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# QIAwave DNA/RNA Mini Handbook

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

Sample to Insight

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# Kit Contents

| QIAwave DNA/RNA Mini Kit<br>Catalog no.<br>Number of preps | (50)<br>80504<br>50 |
|---|---|
| AllPrep® DNA Mini Spin Columns (uncolored)<br>(each in a 2 mL Collection Tube) | 50 |
| RNeasy® Mini Spin Columns (pink) | 50 |
| Waste Tubes (2 mL) | 100 |
| Buffer RLT Plus* | 45 mL |
| Buffer RW1* | 45 mL |
| Buffer RPE/C† (concentrate) | 1 mL |
| RNase-Free Water | 10 mL |
| Buffer AW1/C*† (concentrate) | 15 mL |
| Buffer AW2/C† (concentrate) | 1.5 mL |
| Buffer EB/C† (concentrate) | 2 mL |

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for "Safety Information".

† Before using for the first time, mix with ultrapure water and/ or ethanol (96–100%) according to the instructions given in the protocols section and/or on the label.

# Storage

The QIAwave 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, if not otherwise stated on the label.

# Intended use

The QIAwave 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.

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

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

# Safety Information

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

| | |
|---|---|
| **CAUTION**  | Do not add bleach or acidic solutions directly to the sample-preparation waste. |

Buffer AW1 contains guanidine hydrochloride, Buffer RLT 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.

# Quality Control

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

# Introduction

The QIAwave 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. 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 QIAwave 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 QIAwave DNA/RNA procedure.

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

- Next-generation sequencing
- PCR, qPCR, and digital PCR
- Southern, dot, and slot blot analyses
- Comparative genome hybridization
- Genotyping, SNP analysis

For purification of high-molecular-weight DNA, we recommend using either MagAttract® HMW DNA Kit or QIAGEN Genomic-tips or Blood & Cell Culture DNA Kits. Both allow purification of DNA of up to 150 kb in size. See Ordering Information, starting on page 66.

With the QIAwave DNA/RNA procedure, all RNA molecules longer than approximately 200 nucleotides are isolated. The procedure provides an enrichment for mRNA because 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:

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

## Principle and procedure

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

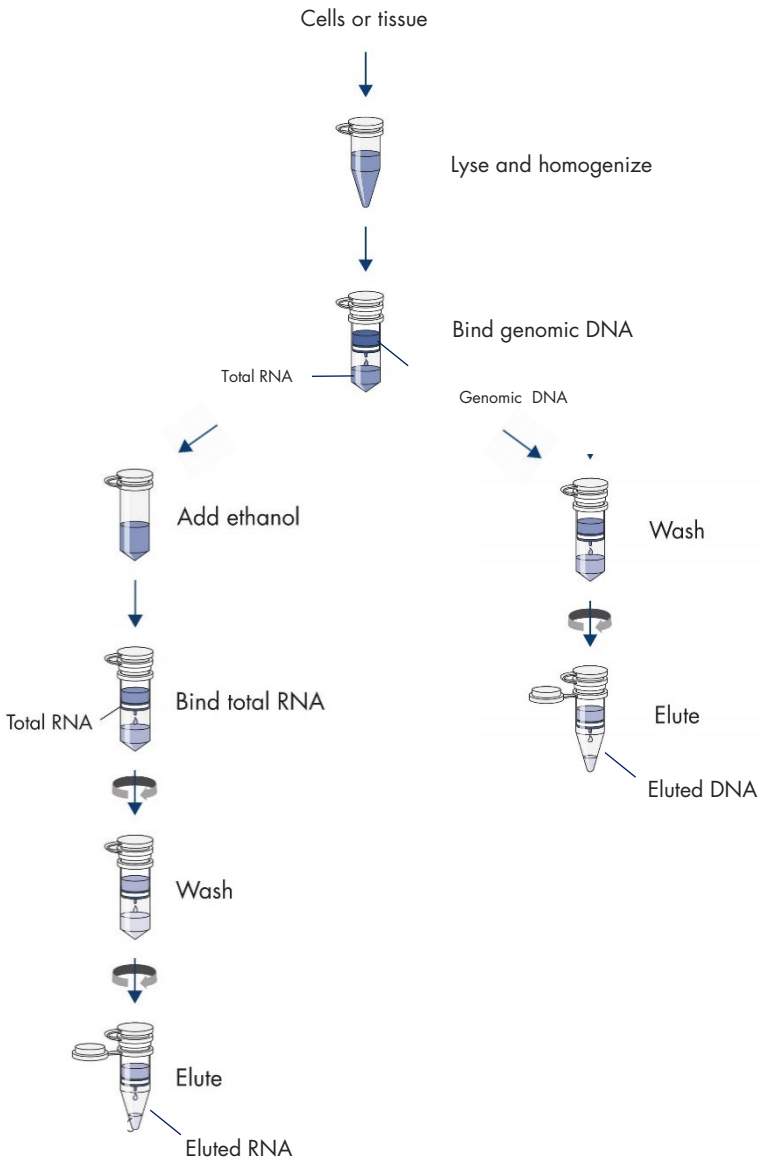
Biological samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate–containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysate is then passed through an AllPrep DNA spin column. This column, in combination with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

