March 2021

RNAprotect® Cell Reagent Handbook

RNAprotect Cell Reagent
For immediate stabilization of nucleic acids in cells

RNeasy® Protect Cell Mini Kit
For immediate stabilization of nucleic acids in cells and subsequent total RNA (>200 nt) purification

Sample to Insight

QIAGEN

Contents

Kit Contents

RNAprotect Cell Reagent **Catalog no.**	**(250 ml)** **76526**
RNAprotect Cell Reagent	250 ml
Quick-start Protocol	1

RNeasy Protect Cell Mini Kit **Catalog no.** **Number of preps**	**(50)** **74624** **50**
RNAprotect Cell Reagent (box 1 of 2):	
RNAprotect Cell Reagent	50 ml
Quick-Start Protocol*	1
RNeasy Plus Mini Kit (box 2 of 2):	
gDNA Eliminator Mini Spin Columns (uncolored) (each in a 2 ml Collection Tube)	50
RNeasy Mini Spin Columns (pink) (each in a 2 ml Collection Tube)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	50
Buffer RLT Plus†	45 ml
Buffer RW1†	45 ml
Buffer RPE‡ (concentrate)	11 ml
RNase-Free Water	10 ml
Quick-Start Protocol*	1

* Follow the instructions in the *RNAprotect Cell Reagent Handbook* when stabilizing and purifying RNA from cells.

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

‡ Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Storage

Store RNAprotect Cell Reagent at room temperature (15–25°C). Under this condition, the reagent is stable for at least 12 months, if not otherwise stated on the label.

The RNeasy Plus Mini Kit should be stored dry at room temperature (15–25°C). Under this condition, all kit components are stable for at least 9 months if not otherwise stated on the label.

Intended Use

The RNAprotect Cell Reagent and the RNeasy Protect Cell Mini Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of RNAprotect Cell Reagent and RNeasy Protect Cell Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Safety Information

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

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

Buffer RLT Plus contains guanidine thiocyanate and Buffer RW1 contains a small amount of guanidine thiocyanate. This chemical 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.

Introduction

The RNeasy Protect Cell procedure provides a complete solution for the stabilization and purification of total RNA from sorted or cultured cells. RNA in cells is immediately stabilized using RNAprotect Cell Reagent and then rapidly purified using the RNeasy Plus Mini Kit, which includes gDNA eliminator columns for rapid and effective removal of genomic DNA contamination. The stabilized cells can be shipped at ambient temperature prior to RNA purification; no dry ice is required.

RNAprotect Cell Reagent also stabilizes DNA in cells. The stabilized DNA can be purified using QIAGEN kits for DNA purification, such as DNeasy® kits and QIAamp® kits. Alternatively, stabilized DNA and RNA can be purified simultaneously from the same cell sample using an AllPrep® Kit.

Principle and procedure

RNA stabilization using RNAprotect Cell Reagent

Immediate stabilization of RNA in sorted or cultured cells is generally a prerequisite for reliable gene expression analysis using microarray, real-time RT-PCR, or other nucleic acid-based technology. This is because changes in the gene expression pattern occur immediately after harvesting due to unspecific and specific RNA degradation as well as to transcriptional induction.

RNAprotect Cell Reagent uses a patent-pending technology to immediately stabilize the gene expression profile in cells. With other technologies, it is necessary to wash or process the cells (e.g., trypsin treatment) prior to adding RNA stabilization reagent. However, changes in gene expression may occur during the washing or processing steps. In contrast, RNAprotect Cell Reagent can be added directly to cells in culture medium or buffer, providing instantaneous RNA stabilization. The reagent can also be used to treat cells not in medium or storage solution, such as pelleted cells. After stabilization in RNAprotect Cell Reagent, cells can be conveniently stored and transported at ambient temperature with the cellular RNA remaining intact and undegraded.

RNAprotect Cell Reagent also enables stabilization of DNA, providing simultaneous stabilization of RNA and DNA in the same cell sample.

RNA purification using the RNeasy Plus Mini Kit

The RNeasy Plus Mini Kit integrates QIAGEN's patented technology for selective removal of double-stranded DNA with well-established RNeasy technology. Efficient purification of high-quality RNA is guaranteed, without the need for additional DNase digestion.

