix A (page 46) and "Handling and storing starting material" (page 14).
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 AllPrep DNA/RNA/Protein procedure or later handling. See Appendix A (page page 46) for general remarks on handling RNA.
DNA fragmented		
a)	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 spin column or less than 30 µl RNase-free water for the RNeasy spin column. Although eluting in smaller volumes results in increased nucleic acid concentrations, yields may be reduced.
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.

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

		Comments and suggestions
b)	Ethanol carryover	During the second wash with Buffer RPE, be sure to centrifuge at 8000 x *g* (10,000 rpm) for 2 min at 20–25°C to dry the RNeasy 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 spin column membrane if any flowthrough is present on the outside of the column (step 11 of the protocols
No protein detected on western blot or Coomassie stained gel		
	Protein pellet lost	In the protein precipitation procedure, the protein pellet is only loosely attached to the side of the tube. Be sure to decant the supernatant gently. See also "Protein pellet does not completely dissolve" below.
Protein pellet does not completely dissolve		
a)	Protein pellet not soluble in the resuspension buffer used	Due to the different isoelectric points of proteins, it is impossible to dissolve all proteins at a certain pH. If your protein(s) of interest do not dissolve in the resuspension buffer, change the pH. In addition, several types of protein are very difficult to solubilize, especially membrane proteins. To improve solubility, use a different resuspension buffer containing other detergent(s) more suitable for your protein of interest.
b)	Protein pellet not completely solubilized	The protein pellet may contain other cellular components that are insoluble in the resuspension buffer. Resuspend/disturb the pellet by pipetting up and down several times. Then briefly centrifuge the sample, and use the supernatant for downstream analysis. For greater solubilization of proteins, dissolve the protein pellet in 5% (w/v) SDS or 8 M urea or increase the volume of resuspension buffer.
Protein bands on SDS-PAGE gel or western blot show a zig-zag pattern or smear		
a)	Protein sample contains insoluble material	Insoluble material may influence the running behavior of the gel. Repeat steps 19 and 20 of the protein precipitation procedure, making sure that no insoluble material is transferred to your downstream application.
b)	Protein shows no clear pattern in SDS-PAGE	The quality of SDS-PAGE can be influenced by several parameters independent of protein quality. Vary the protein load and/or the polyacrylamide concentration of the gel (which should be according to molecular mass of the protein of interest). Incubation of the sample for 10 min at 46°C before loading (instead of 95°C) can improve the resolution.

Appendix A: General Remarks on Handling RNA

Handling RNA

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

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 47), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant) and allow to dry.

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

Disposable plasticware

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

Solutions

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

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

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

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

Storage of RNA

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

Quantification of RNA

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

Spectrophotometric quantification of RNA

Using the QIAxpert UV/VIS Spectrophotometer for microvolume analysis

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

Using a standard spectrophotometer

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

this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 50), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 47). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

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

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

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

Purity of RNA

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

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

For standard photometric measurements, the ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution. For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 49).

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

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect® Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic

* 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 cannot be avoided, we recommend using the QuantiNova® Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see ordering information, page 62).

Integrity of RNA

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

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

Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

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

FA gel preparation

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

1.2 g agarose*

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

Add RNase-free water to 100 ml

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

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

RNA sample preparation for FA gel electrophoresis

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

* 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.

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

Gel running conditions

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

Composition of FA gel buffers

10x FA gel buffer

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

1x FA gel running buffer

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

5x RNA loading buffer

16 µl	saturated aqueous bromophenol blue solution*†
80 µl	500 mM EDTA, pH 8.0
720 µl	37% (12.3 M) formaldehyde
2 ml	100% glycerol*
3.084 ml	formamide*
4 ml	10x FA gel buffer
RNase-free water to 10 ml	
Stability: approximately 3 months at 4°C	

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

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

Appendix D: Storage, Quantification and Determination of Quality of Genomic DNA

Storage of DNA

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

Quantification of DNA

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

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

Measure absorbance of diluted sample in a 0.2 ml cuvette

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

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

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

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

Determination of DNA purity

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

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

Determination of DNA length

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

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

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

Appendix E: Optional On-Column DNase Digestion using the RNase-Free DNase Set

Although DNA binds very efficiently to the AllPrep DNA spin column, some tissues contain very high amounts of DNA (e.g., spleen and thymus) and will overload the AllPrep DNA spin column (unless the amount of starting material is very small). For these tissues, we recommend performing DNase digestion on the RNeasy spin column membrane if the eluted RNA will be used in downstream applications sensitive to very small amounts of DNA. Tissues containing moderate amounts of DNA and cultured cells do not require DNase digestion.