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

**QIAwave 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

- Ultrapure water
- Glassware for reconstitution of buffers (see "Important Notes" for more information)
- 14.3 M β-mercaptoethanol (β-ME, commercially available solutions are usually 14.3 M), alternatively dithiothreitol (DTT, 2 M stock solution)
- Sterile, RNase-free pipette tips
- Microcentrifuge (with rotor for 2 mL tubes)
- Microcentrifuge tube for elution (1.5 or 2 mL)
- **Optional**: Waste Tubes (2 mL) (cat. no. 19211), in case the user prefers to use a new Waste Tube for each washing step. We recommend reusing the same Waste Tube throughout the procedure to reduce plastic consumption.
- 96–100% ethanol*
- 70% ethanol* in water
- Disposable gloves
- For tissue samples: RNAprotect® Tissue Reagent (see Ordering Information starting on page 66) or liquid nitrogen
- Equipment for sample disruption and homogenization (see pages 19–22).

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

- Depending on the method chosen, one or more of the following are required:
    - Trypsin and PBS
    - QIAshredder homogenizer (see Ordering Information starting on page 66)
    - Blunt-ended needle and syringe
    - Mortar and pestle
    - TissueLyser® II, TissueLyser III or TissueLyser LT (see Ordering Information starting on page 66)
    - TissueRuptor® II homogenizer

# Important Notes

## Working with QIAwave products

### Preparation of functional buffers

**Preparation of final buffers from buffer concentrates**

| Kit (cat. no.) | Final buffer | Buffer* | Ultrapure water | Ethanol (96–100%) | Final volume |
|---|---|---|---|---|---|
| 80504 | RPE | RPE/C | 12 mL | 52 mL | 65 mL |
| | AW1 | AW1/C | – | 20 mL | 35 mL |
| | AW2 | AW2/C | 15 mL | 40 mL | 56.5 mL |
| | EB | EB/C | 20 mL | – | 22 mL |

*Use entire volume

Selected buffers are provided as concentrates in 15 mL bottles or 2 mL tubes to shrink buffer bottles and reduce the amount of plastics used. Before using the kit for the first time, concentrates have to be reconstituted to receive the functional buffer. This is done with either water or water and ethanol. To reconstitute, the entire volume of the buffer concentrate should be transferred from the 15 mL bottle or 2 mL tube into a suitably sized glass bottle (see page 14), either by using a pipette or by pouring. Subsequently, the appropriate volume of water or water and ethanol should be added as indicated in the table above. Afterwards, the glass bottle should be capped tightly and the reconstituted buffer mixed thoroughly by inverting.

In case of QIAwave DNA/RNA Mini Kit, the above is true for Buffer AW1/C, AW2/C, RPE/C and EB/C. For detailed instructions see “Things to do before starting” on page 27 and 37.

## Water quality used for preparation of functional buffers

We recommend using highly pure water for reconstitution. Ultrapure water (also known as type 1 water) with a resistivity of 18.2 MΩ-cm at 25°C (such as the one from a MilliQ system) works well. In case the users do not have access to type 1 water, QIAGEN offers Nuclease-Free Water (5 liters, cat. no. 129117)*; and Nuclease-Free Water (1000 mL, cat. no. 129115) *.

**Important**: Avoid using tap water as this can have detrimental impact on the extraction of the target analyte.

## Glassware

We suggest the use of glass bottles* for the reconstitution of buffers. Glass bottles can be cleaned, sterilized, and reused more easily than plastic bottles, which will further reduce the plastic footprint of the kit.

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.

* This item needs to be purchased separately.

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

## Labeling of functional buffers in glass bottles

Reconstituted buffers from buffer concentrates can be labelled with the additional label supplied with the kit. Use the enclosed label and transfer it onto the glass bottle containing the functional buffer prepared before using the kit for the first time.

## Waste Tubes

The newly introduced Waste Tube is made of recycled plastic recovered from post-consumer plastic waste. Due to the slight composition differences of the raw material, the color may differ from lot to lot. However, this has no effect on its intended use to collect the flow-through from sample binding and membrane washing. After each step, the flow-through is discarded and the Waste Tube can be reused. The Waste Tube is only used to process waste and should never come into direct contact with the analyte of interest. For detailed instructions, watch our instructional video at **www.qiagen.com/qiawavewastetube**

## Elution tubes

Elution tubes are not included in the kit. This allows the flexibility to use elution tubes of one's own choice and purchase them in, for example, eco-friendlier big packs.