Cells stabilized in RNAprotect Cell Reagent are centrifuged, and the resulting pellet is lysed and homogenized in a highly denaturing guanidine-thiocyanate–containing buffer, which immediately inactivates ribonucleases (RNases) to ensure isolation of intact RNA.

The lysate is then passed through a gDNA eliminator spin column. This column, in combination with the optimized high-salt buffer, allows efficient removal of genomic DNA.

Ethanol is added to the flow-through to provide appropriate binding conditions for RNA, and the sample is then applied to an RNeasy spin column, where total RNA (>200 nt) binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30 µl, or more, of RNase-free water.

With the RNeasy Plus Mini Kit, all RNA molecules longer than 200 nucleotides are purified. 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 size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently.

The RNeasy Plus Mini Kit provides RNA purification from up to 1 x 10^7 cells. For very small cell samples, we recommend using the RNeasy Plus Micro Kit (see page 41 for Ordering Information), which enables RNA purification from up to 5 x 10^5 cells with an elution volume of as low as 10 µl.

Description of protocols

Stabilization of nucleic acids in sorted or cultured cells using RNAprotect Cell Reagent

This protocol describes how to add RNAprotect Cell Reagent to sorted or cultured cells for immediate, simultaneous stabilization of RNA and DNA.

Purification of RNA from RNAprotect stabilized cells using the RNeasy Plus Mini Kit

This protocol describes how to purify RNA from sorted or cultured cells that have been stabilized in RNAprotect Cell Reagent. The protocol requires use of the RNeasy Plus Mini Kit (included in the RNeasy Protect Cell Mini Kit).

If purifying RNA from very small cell samples using the RNeasy Plus Micro Kit (see page 41 for Ordering Information), follow the cell protocol in the *RNeasy Plus Micro Handbook*.

Specialized protocols

The appendices contain specialized protocols for preparing sorted or cultured cells stabilized in RNAprotect Cell Reagent for subsequent DNA purification. Appendix C (page 35) describes how to prepare RNAprotect stabilized cells for simultaneous purification of DNA and RNA using an AllPrep Kit. Appendix D (page 38) describes how to prepare RNAprotect stabilized cells for DNA purification using a DNeasy Kit or QIAamp Kit.

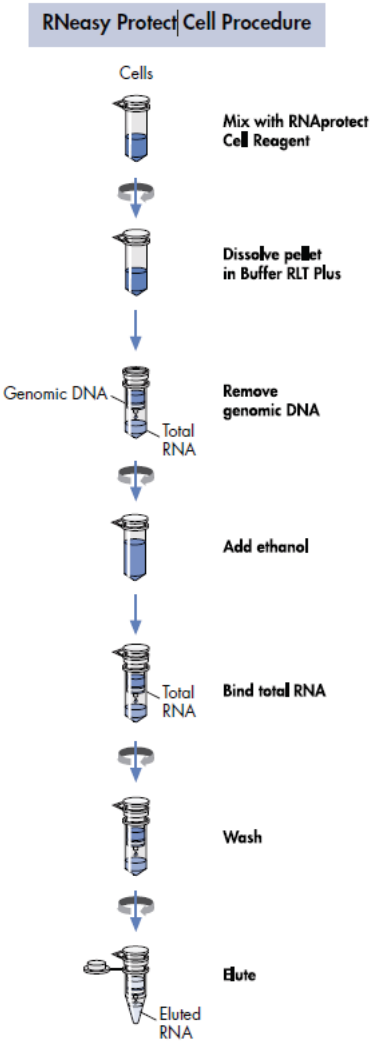
If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) when working with RNA. In some cell, lines are but we would still recommend adding it. Add 10 µl β-ME per 1 ml Buffer RLT Plus before use. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT 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 has to be prepared freshly before each isolation and can be stored at room temperature for up to 1 month.

Automated Purification of RNA on QIAcube® Instruments

Purification of RNA 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 RNAprotect Cell Reagent for purification of high-quality RNA.

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

QIAcube Connect.


