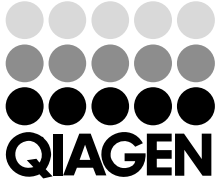
November 2005

AllPrep DNA/RNA Mini Handbook

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



Contents

Kit Contents

AllPrep DNA/RNA Mini Kit	**(50)**
Catalog no.	**80204**
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)	100
Buffer RLT Plus*	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
Handbook	1

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

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

Storage

The AllPrep DNA/RNA Mini Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions.

Quality Control

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

Product Use Limitations

The AllPrep DNA/RNA Mini Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the AllPrep DNA/RNA Mini Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Safety Information

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

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

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

The following risk and safety phrases apply to the components of the AllPrep DNA/RNA Mini Kit.

Buffer AW1

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

Buffer RLT Plus

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

Buffer RW1

Contains ethanol: flammable. Risk phrase:* R10

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36/38: Irritating to eyes and skin; S13: Keep away from food, drink and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show the container or label.

Introduction

The AllPrep DNA/RNA Mini Kit is designed to purify genomic DNA and total RNA simultaneously from a single biological sample. Lysate is first passed through an AllPrep DNA spin column to selectively isolate DNA and then through an RNeasy spin column to selectively isolate RNA. Pure DNA and RNA are purified from the entire sample, in contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately. The kit is compatible with small amounts of a wide range of animal cells and tissues.

The AllPrep DNA/RNA Mini Kit allows the parallel processing of multiple samples in less than 40 minutes. Time-consuming and tedious methods such as CsCl step-gradient ultracentrifugation and alcohol precipitation steps, or methods involving the use of toxic substances such as phenol and/or chloroform, are replaced by the AllPrep DNA/RNA procedure.

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

- PCR
- Southern, dot, and slot blot analyses
- Comparative genome hybridization (CGH)
- Genotyping, SNP analysis

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 50 for ordering information.

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

- RT-PCR
- Quantitative, real-time RT-PCR*
- Differential display
- cDNA synthesis

* Visit www.qiagen.com/geneXpression for information on standardized solutions for gene expression analysis, including QuantiTect® Kits and Assays for quantitative, real-time RT-PCR.

- Northern, dot, and slot blot analyses
- Primer extension
- Poly A^+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

Principle and procedure

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

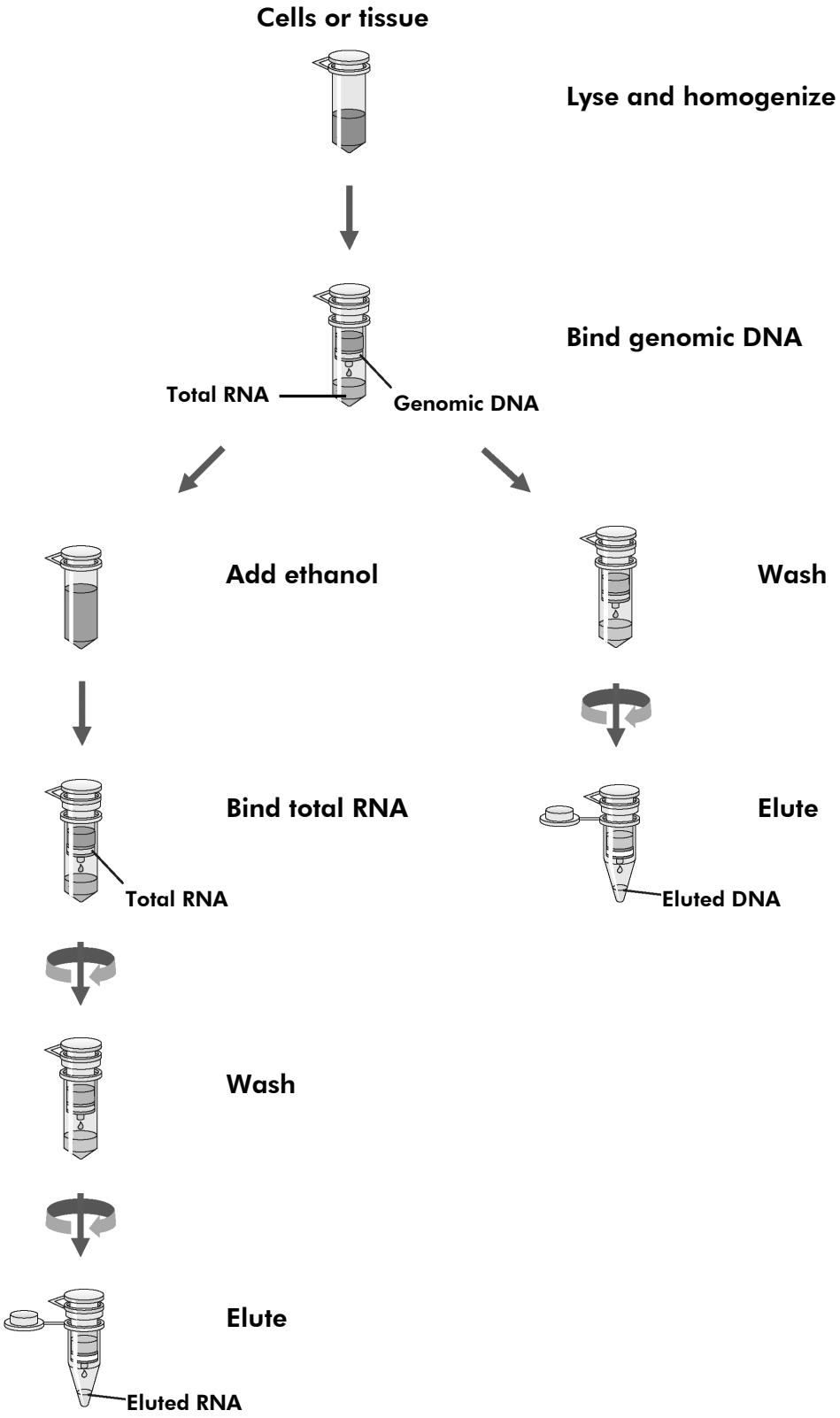
Biological samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate–containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysate is then passed through an AllPrep DNA spin column. This column, in combination with 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.