## Recycling information

Please visit **www.qiagen.com/recycling-card** to learn more about how to recycle kit components.

## Determining the amount of starting material

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

- Type of sample and its DNA and RNA content
- Volume of Buffer RLT Plus required for efficient lysis and the maximum loading volume of the AllPrep DNA and RNeasy spin columns
- DNA binding capacity of the AllPrep DNA spin column
- 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 QIAwave 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 QIAwave 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. Because the QIAwave 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 ($cm^2$)* | Number of cells† |
|---|---|---|
| **Multiwell plates** | | |
| 96-well | 0.32–0.6 | 4–5 x $10^4$ |
| 48-well | 1 | 1 x $10^5$ |
| 24-well | 2 | 2.5 x $10^5$ |
| 12-well | 4 | 5 x $10^5$ |
| 6-well | 9.5 | 1 x $10^6$ |
| **Dishes** | | |
| 35 mm | 8 | 1 x $10^6$ |
| 60 mm | 21 | 2.5 x $10^6$ |
| 100 mm | 56 | 7 x $10^6$ |
| 145–150 mm | 145 | 2 x $10^7$ |
| **Flasks** | | |
| 40–50 mL | 25 | 3 x $10^6$ |
| 250–300 mL | 75 | 1 x $10^7$ |
| 650–750 mL | 162–175 | 2 x $10^7$ |

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

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Cell numbers will vary for different kinds of animal 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 RNAprotect Tissue Reagent, flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C, or immediately immersed in RNAprotect Tissue 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 III* | TissueLyser III* |
| | TissueRuptor II† | TissueRuptor II † |
| | 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 TissueRuptor II.

† Simultaneously disrupts and homogenizes individual samples.

## Homogenization and DNA yield

In most cases, homogenization is crucial to achieve optimal DNA yields with the QIAwave DNA/RNA Mini Kit. Even a very short mechanical homogenization step improves DNA elution from the AllPrep DNA column. We recommend homogenization using bead milling or a rotor-stator device to ensure maximum DNA yields.

## Disruption and homogenization using the TissueLyser systems

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 system
- 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 systems, refer to the *TissueLyser III 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, such as TissueRuptor II, 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 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 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 homogenizers.

### Homogenization using a syringe and needle

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

## Automated purification of nucleic acids on QIAcube Instruments

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

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

The QIAwave DNA/RNA Mini Kit can be automated on the QIAcube Connect using the AllPrep DNA/RNA Mini Kit protocols.


**QIAcube Connect.**

# 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 to obtain optimal nucleic acid yield and purity. The minimum amount is generally 100 cells, while the maximum amount depends on:

- RNA content of the cell type
- DNA binding capacity of the AllPrep DNA spin column
- RNA binding capacity of the RNeasy spin column (100 µg RNA)
- Volume of Buffer RLT Plus required for efficient lysis (the maximum volume of Buffer RLT Plus that can be used limits the maximum amount of starting material to 1 x $10^7$ cells)
- Homogenization; see “Homogenization and DNA yield”, page 20

RNA content can vary greatly between cell types and according to the growth rate. The following examples illustrate how to determine the maximum amount of starting material:

- COS cells have high RNA content (approx. 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 (approx. 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 (approx. 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 17) and if there is no information about its RNA content, we recommend starting with no more than 3 to 4 x $10^6$ cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

**Important**: Do not overload the AllPrep DNA spin column, as this will lead to copurification of DNA with RNA.

**Important**: Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and purity.

As a guide, Table 3 (page 18) shows the expected numbers of HeLa cells in different cell-culture vessels.

## Important points before starting

- If using the QIAwave DNA/RNA Mini Kit for the first time, read the "Important Notes" (page 13).
- If preparing RNA for the first time, read Appendix A (page 50).
- 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.
- If purifying RNA from cell lines rich in RNases, we recommend adding β-ME 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 containing β-ME can be stored at room temperature for up to 1 month. Alternatively, add 20 µL of 2 M DTT per 1 mL Buffer

RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.

- Buffer RLT Plus 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.

## Things to do before starting

**Table 5. Preparation of final buffers from buffer concentrates**

| Kit (cat. no.) | Final buffer | Buffer* | Ultrapure water | Ethanol (96–100%) | Final volume |
|---|---|---|---|---|---|
| 80504 | RPE | RPE/C | 12 mL | 52 mL | 65 mL |
| | AW1 | AW1/C | – | 20 mL | 35 mL |
| | AW2 | AW2/C | 15 mL | 40 mL | 56.5 mL |
| | EB | EB/C | 20 mL | – | 22 mL |

*Use entire volume.