RNeasy Protect Cell Procedure
Cells
Mix with RNAprotect Cell Reagent
Dissolve pellet in Buffer RLT Plus
Genomic DNA
Total RNA
Remove genomic DNA
Add ethanol
Total RNA
Bind total RNA
Wash
Elute
Eluted RNA

Equipment and Reagents to Be Supplied by User

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

- Sterile, RNase-free pipet tips
- Centrifuge and appropriately sized centrifuge tubes for centrifugation of stabilized cells
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- Ethanol (70% and 96–100%)*
- Disposable gloves
- Equipment for cell homogenization: we recommend either QIAshredder homogenizers or the TissueRuptor® II with TissueRuptor Disposable Probes (see page 41 for Ordering Information)
- **Optional**: 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M) or, alternatively, 2 M DTT in water

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

Important Notes

Determining the amount of starting material

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

- The volume of RNAprotect Cell Reagent required for cell stabilization
- The cell type and its RNA content
- The volume of Buffer RLT Plus required for efficient cell lysis
- The DNA removal capacity of the gDNA Eliminator spin column
- The RNA binding capacity of the RNeasy spin column

Effective cell stabilization requires the mixing of 5 volumes of RNAprotect Cell Reagent with 1 volume of cell culture or buffer, or 1 volume of pelleted cells. The maximum volume of Buffer RLT Plus that can be used within the RNeasy Plus Mini Kit limits the maximum amount of starting material to 1 x 10^7 cells. Since RNA content can vary greatly between cell types, we recommend starting with no more than 3–4 x 10^6 cells. Depending on RNA integrity, yield, and purity, it may be possible to increase the cell number up to 1 x 10^7 cells in subsequent preparations.

The amount of starting material must not exceed the maximum of 1 x 10^7 cells, even if the RNA binding capacity of the RNeasy spin column (100 µg RNA) will not be reached. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy spin column membrane, resulting in lower, inconsistent RNA yield and purity. If the binding capacity of the RNeasy spin column is exceeded, RNA yields will not be consistent and may also be reduced.

If the DNA removal capacity of the gDNA eliminator spin column is exceeded, the purified RNA will be contaminated with DNA. Although the gDNA eliminator spin column can bind more than 100 µg DNA, we recommend using samples containing less than 20 µg DNA to ensure removal of virtually all genomic DNA.

Counting cells is the most accurate way to quantitate the amount of starting material. As a guide, Table 1 shows the number of HeLa cells obtained in various culture vessels after confluent growth.

Table 1. 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.

Protocol: Stabilization of Nucleic Acids in Sorted or Cultured Cells Using RNAprotect Cell Reagent

This protocol describes how to stabilize RNA and DNA in different cell formats. RNAprotect Cell Reagent can be added directly to cells (adherent or suspension) in medium or storage solution (e.g., PBS). Alternatively, the reagent can be added to cells not in medium or storage solution, such as pelleted cells.

Important points before starting

- If using RNAprotect Cell Reagent for the first time, read "Important Notes" (page 14). If DNA or DNA/RNA will be purified, refer also to the relevant section in the DNeasy, QIAamp, or AllPrep handbook on determining the amount of starting material.
- Nucleic acids in cells are not protected until the cells are mixed with a sufficient volume of RNAprotect Cell Reagent. The reagent should be added immediately to pelleted cells or should be added directly to cells in medium or storage solution.
- Be sure to mix cells with a sufficient volume of RNAprotect Cell Reagent, as described in the procedure below. Smaller volumes may lead to nucleic acid degradation during storage and reduced RNA and DNA yields. Larger volumes can be used if necessary or desired.
- If adding RNAprotect Cell Reagent directly to cells that are in a large volume of medium or storage solution, the pelleting of the cells for nucleic acid purification (page 19, 35, or 38) may be incomplete, leading to reduced RNA and DNA yields. When processing high numbers of cells, we recommend a volume of 1 ml or less for the medium or storage solution. In general, we recommend lower volumes, especially for low numbers of cells.

- Standard cell lines, such as HeLa cells, Jurkat cells, and macrophages, do not lyse in RNAprotect Cell Reagent. However, some cell lines may lyse in the reagent, releasing nucleic acids into solution. Although the released RNA and DNA remain stabilized, they need to be collected in a cell pellet prior to nucleic acid purification (page 19, 35, or 38). To maximize recovery of the released RNA and DNA, we recommend using lower volumes of medium or storage solution if possible.
- Only fresh, unfrozen cells can be stabilized using RNAprotect Cell Reagent.
- Perform the procedure described below as quickly as possible.