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).

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

AllPrep DNA/RNA Procedure



Equipment and Reagents to Be Supplied by User

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

For all protocols

- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- 96–100% ethanol*
- 70% ethanol* in water
- Disposable gloves
- For tissue samples: RNA*later*™ RNA Stabilization Reagent (see ordering information, page 50) or liquid nitrogen
- Equipment for sample disruption and homogenization (see pages 16–18). Depending on the method chosen, one or more of the following are required:
  - Trypsin and PBS
  - QIAshredder homogenizer (see ordering information, page 50)
  - Blunt-ended needle and syringe
  - Mortar and pestle
  - TissueLyser (see ordering information, page 50)
  - Rotor–stator homogenizer

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

Suppliers of rotor–stator homogenizers*

- BioSpec Products, Inc. (www.biospec.com): Tissue-Tearor™ homogenizer
- Charles Ross & Son Company (www.mixers.com)
- IKA (www.ika.de): ULTRA-TURRAX® dispersers
- KINEMATICA AG (www.kinematica.ch) or Brinkmann Instruments, Inc. (www.brinkmann.com): POLYTRON® laboratory dispersing devices
- Omni International, Inc. (www.omni-inc.com)
- Silverson (www.silverson.com)
- VirTis (www.virtis.com)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Determining the amount of starting material

It is essential to use the correct amount of starting material in order 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 Plus required for efficient lysis and the maximum loading volume of the AllPrep DNA and RNeasy spin columns
- 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 Mini Kit

Specification	AllPrep DNA spin column	RNeasy spin column
Maximum binding capacity	100 µg DNA*	100 µg RNA
Maximum loading volume	700 µl	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 cells	1 x 10^7 cells	Entire flow-through from AllPrep DNA spin column
■ Animal 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.

Table 2. Yields of Genomic DNA and Total RNA with the AllPrep DNA/RNA Mini Kit

Sample type	Average yield of genomic DNA (µg)	Average yield of total RNA* (µg)
Cell cultures (1 x 10^6 cells)		
NIH/3T3	8	10
HeLa, Jurkat	6	15
COS-7	7	35
Mouse/rat tissues (10 mg)		
Brain	5–10	5–10
Heart	5–10	4–8
Kidney	15–25	20–30
Liver	15–25	40–60
Spleen	50–70	30–80
Thymus	50–100	40–80
Lung	15–20	10–20

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

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

Animal cells

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

Table 3. Growth Area and Number of HeLa Cells in Various Culture Vessels

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

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

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

Animal tissues

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

Handling and storing starting material

RNA in harvested tissue is not protected until the sample is treated with RNA*later* RNA Stabilization Reagent, flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C, or immediately immersed in RNA*later* RNA Stabilization Reagent.