- If purifying RNA from cell lines rich in RNases, or tissue, add either 10 µL β-ME, or 20 µL 2 M DTT, to 1 mL Buffer RLT Plus. Buffer RLT Plus with β-ME or DTT can be stored at room temperature for up to 1 month.
- **Preparation of Buffer RPE**: Transfer the entire volume of Buffer RPE/C from the 2 mL tube into a glass bottle larger than 65 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 52 mL ethanol (96–100%) to obtain a final volume of 65 mL. Cap

the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- **Preparation of Buffer AW1**: Transfer the entire volume of Buffer AW1/C from the 15 mL bottle into a glass bottle larger than 35 mL, either by using a pipette or by pouring. Add 20 mL ethanol (96–100%) to Buffer AW1/C to obtain a final volume of 35 mL. Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer AW2**: Transfer the entire volume of Buffer AW2/C from the 2 mL tube into a glass bottle larger than 57 mL, either by using a pipette or by pouring. Add 15 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 40 mL ethanol (96–100%) to obtain a final volume of 56.5 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer EB**: Transfer the entire volume of Buffer EB/C from the 2 mL tube into a glass bottle larger than 22 mL, either by using a pipette or by pouring. Add 20 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) to Buffer EB/C to obtain a final volume of 22 mL. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.
- Preassemble RNeasy Mini spin columns with Waste Tubes.

## 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 6). 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.

**Table 6. Volumes of Buffer RLT Plus for lysing pelleted cells**

| Number of pelleted cells | Volume of Buffer RLT Plus (µL) |
|---|---|
| <5 x $10^6$ | 350 |
| 5 x $10^6$ – 1 x $10^7$ | 600 |

For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RLT Plus (see Table 7) 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 7. Volumes of Buffer RLT Plus for direct cell lysis**

| Dish diameter | Volume of Buffer RLT Plus* (µL) |
|---|---|
| <6 cm | 350 |
| 6–10 cm | 600 |

* 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 19, for more details on homogenization. If processing ≤1 x $10^5$ cells, they can be homogenized by vortexing for 1 min. After homogenization, proceed to step 4.

   **Note**: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep DNA and RNeasy spin columns. Homogenization with 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 Waste Tube (supplied), and store at room temperature or at 4°C for later DNA purification in steps 13–16. Use the flow-through for RNA purification in steps 6–12.

**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 flowthrough 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 Waste Tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through*.

   Reuse the Waste 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 Waste Tube in step 9.

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

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 Waste Tube in step 10.

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

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

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

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

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

**Optional**: Discard the flow-through and reuse the Waste Tube. Centrifuge at full speed for 1 min. Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 10.

11. Place the RNeasy spin column in a new 1.5 mL collection tube (not supplied). Add 30–50 µL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.
12. If the expected RNA yield is >30 µg, repeat step 11 using another 30–50 µL of RNase-free water, or using the eluate from step 11 (if high RNA concentration is required). Reuse the collection tube from step 11.

    If using the eluate from step 11, 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

13. 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 Waste Tube in step 14.

    **Note**: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1/C before use (see "Things to do before starting").
14. 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.

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

**Note**: Buffer AW2 is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer AW2/C before use (see "Things to do before starting").

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

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

15. Place the AllPrep DNA spin column in a new 1.5 mL collection tube (not supplied). Add 100 µL Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

    **Note**: Buffer EB is supplied as a concentrate. Ensure that ultrapure water is added to Buffer EB/C before use (see "Things to do before starting").

16. Repeat step 15 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 15.

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

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

## Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal nucleic acid yield and purity. A maximum amount of 30 mg fresh or frozen tissue or 15–20 mg RNAprotect Tissue-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 17).

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 63).

RNA yields from skeletal muscle, heart, and skin tissue may be low due to the abundance of contractile proteins, connective tissue and collagen. For purification of genomic DNA and total RNA from these tissues, we recommend using the DNeasy Blood and Tissue Kit and the RNeasy Fibrous Tissue Mini Kit, respectively (see Ordering Information, starting on page 66).

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.

**Important**: Do not overload the AllPrep DNA spin column, as this will lead to copurification of DNA with RNA.

**Important**: Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and purity.

## Important points before starting

- If using the QIAwave DNA/RNA Mini Kit for the first time, read the "Important Notes" (page 13).
- If preparing RNA for the first time, read Appendix A (page 50).
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect 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°; or archived at –20°C or –80°C.
- Fresh, frozen, or RNAprotect-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-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 for 1 month after addition of β-ME. Alternatively, add 20 µL 2 M DTT per 1 mL Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.