Procedure

1. Add RNAprotect Cell Reagent to cells according to step 1a or 1b.

   1a. Cells in solution or cells covered with solution:

   Add 5 volumes of RNAprotect Cell Reagent to 1 volume of cell-culture medium or storage solution. Mix by shaking, pipetting, or vortexing.

   Note: The medium or storage solution must not exceed 1 ml. We recommend lower volumes, especially if the cells are susceptible to lysis in RNAprotect Cell Reagent.

   Adherent cells will detach after addition of RNAprotect Cell Reagent. No trypsin treatment is necessary.

   During storage of the stabilized cells, material may sink to the bottom of the storage vessel. To avoid having to resuspend this material, we recommend transferring the cells to a centrifuge tube (for use in step 1 of the protocol on page 19, 35, or 38) after adding the reagent.

1b. Pelleted cells or other cells not covered with solution (e.g., smears):

Add 300 µl RNAprotect Cell Reagent to the cell pellet in a centrifuge tube (not supplied). Resuspend the cells completely by vortexing.

Note: Be sure to add at least 5 volumes of RNAprotect Cell Reagent to the cell pellet. If residual culture medium or buffer increases the overall volume to over 60 µl, increase the amount of RNAprotect Cell Reagent accordingly.

Note: Larger volumes of RNAprotect Cell Reagent can be added if desired.

2. Store the mix of cells and RNAprotect Cell Reagent for up to 1 day at 30°C, up to 7 days at room temperature (15–25°C), or up to 4 weeks at 2–8°C, or archive at –20 or –80°C.

   Note: We recommend lower storage temperatures whenever possible (e.g., 2–8°C instead of room temperature, or room temperature instead of 30°C).

   Note: A precipitate may form during storage, especially at lower temperatures. This does not affect RNA and DNA purification.

Protocol: Purification of RNA from RNAprotect Stabilized Cells Using the RNeasy Plus Mini Kit

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA 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 RNA contained in the sample. Incomplete disruption results in significantly reduced RNA yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the RNeasy spin column membrane and therefore significantly reduced RNA yields.

With the RNeasy procedure, disruption of cultured cells is achieved by thorough vortexing in Buffer RLT Plus. Homogenization is then carried out using either QIAshredder homogenizers or the TissueRuptor II:

- **QIAshredder homogenizers** provide a fast and efficient way to homogenize cell 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 full speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column.

- The **TissueRuptor II** is a rotor–stator homogenizer that can be used to homogenize cell lysates. The blade of the TissueRuptor disposable probe rotates at a very high speed, causing the sample to be homogenized by a combination of turbulence and mechanical shearing. Be sure to use a suitably sized vessel to hold the sample and to keep the tip of the probe submerged during homogenization. Generally, round-bottomed tubes allow more efficient homogenization than conical-bottomed tubes. For guidelines on homogenization using the TissueRuptor II, refer to the *TissueRuptor Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.

Important points before starting

- If using the RNeasy Protect Cell Mini Kit for the first time, read "Important Notes" (page 14).
- If working with RNA for the first time, read Appendix A (page 29).
- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- Buffer RLT Plus may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).
- Buffer RLT Plus and Buffer RW1 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 spin column 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

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- 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 (15–25°C) 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.

Procedure

1. Centrifuge the mix of cells and RNAprotect Cell Reagent for 5 min at 5000 x *g* in an appropriately sized centrifuge tube.

   If transferring the sample to the centrifuge tube from a storage vessel, be sure to resuspend any material deposited at the bottom of the vessel by vortexing or by pipetting up and down. It is important to transfer all sample material to the centrifuge tube, otherwise RNA yields will be reduced.

   Note: If the sample was stored at below room temperature (e.g., –20°C), thaw it completely before starting centrifugation.

   Note: A precipitate may form during storage, especially at lower temperatures.

   This does not affect RNA purification.

2. Remove the supernatant completely by pipetting.

3. Loosen the pellet by flicking the tube.

   Loosening the pellet facilitates dissolving in Buffer RLT Plus in step 4.

4. Add 350 or 600 µl Buffer RLT Plus (see Table 2). Dissolve the pellet completely by vortexing and proceed immediately to step 5.