The QIAGEN RNase-Free DNase Set (see page 62 for ordering information) provides efficient on-column digestion of DNA during RNA purification. The DNase is efficiently removed in subsequent wash steps.

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

Preparation of tissue homogenates and binding of RNA to the RNeasy spin column membrane are performed according to the protocol starting on page 30. After washing with a reduced volume of Buffer RW1, RNA is treated with DNase I while bound to the spin column membrane. DNase I is removed by a second wash with Buffer RW1. Washing with Buffer RPE and elution are then performed according to the protocol on page 30.

Important points before starting

- Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

Things to do before starting

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

Procedure

Carry out the protocol starting on page 30 up to and including step 7. Instead of performing step 8 (the wash with Buffer RW1), follow steps E1–E4 below.

1. Add 350 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flowthrough.*

   Reuse the collection tube in step E4.

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

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

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

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 8 for safety information.

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

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

4. Add 350 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at 8000 x *g* (10,000 rpm). Discard the flow-through.* Continue with step 9 of the protocol on page 30 (i.e., the first wash with Buffer RPE).

   Reuse the collection tube in step

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 8 for safety information.

Appendix F: Quantification of Protein in SDS-PAGE Sample Buffer

The strong denaturation conditions in the AllPrep DNA/RNA/Protein procedure mean that the precipitated protein is highly denatured and shows reduced solubility in water. Resolubilization of the protein is possible in Buffer ALO or SDS-PAGE sample buffer or in other solutions such as 5% (w/v) SDS or 8 M urea. Protein dissolved in SDS or urea solutions can be quantified using the BCA (bicinchoninic acid) method, but there must be no dye in the solutions. Protein in 5% (w/v) SDS can be used directly with the method, but protein dissolved in 8 M urea must be diluted to give 3 M urea.

Ordering Information

Product	Contents	Cat. no.
AllPrep DNA/RNA/Protein Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80004
Accessories		
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
QIAshredder (50)	50 disposable cell-lysate homogenizers	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers	79656
TissueRuptor II	Handheld rotor–stator homogenizer 5 TissueRuptor Disposable Probes	Varies*
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor System	990890
TissueLyser II	Universal laboratory mixer-mill disruptor	Varies*
TissueLyser Adapter Set 2 x24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser System	69982
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser System	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
RNase-Free DNase Set (50)	For 50 RNA minipreps: DNase I, Buffer RDD and Water (all RNase-Free)	79254

Product	Contents	Cat. no.
Related products for sample preparation for systems biology		
AllPrep DNA/RNA Kits — for simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample		
AllPrep DNA/RNA Micro Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy MinElute® Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Reagents and Buffers	80284
AllPrep DNA/RNA Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80204
AllPrep RNA/Protein Kit — for simultaneous purification of total RNA and native protein from the same cultured cell sample		
AllPrep RNA/Protein Kit (50)	50 AllPrep Mini Spin Columns, 50 RNeasy Mini Spin Columns, 50 Protein Cleanup Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80404
Related products for genomic DNA purification		
DNeasy Blood & Tissue Kit — for purification of total DNA from animal blood and tissues and from cells, yeast, bacteria or viruses		
DNeasy Blood & Tissue Kit (50)†	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504

Product	Contents	Cat. no.
QIAGEN Genomic-tips — for purification of high-molecular-weight DNA from a wide range of samples		
QIAGEN Genomic-tip 20/G†	25 columns (maximum DNA binding capacity of 20 µg)	10223
Genomic DNA Buffer Set	Buffers, including specific lysis buffers for yeast, bacteria, cells, blood and tissue	19060
Blood & Cell Culture DNA Kits — for purification of high-molecular weight DNA from blood and cultured cells		
Blood & Cell Culture DNA Mini Kit (25)†	25 QIAGEN Genomic-tip 20/G, QIAGEN Protease, Buffers	13323
Related products for total RNA purification		
RNAprotect Tissue Reagent — for immediate stabilization of the gene expression profile in harvested tissues		
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