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

Disrupting and homogenizing starting material

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

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

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 4 gives an overview of various disruption and homogenization methods, and is followed by a detailed description of each method.

Table 4. Disruption and Homogenization Methods

Sample	Disruption method	Homogenization method
Animal cells	Addition of lysis buffer	Rotor–stator homogenizer or QIAshredder homogenizer or syringe and needle
Animal tissues	TissueLyser*	TissueLyser*
	Rotor–stator homogenizer†	Rotor–stator homogenizer†
	Mortar and pestle	QIAshredder homogenizer or syringe and needle

* Simultaneously disrupts and homogenizes up to 192 samples in parallel. Results are comparable to those obtained using a rotor–stator homogenizer.

† Simultaneously disrupts and homogenizes individual samples.

Disruption and homogenization using the TissueLyser system

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

- Size and composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed and configuration of the TissueLyser
- Disintegration time

For animal tissues, the optimal beads are 3–7 mm diameter stainless steel beads. All other disruption parameters must be determined empirically for each application. For guidelines on disruption and homogenization of tissues using the TissueLyser system, refer to the *TissueLyser Handbook*. For other bead mills, please refer to suppliers' guidelines for further details.

Note: Do not use Buffer RLT Plus with tungsten carbide beads. Buffer RLT Plus reacts with tungsten carbide and can damage the surface of the beads.

Disruption and homogenization using rotor–stator homogenizers

Rotor–stator homogenizers thoroughly disrupt and simultaneously homogenize, in the presence of lysis buffer, single samples of animal tissues in 15–90 seconds depending on the toughness and size of the sample. Rotor–stator homogenizers can also be used to homogenize cell lysates. The rotor turns at a very high speed, causing the sample to be disrupted and homogenized by a

combination of turbulence and mechanical shearing. Foaming of the sample should be kept to a minimum by using properly sized vessels, keeping the tip of the homogenizer submerged, and holding the immersed tip to one side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes. Probes with diameters of 5 mm and 7 mm are suitable for volumes of up to 300 µl and can be used for homogenization in microcentrifuge tubes. Probes with a diameter of 10 mm or above require larger tubes. In addition, round-bottomed tubes allow more efficient homogenization than conical-bottomed tubes.

Longer homogenization times with rotor–stator homogenizers result in greater DNA fragmentation. Therefore, the homogenization time should be kept as short as possible if the DNA will be used in downstream applications that require long DNA fragments.

Disruption 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 two 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 homgenizers.

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.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA from Animal Cells

Determining the correct amount of starting material

It is essential to use the correct amount of starting material in order 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 Plus required for efficient lysis (the maximum volume of Buffer RLT Plus that can be used limits the maximum amount of starting material to 1 x 10^7 cells)

RNA content can vary greatly between cell types. The following 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.

As a guide, Table 3 (page 15) shows the expected numbers of HeLa cells in different cell-culture vessels.

Important points before starting

- If using the AllPrep DNA/RNA Mini Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 38).
- 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.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25°C) for 1 month after addition of β-ME.
- 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 Plus may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

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 Plus.

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

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

Table 5. Volumes of Buffer RLT Plus for Lysing Pelleted Cells

Number of pelleted cells	Volume of Buffer RLT Plus
$<5 \times 10^6$	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 Plus (see Table 6) to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

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

Table 6. Volumes of Buffer RLT Plus for Direct Cell Lysis

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

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

3. Homogenize the lysate according to step 3a, 3b, or 3c.

See "Disrupting and homogenizing starting material", page 16, for more details on homogenization. If processing $\leq 1 \times 10^5$ cells, they can be homogenized by vortexing for 1 min. After homogenization, proceed to step 4.

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

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

3b. Homogenize the lysate for 30 s using a rotor–stator homogenizer. Proceed to step 4.

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

4. **Transfer the homogenized lysate to 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.

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 14–17. 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. **Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the flow-through from step 5, and 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.

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). Discard the flow-through.***

   Reuse the collection tube in step 8.