- Buffer RLT Plus 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.

## Things to do before starting

**Table 8. Preparation of final buffers from buffer concentrates**

| Kit (cat. no.) | Final buffer | Buffer* | Ultrapure water | Ethanol (96–100%) | Final volume |
|---|---|---|---|---|---|
| 80504 | RPE | RPE/C | 12 mL | 52 mL | 65 mL |
| | AW1 | AW1/C | – | 20 mL | 35 mL |
| | AW2 | AW2/C | 15 mL | 40 mL | 56.5 mL |
| | EB | EB/C | 20 mL | – | 22 mL |

*Use entire volume.

- If purifying RNA from cell lines rich in RNases, or tissue, add either 10 µL β-ME, or 20 µL 2 M DTT, to 1 mL Buffer RLT Plus. Buffer RLT Plus with β-ME or DTT can be stored at room temperature for up to 1 month.
- **Preparation of Buffer RPE:** Transfer the entire volume of Buffer RPE/C from the 2 mL tube into a glass bottle larger than 65 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters,

cat. no. 129117) and 52 mL ethanol (96–100%) to obtain a final volume of 65 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- **Preparation of Buffer AW1**: Transfer the entire volume of Buffer AW1/C from the 15 mL bottle into a glass bottle larger than 35 mL, either by using a pipette or by pouring. Add 20 mL ethanol (96–100%) to Buffer AW1/C to obtain a final volume of 35 mL. Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer AW2**: Transfer the entire volume of Buffer AW2/C from the 2 mL tube into a glass bottle larger than 57 mL, either by using a pipette or by pouring. Add 15 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) and 40 mL ethanol (96–100%) to obtain a final volume of 56.5 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer EB**: Transfer the entire volume of Buffer EB/C from the 2 mL tube into a glass bottle larger than 22 mL, either by using a pipette or by pouring. Add 20 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 liters, cat. no. 129117) to Buffer EB/C to obtain a final volume of 22 mL. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.
- Preassemble RNeasy Mini spin columns with Waste Tubes.

## Procedure

### Sample disruption and homogenization

1. Excise the tissue sample from the animal or remove it from storage. Remove RNAprotect-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 RNAprotect-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 RNAprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (18–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect Tissue Reagent. 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 RNAprotect Tissue Reagent, flash-frozen or disrupted and homogenized in step 3. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

   **Note**: Remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA (see the *RNAprotect 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 19, for more details on disruption and homogenization.

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

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

**Note**: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep DNA 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 9. Volumes of Buffer RLT Plus for tissue disruption and homogenization**

| Amount of starting material (mg) | Volume of Buffer RLT Plus (µL) |
|---|---|
| <20 | 350 or 600* |
| 20–30 | 600 |

* Use 600 µL Buffer RLT Plus for tissues stabilized in RNAprotect Tissue Reagent or for difficult-to-lyse tissues.

3a. **Disruption and homogenization using a rotor–stator homogenizer**:
Place the weighed (fresh, frozen, or RNAprotect-stabilized) tissue in a suitably sized vessel. Add the appropriate volume of Buffer RLT Plus (see Table 9). 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 RNAprotect-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 9). 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 RNAprotect-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 9), 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 Waste Tube (supplied), and store at room temperature or at 4°C for later DNA purification in steps 13–16. Use the flow-through for RNA purification in steps 6–12.

**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 or 600 µL) of 70% ethanol to the flowthrough 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 Waste Tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flowthrough*.

   Reuse the Waste 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 Waste Tube in step 9.

   **Note**: After centrifugation, carefully remove the RNeasy spin column from the Waste Tube so that the column does not contact the flow-through. Be sure to empty the Waste 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 63) 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 Waste Tube in step 10.

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 6 for safety information.

**Note**: Buffer RPE is supplied as a concentrate. Ensure that ultrapure water and ethanol are added to Buffer RPE/C before use (see "Things to do before starting").

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

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

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

    **Optional**: Discard the flow-through and reuse the Waste Tube. Centrifuge at full speed for 1 min. Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 10.

11. Place the RNeasy spin column in a new 1.5 mL collection tube (not supplied). Add 30–50 µL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

12. If the expected RNA yield is >30 µg, repeat step 11 using another 30–50 µL of RNase-free water, or using the eluate from step 11 (if high RNA concentration is required). Reuse the collection tube from step 11.

    If using the eluate from step 11, 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

13. 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 Waste Tube in step 14.

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

14. 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 ultrapure water and ethanol are added to Buffer AW2/C before use (see "Things to do before starting").