Product	Contents	Cat. no.
RNeasy Mini Kit — for purification of up to 100 µg total RNA from animal cells or tissues		
RNeasy Plus Mini Kit (50)	For 50 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-Free Water and Buffers	74134
RNeasy Fibrous Tissue Mini Kit — for purification of up to 100 µg total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)†	50 RNeasy Mini Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, RNase-Free Reagents and Buffers	74704
Related products for total protein preparation		
Qproteome® Mammalian Protein Prep Kit — for total protein preparations from mammalian cells and tissues		
Qproteome Mammalian Protein Prep Kit	For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase®	37901
Related products for PCR and RT-PCR applications		
QIAGEN Fast Cycling PCR Kit — for fast and specific PCR on any thermal cycler		
QIAGEN Fast Cycling PCR Kit (200)†	For 200 x 20 µl reactions: 2 x 1 ml QIAGEN Fast Cycling PCR Master Mix, 10x CoralLoad Fast Cycling Dye, Q-Solution, RNase-Free Water	203743

Product	Contents	Cat. no.
QIAGEN Multiplex PCR Kit — for highly specific and sensitive multiplex PCR without optimization		
QIAGEN Multiplex PCR Kit (100)†	For 100 x 50 µl reactions: 1.7 ml 2x Master Mix, 5x Q-Solution, 2 x 1.7 ml RNase-Free Water	206143
QIAGEN OneStep Ahead RT-PCR Kit — for fast and successful one-step RT-PCR		
QIAGEN OneStep Ahead RT-PCR Kit (50)	6 vials for 50 reactions: 1 x 500 µl OneStep Ahead RT-PCR Master Mix, 1 x 50 µl OneStep Ahead RT Mix, 1 x 200 µl Template Tracer, 1 x 50 µl Master Mix Tracer, 1 x 1.9 ml water, 1 x 400 µl Q-Solution	220211
QuantiNova Reverse Transcription Kit for fast cDNA synthesis and reproducible real-time two-step RT-PCR		
QuantiNova Rev. Transcription Kit (50)†	For 10 x 20 µl reactions: 20 µl 8x gDNA Removal Mix, 10 µl Reverse Transcription Enzyme, 40 µl Reverse Transcription Mix (containing RT primers), 20 µl Internal Control RNA, 1.9 ml RNase-Free Water	205410
QuantiNova Reverse Transcription Kit for fast cDNA synthesis and reproducible real-time two-step RT-PCR		
QuantiNova Rev. Transcription Kit (10)†	For 10 x 20 µl reactions: 20 µl 8x gDNA Removal Mix, 10 µl Reverse Transcription Enzyme, 40 µl Reverse Transcription Mix (containing RT primers), 20 µl Internal Control RNA, 1.9 ml RNase-Free Water	205410

Product	Contents	Cat. no.
QuantiNova SYBR Green PCR Kit— for unparalleled result using SYBER Green based qPCR		
QuantiNova SYBR Green PCR Kit (100)†	For 100 x 20 µl reactions: 1 ml 2x QuantiNova SYBR Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml Water	208052
QuantiNova SYBR Green RT-PCR Kit for one-step qRT-PCR using SYBR Green I for gene expression analysis		
QuantiNova SYBR Green RT-PCR Kit (100)†	For 100 x 20 µl reactions: 1 ml QuantiNova SYBR Green RT-PCR Master Mix, 20 µl QuantiNova SYBR Green RT Mix, 20 µl Internal Control RNA, 500 µl Yellow Template Dilution Buffer, 250 µl ROX Reference Dye, 1.9 µl RNase-Free Water	208152

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

† Larger kit sizes also available; see www.qiagen.com.

‡ For real-time PCR, two-step RT-PCR and one-step RT-PCR with sequence-specific probes, QuantiFast Probe Kits are available; see www.qiagen.com/FastPCR.

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

Document Revision History

Date	Changes
November 2020	Updated branding of RNA protection products.

Limited License Agreement

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

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