   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

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.

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 3 for safety information.

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 "Important points 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.

Genomic DNA purification

14. 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 15.

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

15. 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 "Important points 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.

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

17. Repeat step 16 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 16.

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

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 3 for safety information.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA from Animal Tissues

Determining the correct amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal nucleic acid yield and purity. A maximum amount of 30 mg fresh or frozen tissue or 15–20 mg RNA*later* stabilized tissue (which is partially dehydrated) 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 Plus 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 14).

For maximum RNA yields from liver, 50% ethanol (instead of 70% ethanol) should be used in step 6 of the procedure.

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 47).

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 Tissue Kit and the RNeasy Fibrous Tissue Mini Kit, respectively (see page 50 for ordering information).

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

Important points before starting

- If using the AllPrep DNA/RNA Mini Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 38).
- For optimal results, stabilize harvested tissues immediately in RNA*later* RNA Stabilization Reagent (see the *RNA*later *Handbook*). Tissues can be stored in the reagent for up to 1 day at 37°C, 7 days at 18–25°C, or 4 weeks at 2–8°C, or archived at –20°C or –80°C.
- Fresh, frozen, or RNA*later* stabilized tissues can be used. Tissues can be stored at –70°C for several months. Flash-freeze tissues in liquid nitrogen, and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT Plus. Homogenized tissue lysates from step 3 can also be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- If desired, more than 30 mg tissue can be disrupted and homogenized at the start of the procedure (increase the volume of Buffer RLT Plus proportionately). Use a portion of the homogenate corresponding to no more than 30 mg tissue for nucleic acid purification, and store the rest at –80°C.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25°C) for 1 month after addition of β-ME.
- 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 Plus may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Procedure

Sample disruption and homogenization

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

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

2. **Follow either step 2a or 2b.**

2a. **For RNA*later* stabilized tissues:**
If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed to step 3.

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

RNA in RNA*later* stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (18–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNA*later* Reagent. Previously stabilized tissues can be stored at –80°C without the reagent.

2b. **For unstabilized fresh or frozen tissues:**
If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately to step 3.

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

RNA in harvested tissues is not protected until the tissues are treated with RNA*later* 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 RNA*later* Reagent to stabilize RNA (see the *RNA*later *Handbook*). However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

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

   See "Disrupting and homogenizing starting material", page 16, for more details on disruption and homogenization.

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

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

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

Table 7. Volumes of Buffer RLT Plus for Tissue Disruption and Homogenization

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

* Use 600 µl Buffer RLT Plus for tissues stabilized in RNA*later* Reagent or for difficult-to-lyse tissues.

3a. Disruption and homogenization using a rotor–stator homogenizer: Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a suitably sized vessel. Add the appropriate volume of Buffer RLT Plus (see Table 7). Immediately disrupt and homogenize the tissue using a conventional rotor–stator homogenizer until it is uniformly homogeneous (usually 20–40 s). Proceed to step 4.

3b. Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer:
Immediately place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add the appropriate volume of Buffer RLT Plus (see Table 7). Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed. Proceed to step 4.

3c. Disruption using a mortar and pestle followed by homogenization using a needle and syringe:
Immediately place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add the appropriate volume of Buffer RLT Plus (see Table 7), and homogenize by passing lysate at least 5 times through a 20-gauge needle fitted to an RNase-free syringe. Proceed to step 4.

3d. Disruption and homogenization using the TissueLyser:
See the *TissueLyser Handbook*. Then proceed to step 4.

4. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting, and transfer it to the 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 14–17. 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. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the flow-through from step 5, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.

If some lysate was lost during homogenization and DNA removal, adjust the volume of ethanol accordingly.

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

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

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). Discard the flowthrough.***

   Reuse the collection tube in step 8.

   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

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 47) instead of step 8.

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 "Important points 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.

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 3 for safety information.

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.

Genomic DNA purification

14. **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). Discard the flow-through.***

    Reuse the spin column in step 15.

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

15. **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 "Important points 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.

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 3 for safety information.

16. **Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.**
17. **Repeat step 16 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 16.

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

	Comments and suggestions
Clogged AllPrep DNA or RNeasy spin column	
a) Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 16) 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 26) 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 16) for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material (see protocols, pages 19 and 26) 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).