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

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

15. Place the AllPrep DNA spin column in a new 1.5 mL collection tube (not supplied). Add 100 µL Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

    **Note**: Buffer EB is supplied as a concentrate. Ensure that ultrapure water is added to Buffer EB/C before use (see "Things to do before starting").

16. Repeat step 15 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 15.

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

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

| | Comments and suggestions |
|---|---|
| **Clogged AllPrep DNA or RNeasy spin column** | |
| a) Inefficient disruption and/or homogenization | See "Disrupting and homogenizing starting material" (page 19) for details on disruption and homogenization methods.<br>Increase *g* force and centrifugation time if necessary.<br>In subsequent preparations, reduce the amount of starting material (see the protocols, pages 25 and 35) and/or increase the homogenization time. |
| b) Too much starting material | Reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 19). |
| c) Centrifugation temperature too low | The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the 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 19) for details on disruption and homogenization methods.<br>In subsequent preparations, reduce the amount of starting material (see the protocols, pages 25 and 35) and/or increase the volume of lysis buffer and the homogenization time. |
| b) Too much starting material | Overloading the spin columns significantly reduces nucleic yields. Reduce the amount of starting material (see page 19). |
| c) RNA still bound to RNeasy spin column membrane | Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifuging. |

| | Comments and suggestions |
|---|---|
| d) DNA still bound to AllPrep DNA column membrane | Repeat DNA elution, but incubate the AllPrep DNA spin column on the benchtop for 10 min with Buffer EB before centrifuging.<br>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.<br>Perform the optional centrifugation to dry the RNeasy spin column membrane if any flow-through is present on the outside of the column (step 10 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 25). |
| g) Buffer RPE or Buffer AW1 or Buffer AW2 or Buffer EB prepared incorrectly | Make sure that ultrapure water and/or ethanol has been added to Buffer RPE/C, Buffer AW1/C, Buffer AW2/C, and Buffer EB/C before use (see "Things to do before starting", pages 27 and 37). |

### 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. |
| 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 63), or perform DNase digestion of the eluted RNA followed by RNA cleanup. |

### Low $A_{260}/A_{280}$ value

| | Comments and suggestions |
|---|---|
| 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 53). |
| **RNA degraded** | |
| a) Inappropriate handling of starting material | Ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent.<br>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.<br>See Appendix A (page 49) and "Handling and storing starting material" (page 19). |
| b) RNase contamination | Although all QIAwave DNA/RNA buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the QIAwave DNA/RNA procedure or later handling. See Appendix A (page 49) for general remarks on handling RNA. |
| c) 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 acid does not perform well in downstream experiments** | |
| a) Salt carryover during elution | Ensure that Buffers are at 20–30°C.<br>Ensure that the correct buffer is used for each step of the procedure.<br>When reusing Waste Tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels. |

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

| | Comments and suggestions |
|---|---|
| b) Ethanol carryover | During the second wash with Buffer RPE, be sure to centrifuge at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. After centrifugation, carefully remove the column from the Waste Tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.<br><br>Perform the optional centrifugation to dry the RNeasy spin column membrane if any flow-through is present on the outside of the column (step 10 of the protocols). |

# References

1. 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.

# Appendix A: General Remarks on Handling RNA

## Handling RNA

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

## General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, non-disposable plasticware and laboratory equipment (e.g., pipettes and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA*, followed by RNase-free water (see "Solutions", page 51), or rinse with chloroform* if the plasticware is chloroform-resistant. To

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

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

## Disposable plasticware

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

## Solutions

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

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

of the purine residues has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

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

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

## Storage of RNA

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

## Quantification of RNA

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

## Spectrophotometric quantification of RNA

### Using a standard spectrophotometer

To ensure significance, $A_{260}$ readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per mL ($A_{260}$ = 1 → 4 µg/mL). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 54), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA*, followed by washing with RNase-free water (see

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

"Solutions", page 51). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

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

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

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

## Purity of RNA

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

* 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.

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 53).

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

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiNova Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, we recommend using the QuantiNova Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see Ordering Information, page 66).

## Integrity of RNA

The integrity and size distribution of total RNA purified with QIAwave RNA Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel® Connect system or Agilent® 2100 Bioanalyzer. Ribosomal RNAs should

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

appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification. As a useful measure of RNA integrity, the QIAxcel Connect system and the Agilent 2100 Bioanalyzer provide an RNA integrity score (RIS) and an RNA integrity number (RIN), respectively. Ideally, the value should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

# Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

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

## FA gel preparation

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

1.2 g agarose*
10 mL 10x FA gel buffer (see composition below)
Add RNase-free water to 100 mL

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

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

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

## RNA sample preparation for FA gel electrophoresis

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

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

## Gel running conditions

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

## Composition of FA gel buffers

**10x FA gel buffer**

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

* 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.

**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 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:

* 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.

| | |
|---|---|
| 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.