   If necessary, add β-ME to Buffer RLT Plus before use (see "Things to do before starting").

   Note: Be sure to dissolve the pellet completely. This can take about 1 min. Incomplete dissolving may lead to inefficient lysis and reduced RNA yields.

   Note: The dissolved pellet may be turbid. This does not affect RNA purification.

   The dissolved pellet can be stored at –70°C for several months. After removal from storage, incubate the dissolved pellet at room temperature or at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation at 37°C, which can cause RNA degradation. Proceed to step 5.

Table 2. Volumes of Buffer RLT Plus for lysing cells

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

5. Homogenize the lysate according to step 5a or 5b.

   See "Disrupting and homogenizing starting material", page 19, for more details on homogenization.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy spin column.

   5a. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Proceed to step 6.

   5b. Homogenize the lysate for 30 s using the TissueRuptor II. Proceed to step 6.

6. Transfer the homogenized lysate to a gDNA eliminator spin column placed in a 2 ml collection tube (supplied). Centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm).

   Discard the column, and save the flow-through.

   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.

7. Add 1 volume of 70% ethanol to the flow-through, and mix well by pipetting. Do not centrifuge.

   The volume of flow-through may be less than 350 or 600 µl due to loss during homogenization and DNA removal.

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

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

   Reuse the collection tube in step 9.

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

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

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

   Reuse the collection tube in step 10.

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

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

    Reuse the collection tube in step 11.

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

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

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

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

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

12. **Optional**: Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min.

    Perform this step to eliminate any possible carryover of Buffer RPE or if residual flow-through remains on the outside of the RNeasy spin column after step 11.

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

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

    If using the eluate from step 13, 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.

    For RT-PCR and real-time RT-PCR with the purified RNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit **www.qiagen.com/PCR**.

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 in 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 or protocols in this handbook (for contact information, see back cover or visit **www.qiagen.com**).

		Comments and suggestions
Nucleic acids degraded		
a)	Cells not immediately stabilized	Mix the cells immediately with RNAprotect Cell Reagent.
b)	Prolonged storage	Cells mixed with RNAprotect Cell Reagent can be stored for up to 1 day at 30°C, up to 7 days at 15–25°C, or up to 4 weeks at 2–8°C or archived at –20 or –80°C. We recommend lower storage temperatures whenever possible.
c)	RNA degradation during RNA purification	Although all RNeasy buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be careful not to introduce any RNases during RNA purification or later handling. See Appendix A (page 29) for general remarks on handling RNA.
Clogged gDNA eliminator spin column		
a)	Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 19) for details on disruption and homogenization. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see page 14) 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 14).
c)	Centrifugation temperature too low	The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the gDNA eliminator spin column. If this happens, set the centrifugation temperature to 25°C. Warm the lysate to 37°C before transferring it to the gDNA Eliminator spin column.

		Comments and suggestions
Low RNA yield		
a)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 19) for details on disruption and homogenization. In subsequent preparations, reduce the amount of starting material (see page 14) and/or increase the volume of lysis buffer and the homogenization time.
b)	Too much starting material	Overloading the RNeasy spin column significantly reduces RNA yield. Reduce the amount of starting material (see page 14).
c)	Ethanol added to lysate before DNA removal	Pass the lysate through the gDNA eliminator spin column before adding ethanol to it.
d)	RNA still bound to column membrane	Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifuging.
e)	Ethanol carryover	During the second wash with Buffer RPE, be sure to centrifuge at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. Perform the optional centrifugation to dry the RNeasy spin column membrane if any flow-through is present on the outside of the column (step 12 of the second protocol, page 25).
f)	Incomplete removal of RNAprotect Cell Reagent	Be sure to remove any excess RNAprotect Cell Reagent to prevent significant dilution of Buffer RLT Plus. Sample lysis will be impaired if the lysis buffer is diluted.
g)	Cells in too large a volume of medium or storage solution (e.g., PBS)	In subsequent preparations, reduce the volume of medium or storage solution.
Low A_{260}/A_{280} value		
Water used to dilute RNA for A260/A280 measurement		Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 31).
DNA contamination in downstream experiments		
a)	Cell number too high	For some cell types, the efficiency of DNA removal may be reduced when processing very high cell numbers (containing more than 20 µg genomic DNA). If the eluted RNA contains substantial DNA contamination, try processing smaller cell numbers.
b)	Incomplete removal of RNAprotect Cell Reagent	Be sure to remove any excess RNAprotect Cell Reagent to prevent significant dilution of Buffer RLT Plus. The gDNA eliminator spin column will not work effectively if the lysis buffer is diluted.