	Comments and suggestions
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.
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. Alternatively, heat Buffer EB to 70°C prior to DNA elution.
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 flow-through is present on the outside of the column (step 11 of the protocols).
f) Incomplete removal of cell-culture medium (cell samples)	When processing cultured cells, ensure complete removal of cell-culture medium after harvesting cells (see protocol, page 19).
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.
b) Sample is affecting pH of homogenate	The final homogenate should have a pH of 7. Make sure that the sample is not highly acidic or basic.
Contamination of RNA with DNA affects downstream applications	
a) 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.

	Comments and suggestions
b) Incomplete removal of cell-culture medium or stabilization reagent	Be sure to remove any excess cell-culture medium or stabilization reagent to prevent significant dilution of the lysis buffer. The 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 47), or perform DNase digestion of the eluted RNA followed by RNA cleanup.
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 40).
RNA degraded	
a) Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in RNA*later* RNA Stabilization 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 procedure quickly, especially the first few steps. See Appendix A (page 38) and "Handling and storing starting material" (page 16).
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 procedure or later handling. See Appendix A (page 38) for general remarks on handling RNA.

* 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.

	Comments and suggestions
DNA fragmented	
Homogenization too vigorous	The length of the purified DNA (usually 15–30 kb) depends strongly on the homogenization conditions. If longer DNA fragments are required, keep the homogenization time to a minimum or use a gentler homogenization method if possible (e.g., use a QIAshredder homogenizer instead of a rotor–stator homogenizer).
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 1 x 30 µl of 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.
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 flow-through is present on the outside of the column (step 11 of the protocols).

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. In order 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.

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.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 39). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

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

Glassware

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

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS*), thoroughly rinsed with RNase-free water, and then rinsed with ethanol*† and allowed to dry.

Solutions

Solutions (water and 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 have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

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

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

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

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

Storage of RNA

Purified RNA may be stored at –20°C or –70°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 accurately quantified using an Agilent 2100 bioanalyzer, quantitative RT-PCR, or fluorometric quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml ($A_{260}=1 \rightarrow 44$ µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 41), 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 39). 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

* 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.

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

Purity of RNA

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

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "spectrophotometric quantification of RNA", page 40).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While the the vast majority of cellular DNA will bind to the AllPrep DNA spin column, trace amounts may still remain in the purified RNA, depending on the amount and nature of the sample.

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

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

* 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.

analysis of RNA sequences (without detection of genomic DNA) where possible. For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (see ordering information, page 50).

Integrity of RNA

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

* 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 C: Protocol for Formaldehyde Agarose Gel Electrophoresis

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

FA gel preparation

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

1.2 g agarose*

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

Add RNase-free water to 100 ml

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

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

RNA sample preparation for FA gel electrophoresis

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

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

Gel running conditions

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

* 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.

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	10 x FA gel buffer
RNase-free water to 10 ml	
Stability: approximately 3 months at 4°C	

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

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

Appendix D: 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 in order 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

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.

* 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.

Determination of DNA purity

The ratio of the readings at 260 nm and 280 nm (A_{260}/ A_{280}) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The A_{260}/ A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/ A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/ A_{280} ratio and reduced sensitivity to protein contamination.* For accurate A_{260}/ A_{280} values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5). Make sure to zero the spectrophotometer with the appropriate buffer.

Pure DNA has an A_{260}/ A_{280} ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

Determination of DNA length

The precise length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol† precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,† for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room 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

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

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

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 50 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 26. 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 26.

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 26 up to and including step 7. Instead of peforming step 8 (the wash with Buffer RW1), follow steps E1–E4 below.

E1. 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 flow-through.*

Reuse the collection tube in step E4.

E2. 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.

E3. Add the DNase I incubation mix (80 µl) directly to the RNeasy spin column membrane, and incubate at room temperature (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.

E4. 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 26 (i.e., the first wash with Buffer RPE).