## Determination of DNA purity

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

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

## Determination of DNA length

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

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

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

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

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

The QIAGEN RNase-Free DNase Set (see Ordering Information, starting on page 66) 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 35 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 35.

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

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

   Reuse the Waste Tube in step 4.
2. Add 10 µL DNase I stock solution (see above) to 70 µL Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

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

   **Note**: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
3. Add the DNase I incubation mix (80 µL) directly to the RNeasy spin column membrane, and incubate at room temperature for 15 min.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

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

4. Add 350 µL Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through*.

   Continue with step 9 of the protocol on page 35 (i.e., the first wash with Buffer RPE).

   Reuse the Waste Tube in step 9.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAwave DNA/RNA Mini (50) | 50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, 100 Waste Tubes (2 mL), Buffers, RNase-Free Water (10 mL) | 80504 |
| **Accessories** | | |
| Waste Tubes | 1000 Waste Tubes (2 mL) | 19211 |
| RNAprotect Tissue Reagent (50 mL) | 50 mL RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples | 76104 |
| RNAprotect Tissue Reagent (250 mL) | 250 mL RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples | 76106 |
| RNAprotect Tissue Tubes (50 x 1.5 mL) | For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 mL RNAprotect Tissue Reagent each | 76154 |
| RNAprotect Tissue Tubes (20 x 5 mL) | For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 mL RNAprotect Tissue Reagent each | 76163 |
| QIAshredder (50)* | 50 disposable cell-lysate homogenizers | 79654 |
| TissueLyser LT† | Compact bead mill, 100–240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately) | 85600 |
| TissueLyser LT Adapter, 12-Tube† | Adapter for disruption of up to 12 samples in 2 mL microcentrifuge tubes on the TissueLyser LT | 69980 |

| Product | Contents | Cat. no. |
|---|---|---|
| TissueLyser III† | Bead mill; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately) | 9003240 |
| RNase-Free DNase Set (50) | For 50 RNA minipreps: DNase I, Buffer RDD, and Water (all RNase-Free) | 79254 |
| **QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits** | | |
| QIAcube Connect‡ | Instrument, connectivity package, 1-year warranty on parts and labor | Inquire |
| Starter Pack, QIAcube | Filter-tips, 200 µL (1024), 1000 µL filter-tips (1024), 30 mL reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder | 990395 |
| **MagAttract HMW DNA Kit** | | |
| MagAttract HMW DNA Kit (48) | For 48 DNA preps: MagAttract Suspension G, Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water | 67563 |
| **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** | | |

| Product | Contents | Cat. no. |
|---|---|---|
| Blood & Cell Culture DNA Mini Kit (25)* | 25 QIAGEN Genomic-tip 20/G, QIAGEN Protease, Buffers | 13323 |
| **QIAwave Plasmid Miniprep Kit — for purification of molecular biology–grade DNA** | | |
| QIAwave Plasmid Miniprep Kit (50)* | 50 QIAprep® 2.0 Spin Columns, RNase A, LyseBlue®, 50 Waste Tubes (2 mL), Buffers | 27204 |
| **QIAwave DNA Blood & Tissue Kit – for purification of total DNA from animal blood, animal tissue, rodent tails, ear punches, cultured cells, bacteria, and insects** | | |
| QIAwave DNA Blood&Tissue Kit (50)* | 50 DNeasy Mini Spin Columns, Proteinase K, Buffers, 50 Waste Tubes (2 mL) | 69554 |
| **Related products for total RNA purification** | | |
| **QIAwave RNA Mini — for purification of total RNA from animal cells, animal tissues, bacteria, and yeast, and for RNA cleanup** | | |
| QIAwave RNA Mini Kit (50)* | 50 RNeasy Mini Spin Columns, RNase-free Water, Buffers, 50 Waste Tubes (2 mL) | 74534 |

| Product | Contents | Cat. no. |
|---|---|---|
| **QIAwave RNA Plus — For purification of total RNA including gDNA removal from animal cells, animal tissues, bacteria, and yeast** | | |
| QIAwave RNA Plus Mini (50)* | 50 gDNA Eliminator Mini Spin Columns, 50 RNeasy Mini Spin Columns, 50 Waste Tubes (2 mL), Buffers, RNase-Free Water | 74634 |
| **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 |
| **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 MgCl2 | 203603 |
| dNTP Mix, PCR Grade (200 µL)* | Mix containing 10 mM each of dATP, dCTP, dGTP and dTTP | 201900 |

