April 2021

RNeasy® Protect Saliva Mini Handbook

For immediate stabilization of total RNA in saliva and subsequent RNA purification

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy Protect Saliva Mini Kit **Catalog no** **Number of preps**	**(50)** **74324** **50**
RNAprotect Saliva Reagent (box 1 of 2):	
RNAprotect® Saliva Reagent	50 ml
RNeasy Protect Saliva Mini Handbook*	1
RNeasy Micro Kit (box 2 of 2):	
RNeasy MinElute® Spin Columns (each in a 2 ml Collection Tube)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	100
Buffer RLT†	45 ml
Buffer RW1†	45 ml
Buffer RPE‡ (concentrate)	11 ml
RNase-Free Water	10 ml
Carrier RNA, poly-A	1 vial (300 µg)
RNase-Free DNase Set RNase-Free DNase I (lyophilized) Buffer RDD RNase-Free Water	 1500 units 2 x 2 m 1.5 ml
Handbook*	1

* Follow the instructions in the *RNeasy Protect Saliva Mini Handbook* when stabilizing and purifying RNA from saliva.

† Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 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 Saliva Reagent at room temperature (15–25°C). The reagent is stable for at least 12 months under these conditions, if not otherwise stated on the label.

The RNeasy Micro Kit is shipped at room temperature. Store the RNeasy MinElute spin columns and the RNase-Free DNase Set box (containing RNase-free DNase, Buffer RDD and RNase-free water) immediately upon receipt at 2–8°C. Store the remaining components of the RNeasy Micro Kit dry at room temperature. All components of the RNeasy Micro Kit are stable for at least 9 months under these conditions, if not otherwise stated on the label.

Intended Use

The RNeasy Protect Saliva Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease. All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines.

Safety Information

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

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

Buffer RLT 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.

Quality Control

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

Introduction

The RNeasy Protect Saliva Mini procedure provides a complete solution for the stabilization and purification of total RNA from saliva. RNA in collected saliva is immediately stabilized using RNAprotect Saliva Reagent, and then rapidly purified and concentrated using the RNeasy Micro Kit.

Principle and procedure

RNA stabilization using RNAprotect

Saliva Reagent Immediate stabilization of RNA in a saliva sample is 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 saliva collection due to unspecific and specific RNA degradation as well as to transcriptional induction. Also, since saliva contains bacteria in addition to cells and free-circulating molecules (e.g., nucleic acids) of human origin, it is important to prevent the uncontrolled growth of bacteria, which can also affect the gene expression profile. RNAprotect Saliva Reagent uses a novel patent-pending technology to immediately stabilize the gene expression profile in saliva samples, which can then be stored and transported at ambient temperature.

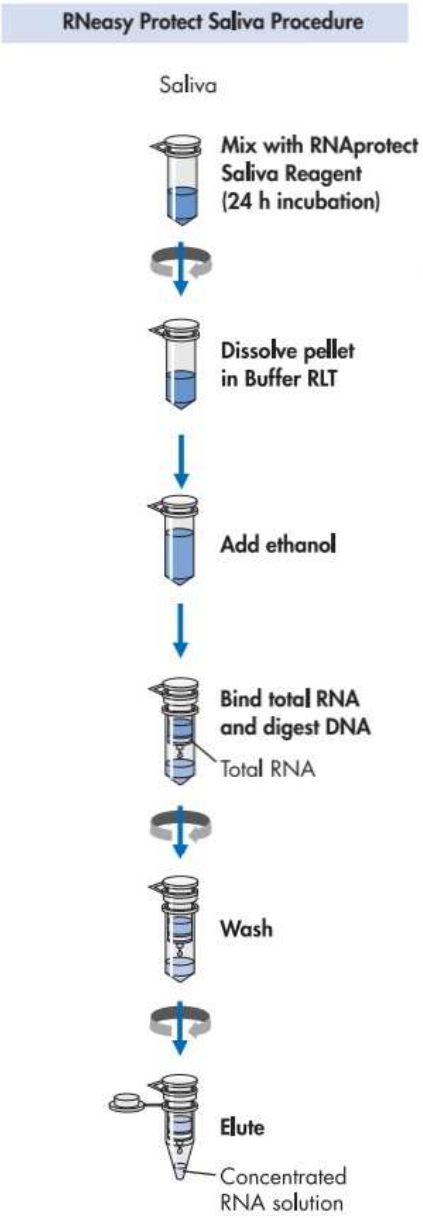
Note: RNA in saliva is already degrading prior to collection and stabilization with RNAprotect Saliva Reagent. This means that RNA purified from saliva samples will be of lower quality than RNA purified from other samples types, such as cultured cells and animal tissues.