Reuse the collection tube in step 9.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 3 for safety information.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
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
Accessories		
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201
RNA*later* RNA Stabilization Reagent (50 ml)	For stabilization of RNA in 25 x 200 mg tissue samples: 50 ml RNA*later* RNA Stabilization Reagent	76104
RNA*later* RNA Stabilization Reagent (250 ml)	For stabilization of RNA in 125 x 200 mg tissue samples: 250 ml RNA*later* RNA Stabilization Reagent	76106
RNA*later* TissueProtect Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA*later* RNA Stabilization Reagent each	76154
RNA*later* TissueProtect Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNA*later* RNA Stabilization Reagent each	76163
QIAshredder (50)*	50 disposable cell-lysate homogenizers	79654
TissueLyser†	Universal laboratory mixer-mill disruptor	Inquire
RNase-Free DNase Set (50)	For 50 RNA minipreps: DNase I, Buffer RDD, and Water (all RNase-Free)	79254
Related products for genomic DNA purification		
DNeasy® Tissue Kit — for purification of up to 40 µg total cellular DNA from animal cells or tissues		
DNeasy Tissue Kit (50)*	50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes	69504

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

† Visit www.qiagen.com/products/accessories for details on the TissueLyser and accessories.

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		
RNeasy Mini Kit — for purification of up to 100 µg total RNA from animal cells or tissues		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74104
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
RNeasy MinElute® Cleanup Kit — for RNA cleanup and concentration with small elution volumes		
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74204

* Larger kit sizes and/or formats available; see www.qiagen.com .

Product	Contents	Cat. no.
Related products for downstream applications		
HotStarTaq® *Plus* DNA Polymerase — for fast and highly specific amplification in all applications		
HotStarTaq *Plus* DNA Polymerase (250 U)*†	250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer, 10x CoralLoad PCR Buffer, 5x Q-Solution, 25 mM $MgCl_2$	203603
dNTP Mix, PCR Grade (200 µl)*	Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP	201900
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
Omniscript® RT Kit — for reverse transcription using 50 ng to 2 µg RNA per reaction		
Omniscript RT Kit (50)*	For 50 x 20 µl reactions: Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix, RNase-Free Water	205111
Sensiscript® RT Kit — for reverse transcription using less than 50 ng RNA per reaction		
Sensiscript RT Kit (50)*	For 50 x 20 µl reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix, RNase-Free Water	205211
QIAGEN OneStep RT-PCR Kit — for fast and successful one-step RT-PCR		
QIAGEN OneStep RT-PCR Kit (25)*	For 25 x 50 µl reactions: Enzyme Mix, 5x PCR Buffer, dNTP Mix, 5x Q-Solution, RNase-Free Water	210210

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

† dNTPs not included.

Product	Contents	Cat. no.
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, RNase-Free Water	205311
QuantiTect SYBR® Green PCR Kit — for quantitative, real-time PCR and two-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204143
QuantiTect SYBR Green RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green RT-PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204243
QuantiTect Probe PCR Kit — for quantitative, real-time PCR and two-step RT-PCR using sequence-specific probes		
QuantiTect Probe PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204343
QuantiTect Probe RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Probe RT-PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204443
QuantiTect Multiplex PCR Kits — for quantitative, multiplex, real-time PCR and two-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex PCR Kit (200)*†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water	204543

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

† Visit www.qiagen.com/GeneGlobe to search for and order primer sets or primer–probe sets.

‡ Recommended for ABI PRISM and Applied Biosystems® cyclers.

Product	Contents	Cat. no.
QuantiTect Multiplex PCR NoROX Kit (200)*†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains no ROX dye), 2 x 2 ml RNase-Free Water	204743
QuantiTect Multiplex RT-PCR Kits — for quantitative, multiplex, real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex RT-PCR Kit (200)*†§	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204643
QuantiTect Multiplex RT-PCR NR Kit (200)*†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains no ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204843

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

† Visit www.qiagen.com/GeneGlobe to search for and order primer–probe sets.

‡ Recommended for all cyclers except ABI PRISM and Applied Biosystems cyclers.

§ Recommended for ABI PRISM and Applied Biosystems cyclers.

QIAGEN Companies

Please see the back cover for contact information for your local QIAGEN office.