	Comments and suggestions
RNA concentration too low	
Elution volume too high	Elute RNA with less than 2 x 50 µl of water. Do not use less than 1 x 30 µl of water. Although eluting with less than 2 x 50 µl of water results in increased RNA concentrations, RNA yields may be reduced.
RNA does not perform well in downstream experiments	
a) Salt carryover during elution	Ensure that Buffer RPE is at 20–30°C. When reusing collection tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels.
b) Ethanol carryover	During the second wash with Buffer RPE, be sure to centrifuge at ≥8000 x *g* (≥10,000 rpm) for 2 min at 20–25°C to dry the RNeasy spin column membrane. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. Perform the optional centrifugation to dry the RNeasy spin column membrane if any flow-through is present on the outside of the column (step 12 of the second protocol, page 25).

Appendix A: General Remarks on Handling RNA

Handling RNA

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

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate nondisposable plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 30), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant), and allow to dry.

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

Disposable plasticware

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

Glassware

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

Solutions

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

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

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

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

Storage of RNA

Purified RNA may be stored at –20 or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

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

Spectrophotometric quantification of RNA

Using the QIAxpert® UV/VIS Spectrophotometer for microvolume analysis

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

Using a standard spectrophotometer

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per milliliter (A_{260} = 1 → 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 33), 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 measurement. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 30). Use the buffer in which the RNA is diluted to blank 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

* 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.

Purity of RNA

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

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 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 31).

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. 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.

* 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.

To prevent any interference by DNA in 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 QuantiTect Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see for Ordering Information, page 41).

Integrity of RNA

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

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

Appendix C: Preparing RNAprotect Stabilized Cells for Simultaneous DNA and RNA Purification

This specialized protocol describes how to prepare cultured or sorted cells stabilized in RNAprotect Cell Reagent for the simultaneous purification of DNA and RNA. The purification procedure requires the use of an AllPrep Kit, such as the AllPrep DNA/RNA Micro Kit or AllPrep DNA/RNA Mini Kit (see page 41 for Ordering Information). Separate eluates of RNA and DNA are simultaneously purified from the same sample.

Important points before starting

- If using the AllPrep Kit for the first time, carefully read the handbook supplied with the kit, especially the "Safety Information", page 6, and "Important Notes", page 14, and determine the appropriate number of cells to be processed.
- Buffer RLT Plus (supplied with the AllPrep Kit) contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the AllPrep handbook for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all spin column centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- Should any problems arise, refer to "Nucleic acids degraded" on page 26 and also to the troubleshooting guide in the *AllPrep Handbook*.

Things to do before starting

- Buffer RLT Plus may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).

- 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 (15–25°C) 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.

Procedure

1. Centrifuge the mix of cells and RNAprotect Cell Reagent for 5 min at 5000 x *g* in an appropriately sized centrifuge tube.

   If transferring the sample to the centrifuge tube from a storage vessel, be sure to resuspend any material deposited at the bottom of the vessel by vortexing or by pipetting up and down. It is important to transfer all sample material to the centrifuge tube; otherwise, DNA and RNA yields will be reduced.

   Note: If the sample was stored at below room temperature (e.g., –20°C), thaw it completely before starting centrifugation.

   Note: A precipitate may form during storage, especially at lower temperatures. This does not affect DNA and RNA purification.

2. Remove the supernatant completely by pipetting.

3. Loosen the pellet by flicking the tube.

   Loosening the pellet facilitates dissolving in Buffer RLT Plus in step 4.

4. Add 350 µl or 600 µl Buffer RLT Plus (see Table 3). Dissolve the pellet completely by vortexing.

   If necessary, add β-ME to Buffer RLT Plus before use (see "Things to do before starting").

   Note: Be sure to dissolve the pellet completely. This can take about 1 min. Incomplete dissolving may lead to inefficient lysis and reduced DNA and RNA yields.