RNA purification using the RNeasy Micro Kit

RNeasy Micro technology combines the selective binding properties of a silica–based membrane with the speed of microspin technology. Guanidine-thiocyanate–containing lysis buffer and ethanol are added to the sample to create conditions that promote selective binding of RNA to the RNeasy MinElute membrane. The sample is then applied to the RNeasy MinElute

spin column. RNA binds to the silica membrane. Traces of DNA that may copurify are removed by a DNase treatment on the spin column. DNase and any contaminants are washed away, and high-quality total RNA is eluted in RNase-free water (see flowchart, next page).

With the RNeasy Micro procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure enriches for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA and tRNAs, which together make up 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.


RNeasy Protect Saliva Procedure
Saliva
Mix with RNAprotect Saliva Reagent (24 h incubation)
Dissolve pellet in Buffer RLT
Add ethanol
Bind total RNA and digest DNA
Total RNA
Wash
Elute
Concentrated RNA solution

Equipment and Reagents to Be Supplied by User

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

For saliva collection and RNA stabilization

- Ice
- Vessels for collecting saliva (e.g., 50 ml polypropylene tubes)
- 2 ml microcentrifuge tubes
- Vortexer

For RNA purification

- Ethanol (70% and 96–100%)*
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- Disposable gloves

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

Protocol: Stabilization of RNA in Saliva using RNAprotect Saliva Reagent

Important points before starting

- The subject should refrain from eating, drinking or oral hygiene procedures for at least 1 hour prior to collection of saliva. Optionally, the mouth can be rinsed with water (without swallowing) 5 minutes before saliva collection.
- Collect saliva using a collection vessel with an appropriate volume and a sufficiently large opening (e.g., a 50 ml polypropylene tube). The subject should spit into the collection vessel, but should not cough up mucus.
- During saliva collection, keep the collection vessel on ice to minimize changes in the gene expression pattern. After saliva collection, the sample should be immediately mixed with RNAprotect Saliva Reagent. RNA is not stabilized until the sample is treated with RNAprotect Saliva Reagent.
- Perform the procedure described below as quickly as possible.

Procedure

1. Collect 200 µl saliva in a collection vessel placed on ice. Proceed immediately to step If processing larger volumes of saliva, collect several samples of 200 µl saliva.

   Note: The RNA in the saliva sample is not stabilized until the sample is treated with RNAprotect Saliva Reagent in step 2.

2. Add 200 µl saliva to 1 ml RNAprotect Saliva Reagent in a 2 ml microcentrifuge tube (not supplied). Mix well by vortexing.

   The RNA in the saliva sample is now stabilized.

3. Store the mix of saliva and RNAprotect Saliva Reagent for up to 1 day at 37°C, up to 14 days at room temperature (15–25°C), or up to 28 days at 2–8°C, or archive at –90°C to —15°C. 0.

   Note: We recommend lower storage temperatures whenever possible (e.g., 2–8°C instead of room temperature or room temperature instead of 37°C).

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

   Note: To ensure maximal RNA yields, store the stabilized saliva sample for at least 24 hours before starting RNA purification. If the stabilized samples have been frozen at -95°C or -15°C before extraction, this incubation step can be skipped.

Protocol: Purification of RNA from Stabilized Saliva using the RNeasy Micro Kit

Important points before starting

- If working with RNA for the first time, read the appendix (page 20).
- Saliva samples generally contain very low amounts of RNA. To ensure maximal RNA yields, store the stabilized saliva sample for at least 24 hours before starting RNA purification. Due to the heterogenous nature of saliva, shorter storage times may be sufficient for some samples. However, we still recommend storage for at least 24 hours.
- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the procedure, including centrifugation, at room temperature. During the procedure, work quickly.