| Product | Contents | Cat. no. |
|---|---|---|
| **QIAGEN Multiplex PCR Plus Kit — for highly specific and sensitive multiplex PCR without optimization** | | |
| QIAGEN Multiplex PCR Plus Kit (100)* | For 100 x 50 µL multiplex PCR reactions: 2x Multiplex PCR Master Mix (3 x 0.85 mL), 5x Q-Solution (1 x 2 mL), RNase-Free Water (2 x 1.9 mL), 10x CoralLoad Dye (1 x 1.2 mL) | 206152 |
| **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 Ahead RT-PCR Kit — for fast and successful one-step RT-PCR** | | |
| QIAGEN OneStep Ahead RT-PCR Kit (25)* | 6 vials for 50 reactions: 1 x 500 µL OneStep Ahead RT-PCR Master Mix, 1 x 50 µL OneStep Ahead RT Mix, 1 x 200 µL Template Tracer, 1 x 50 µL Master Mix Tracer, 1 x 1.9 mL water, 1 x 400 µL Q-Solution | 220211 |
| **QuantiNova Reverse Transcription Kit — for fast cDNA synthesis and reproducible real-time two-step RT-PCR** | | |
| QuantiNova Rev. Transcription Kit (10)* | For 10 x 20 µL reactions: 20 µL 8x gDNA Removal Mix, 10 µL Reverse Transcription Enzyme, 40 µL | 205410 |

| Product | Contents | Cat. no. |
|---|---|---|
| | Reverse Transcription Mix (containing RT primers), 20 µL Internal Control RNA, 1.9 mL RNase-Free Water | |
| **QuantiNova SYBR® Green PCR Kit — for unparalleled results using SYBR® Green-based qPCR** | | |
| QuantiNova SYBR® Green PCR Kit (100)* | For 100 x 20 µL reactions: 1 mL 2x QuantiNova SYBR® Green PCR Master Mix, 500 µL QuantiNova Yellow Template Dilution Buffer, 250 µL QN ROX Reference Dye, 1.9 mL Water | 208052 |
| **QuantiNova SYBR® Green RT-PCR Kit — for one-step qRT-PCR using SYBR® Green I for gene expression analysis** | | |
| QuantiNova SYBR® Green RT-PCR Kit (100)* | For 100 x 20 µL reactions: 1 mL QuantiNova SYBR® Green RT-PCR Master Mix, 20 µL QuantiNova SYBR® Green RT Mix, 20 µL Internal Control RNA, 500 µL Yellow Template Dilution Buffer, 250 µL ROX Reference Dye, 1.9 µL RNase-Free Water | 208152 |
| **QuantiNova Probe PCR Kit — for highly sensitive, specific, and ultrafast, probe-based real-time PCR** | | |
| QuantiNova Probe PCR Kit (100)* | For 100 x 20 µL reactions: 1 mL 2x QuantiNova Probe PCR Master Mix , 250 µL QN ROX Reference Dye, 500 µL QuantiNova Yellow Template Dilution Buffer, 1.9 mL Water | 208252 |
| **QuantiNova Probe RT-PCR Kit — for one-step qRT-PCR using sequence-specific probes for gene expression analysis** | | |
| QuantiNova Probe RT-PCR Kit (100) | For 100 x 20 µL reactions: 1 mL QuantiNova Probe RT-PCR Master Mix, 20 µL QuantiNova Probe RT Mix, 20 µL Internal Control RNA, 500 µL Yellow Template Dilution Buffer, 250 µL ROX Reference Dye, 1.9 µL RNase-Free Water | 208352 |

| Product | Contents | Cat. no. |
|---|---|---|
| **QuantiNova Multiplex PCR Kits — for ultrafast, multiplex, real-time PCR and two-step qRT-PCR using sequence-specific probes** | | |
| QuantiNova Multiplex PCR Kit (100)* | For 100 x 20 µL reactions: 500 µL QuantiNova Multiplex PCR Mastermix, 500 µL yellow template dilution buffer, 250µL ROX reference dye, 1.9 µL RNase-Free water | 208452 |
| **QuantiNova Multiplex RT-PCR Kit — for fast, reliable quantification of up to 5 RNA targets in a single tube by multiplex real-time RT-PCR** | | |
| QuantiNova Multiplex RT-PCR Kit (100) | For 100 x 20 µL reactions: 0.5 mL 4x QuantiNova Multiplex RT-PCR Master Mix, 20 µL QuantiNova Multiplex RT-Mix, 20 µL QuantiNova IC RNA, 500 µL QuantiNova Yellow Template Dilution Buffer, 250 µL QN ROX Reference Dye, 1.9 mL RNase-Free Water | 208552 |

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

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

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

§ dNTPs not included.

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

# Document Revision History

| Date | Changes |
|---|---|
| 06/2023 | Initial release. |

## Notes

## Notes

### Limited License Agreement for QIAwave DNA/RNA Mini Kit

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