   Note: The dissolved pellet may be turbid. This does not affect DNA and RNA purification.

   The dissolved pellet can be stored at –70°C for several months. After removal from storage, incubate the dissolved pellet at room temperature or at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation at 37°C, which can cause RNA degradation. Proceed to step 5.

Table 3. Volumes of Buffer RLT Plus for lysing pelleted cells

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

5. Proceed immediately with homogenization and DNA and RNA purification according to the cell protocol in the AllPrep handbook.

Appendix D: Preparing RNAprotect Stabilized Cells for DNA Purification

This specialized protocol describes how to prepare cultured or sorted cells stabilized in RNAprotect Cell Reagent for the purification of DNA. The purification procedure requires the use of the DNeasy Blood & Tissue Kit, QIAamp DNA Mini Kit, or other similar QIAGEN kit for genomic DNA purification from animal or human cells (see page 41 for Ordering Information).

Important points before starting

- If using the DNeasy Kit or QIAamp Kit for the first time, carefully read the handbook supplied with the kit, especially the "Safety Information", page 6, and "Important Notes", page 14, and determine the appropriate number of cells to be processed.
- Buffer AL (supplied with the DNeasy or QIAamp Kit) contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the DNeasy or QIAamp handbook for safety information.
- The first step of the procedure requires the use of PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2).*
- The fourth step of the procedure requires the optional use of RNase A* (see page 41 for Ordering Information).
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all spin column centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- Vortexing should be performed by pulse-vortexing for 5–10 s.

* 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.

- Should any problems arise, refer to "Nucleic acids degraded" on page 26 and also to the Troubleshooting Guide in the DNeasy or QIAamp handbook.

Things to do before starting

- Buffer AL may form a precipitate during storage. If necessary, warm to 56°C until the precipitate has fully dissolved.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 5.

Procedure

1. Centrifuge the mix of cells and RNAprotect Cell Reagent for 5 min at 5000 x *g* in an appropriately sized centrifuge tube.

   If transferring the sample to the centrifuge tube from a storage vessel, be sure to resuspend any material deposited at the bottom of the vessel by vortexing or by pipetting up and down. It is important to transfer all sample material to the centrifuge tube, otherwise DNA yields will be reduced.

   Note: If the sample was stored at below room temperature (e.g., –20°C), thaw it completely before starting centrifugation.

   Note: A precipitate may form during storage, especially at lower temperatures. This does not affect DNA purification.

2. Remove the supernatant completely by pipetting.

3. Loosen the pellet by flicking the tube.

   Loosening the pellet facilitates dissolving in PBS in step 4.

4. Add 200 µl PBS, and dissolve the pellet completely by vortexing. Add 20 µl Proteinase K (supplied with the DNeasy or QIAamp Kit).

   Ensure that an appropriate number of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), we recommend using less than the maximum number of cells specified in the DNeasy or QIAamp handbook.

   Note: Be sure to dissolve the pellet completely. This can take about 1 min. Incomplete dissolving may lead to inefficient lysis and reduced DNA yields.

   Note: The dissolved pellet may be turbid. This does not affect DNA purification.

   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C) before continuing with step 5.

5. Add 200 µl Buffer AL (without added ethanol). Mix thoroughly by vortexing, and incubate at 56°C for 10 min.

   Ensure that ethanol has not been added to Buffer AL. Buffer AL can be purchased separately (see page 41 for Ordering Information).

   It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

6. Proceed with adding ethanol (200 µl) and applying the sample to the DNeasy or QIAamp spin column according to the blood protocol in the DNeasy or QIAamp handbook.

Ordering Information

Product	Contents	Cat. no.
RNAprotect Cell Reagent (250 ml)	250 ml RNAprotect Cell Reagent	76526
RNeasy Protect Cell Mini Kit (50)	RNAprotect Cell Reagent (50 ml) and RNeasy Plus Mini Kit (50)	74624
RNeasy Plus Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74134
Accessories		
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201
QIAshredder (50)	50 disposable cell-lysate homogenizers	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers	79656
TissueRuptor II (120 V, 60 Hz, US)	Handheld rotor–stator homogenizer	9002755
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II	990890
Buffer AL (264 ml)	264 ml Lysis Buffer for use in DNA purification	19075
QIAGEN Proteinase K (2 ml)	2 ml proteinase K solution (>600 mAU/ml) for use in DNA purification	19131