QIAGEN Distributors

Argentina
Tecnolab S.A.
Tel: (011) 4555 0010
Fax: (011) 4553 3331
E-mail: info@tecnolab.com.ar
Web site: www.tecnolab.com.ar

Brazil
Uniscience do Brasil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

Chile
Biosonda SA
Tel: 562 209 6770
Fax: 562 274 5462
E-mail: ventas@biosonda.cl
Web site: www.biosonda.cl

China
Gene Company Limited
Tel: (852)2896-6283
Fax: (852)2515-9371
E-mail:
Hong Kong:
info@genehk.com
Beijing:
info_bj@genecompany.com
Shanghai:
info_sh@genecompany.com
Chengdu:
gene@public.cd.sc.cn
Guangzhou:
info_gz@genecompany.com

Croatia
INEL Medicinska Tehnika d.o.o.
Tel: (01) 2984-898
Fax: (01) 6520-966
E-mail:
inel-medicinska-tehnika@zg.htnet.hr

Cyprus
Scientronics Ltd
Tel: 02-357 22 765416
Fax: 02-357 22 764614
E-mail:
a.sarpetsas@biotronics.com.cy

Czech Republic
BIO-CONSULT spol. s.r.o.
Tel/Fax: (420) 2 417 29 792
E-mail: info@bioconsult.cz
Web site: www.bioconsult.cz

Egypt
Clinilab
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

Estonia
PEAI-Est OÜ
Tel: (051) 65 830
Fax: (07) 383 360
E-mail: langel@ut.ee

Greece
BioAnalytica S.A.
Tel: (210)-640 03 18
Fax: (210)-646 27 48
E-mail: bioanalyt@hol.gr
Web site: www.bioanalytica.gr

Hungary
Kasztel-Med Co. Ltd.
Tel: (01) 385 3887
Fax: (01) 381 0695
E-mail: info@kasztel.hu
Web site: www.kasztel.hu

India
Genetix
Tel: (011)-2542 1714
or (011)-2515 9346
Fax: (011)-2546 7637
E-mail: genetix@nda.vsnl.net.in

Israel
Westburg (Israel) Ltd.
Tel: 08-6900655
or 1-800 20 22 20 (toll free)
Fax: 08-6900650
E-mail: info@westburg.co.il
Web site: www.westburg.co.il

Korea
LRS Laboratories, Inc.
Tel: (02) 924-86 97
Fax: (02) 924-86 96
E-mail: webmaster@lrslab.co.kr
Web site: www.lrslab.co.kr

Malaysia
RESEARCH BIOLABS SDN. BHD.
Tel: (603)-8070 3101
Fax: (603)-8070 5101
E-mail: biolabs@tm.net.my
Web site: www.researchbiolabs.com

Mexico
Quimica Valaner S.A. de C.V.
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: ventas@valaner.com
Web site: www.valaner.com

New Zealand
Biolab Ltd
Tel: (09) 980 6700
or 0800 933 966
Fax: (09) 980 6788
E-mail:
biosciences@nzl.biolabgroup.com
Web site: www.biolabgroup.com/nzl

Poland
Syngen Biotech Sp.z.o.o.
Tel: (071) 798 58 50 - 52
Fax: (071) 798 58 53
E-mail: info@syngen.pl
Web site: www.syngen.pl

Portugal
IZASA PORTUGAL, LDA
Tel: (21) 424 7312
Fax: (21) 417 2674
E-mail: consultasbiotec@izasa.es

Saudi Arabia
Abdulla Fouad Co. Ltd.
Tel: (03) 8324400
Fax: (03) 8346174
E-mail:
sadiq.omar@abdulla-fouad.com

Singapore
Research Biolabs Pte Ltd
Tel: 6777 5366
Fax: 6778 5177
E-mail: sales@researchbiolabs.com
Web site: www.researchbiolabs.com

Slovak Republic
BIO-CONSULT Slovakia spol. s.r.o.
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@cdicon.sk
Web site: www.bioconsult.cz

Slovenia
MEDILINE d.o.o.
Tel: (01) 830-80-40
Fax: (01) 830-80-70
or (01) 830-80-63
E-mail: mediline@siol.net

South Africa
Southern Cross Biotechnology
(Pty) Ltd
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za

Spain
IZASA, S.A.
Tel: (93) 902.20.30.90
Fax: (93) 902.22.33.66
E-mail: consultasbiotec@izasa.es

Taiwan
TAIGEN Bioscience Corporation
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

Thailand
Theera Trading Co. Ltd.
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

Turkey
Medek Medikal Ürünler
ve Saglik Hizmetleri A. S.
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: makialp@med-ek.com

All other countries
QIAGEN GmbH, Germany

Australia ■ Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 021-51345678 ■ Fax 021-51342500 ■ Technical 021-51345678

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 800 787980

Japan ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)