Product	Contents	Cat. no.
QIAGEN Proteinase K (10 ml)	10 ml proteinase K solution (>600 mAU/ml) for use in DNA purification	19133
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution). Unit definition: That amount of enzyme causing the hydrolysis of RNA at a rate such that k (velocity constant) equals unity (Kunitz units) at 25°C and pH 5.0.	19101
Related products for sample purification		
RNeasy Plus Micro Kit — for purification of total RNA from small cell and tissue samples using gDNA Eliminator columns		
RNeasy Plus Micro Kit (50)	For 50 micropreps: RNeasy MinElute® Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Water and Buffers	74034
AllPrep DNA/RNA Kits — for simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample		
AllPrep DNA/RNA Micro Kit (50)	For 50 micropreps: AllPrep DNA Spin Columns, RNeasy MinElute Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Water and Buffers	80284
AllPrep DNA/RNA Mini Kit (50)	For 50 minipreps: AllPrep DNA Spin Columns, RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Water and Buffers	80204

Product	Contents	Cat. no.
DNeasy Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
DNeasy Blood & Tissue Kit (250)	250 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69506
QIAamp DNA Mini Kit — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA from human samples		
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Mini Kit (250)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51306
Related products for sample stabilization and purification		
Allprotect® Tissue Reagent — for immediate stabilization of DNA, RNA, and protein in animal and human tissues		
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
RNAprotect Tissue Reagent (50 ml)	50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104

Product	Contents	Cat. no.
RNAprotect Tissue Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAprotect Tissue Reagent each	76154
RNeasy Protect Mini Kit — for immediate stabilization of the gene expression profile in tissues and subsequent RNA purification		
RNeasy Protect Mini Kit (50)*	RNAprotect Tissue Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74124
RNAprotect Bacteria Reagent — for in vivo stabilization of the gene expression profile in bacteria		
RNAprotect Bacteria Reagent	RNAprotect Bacteria Reagent (2 x 100 ml)	76506
RNeasy Protect Bacteria Mini Kit — for in vivo stabilization of the gene expression profile in bacteria and subsequent RNA purification		
RNeasy Protect Bacteria Mini Kit (50)*	RNeasy Mini Kit (50) and RNAprotect Bacteria Reagent (2 x 100 ml)	74524
RNeasy Protect Saliva Mini Kit — for immediate stabilization of RNA in saliva and subsequent total RNA purification		
RNeasy Protect Saliva Mini Kit (50)	RNAprotect Saliva Reagent (50 ml) and RNeasy Micro Kit (50)	74324

* Larger kit size and/or format available; see **www.qiagen.com**

Product	Contents	Cat. no.
PAXgene® Blood RNA Kit — for isolation and purification of intracellular RNA from whole blood stabilized in PAXgene Blood RNA Tubes		
PAXgene Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes*	762164
PAXgene Bone Marrow RNA System — for storage and transport of bone marrow samples and stabilization and purification of intracellular RNA		
PAXgene Bone Marrow RNA Tubes (50)	50 Bone Marrow Collection Tubes. To be used in conjunction with the PAXgene Bone Marrow RNA Kit	764114
PAXgene Bone Marrow RNA Kit (30)	For 30 RNA preps: 30 PAXgene Spin Columns, 30 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Bone Marrow RNA Tubes	764133
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiNova Reverse Transcription Kit (50)	For 50 x 20 µl reactions: 100 µl 8x gDNA Removal Mix, 50 µl Reverse Transcription Enzyme, 200 µl Reverse Transcription Mix (containing RT primers), 100 µl Internal Control RNA, 1.9 ml RNase-Free Water	205411

* PAXgene Blood RNA Tubes can be ordered from BD™ and BD authorized distributors (**www.bd.com**).

Product	Contents	Cat. no.
QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at www.qiagen.com/GeneGlobe)		
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions or 400 x25 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies

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
03/2021	Updated some items in the Kit Contents section, the branding of RNA protection products, Appendices A–C, and the Ordering Information section.

Notes

Notes

Notes

Limited License Agreement for RNAprotect Cell Reagent Handbook

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.

1124208 03/2021 HB-0483-003