Things to do before starting

- Before using the RNeasy Micro Kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) with 6 ml RNase-free water (supplied with the RNeasy Micro Kit).
- 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.
- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized 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 –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Centrifuge the mix of saliva and RNAprotect Saliva Reagent for 10 min at 10,000 x *g* in a microcentrifuge.

   Note: The stabilized saliva sample must be stored for at least 24 hours prior to centrifugation (see "Important points before starting", page 10).

   Note: If the sample was stored at below room temperature (e.g., 2–8°C or –30°C to –15°C), thaw it completely and equilibrate it to room temperature 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 in step 4.

4. Add 350 µl Buffer RLT. Dissolve the pellet completely by vortexing.

   Note: Be sure to dissolve the pellet completely. This can take about 1 min.

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

   The dissolved pellet can be stored at –90°C to –15°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.

5. Add 1 volume (350 µl) of 70% ethanol, and mix well by pipetting or vortexing. Do not centrifuge. Proceed immediately to step 6.

   A precipitate may form after addition of ethanol, but this will not affect the procedure.

6. Transfer the sample to an RNeasy MinElute spin column 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 7.

7. Add 350 µl Buffer RW1 to the RNeasy MinElute 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 8.

8. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube.

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

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

   Note: Be sure to add the DNase I incubation mix directly to the RNeasy MinElute 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.

10. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through* and collection tube.

11. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RPE to the 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 12.

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

* Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

12. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to dry the spin column membrane. Discard the flow-through and collection tube.

    Note: Prepare 80% ethanol using ethanol (96–100%) and the RNase-free water supplied with the kit.

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

13. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    Note: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

14. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

    The dead volume of the RNeasy MinElute spin column is 2 µl; elution with 14 µl RNase-free water results in a 12 µl eluate.

    Elution with smaller volumes of RNase-free water leads to higher total RNA concentrations, but lower RNA yields. RNA yield will be reduced by approximately 20% if RNA is eluted in 8 µl RNase-free water. We do not recommend eluting RNA in less than 8 µl RNase-free water, as the spin column membrane may not be sufficiently hydrated.

    Note: When performing RT-PCR with the purified RNA, we recommend using the QIAGEN OneStep Ahead RT-PCR Kit (cat. no. 220213). This kit contains a specially formulated blend of Omniscript® Reverse Transcriptase (designed for >50 ng RNA) and Sensiscript® Reverse Transcriptase (designed for <50 ng RNA). For quantitative, real-time RT-PCR, we recommend QIAGEN QuantiNova® Kits. See page 27 for ordering information.

Troubleshooting Guide

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

		Comments and suggestions
RNA degraded		
a)	RNA in saliva already degraded prior to collection	RNA purified from saliva is usually less intact than total RNA purified from other samples, such as cells, blood and tissues. To check for RNA degradation, purify RNA from a stabilized saliva sample and an unstabilized saliva sample, and compare the intactness of the purified RNA.
b)	Saliva sample not immediately stabilized	Mix the saliva sample immediately with RNAprotect Saliva Reagent. Collect saliva on ice and as quickly as possible.
c)	Prolonged storage	Saliva mixed with RNAprotect Saliva Reagent can be stored for up to 1 day at 37°C, up to 14 days at 15–25°C, or up to 28 days at 2–8°C, or archived at –90°C to –15°C. We recommend lower storage temperatures whenever possible.
d)	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 the appendix (page 20) for general remarks on handling RNA.
Clogged column		
a)	Sample not completely dissolved	Be sure to completely dissolve the saliva-derived pellet in Buffer RLT (see step 4 of the second protocol).
b)	Too much sample	Be sure to purify RNA from 200 µl saliva per RNeasy MinElute spin column.
c)	Centrifugation temperature too low	Centrifugation at low temperatures (e.g., under refrigeration) can cause the formation of precipitates that can clog the RNeasy MinElute spin column. Perform all steps of the procedure, including centrifugation, at room temperature (15–25°C).

		Comments and suggestions
Low RNA yield		
a)	Sample not completely dissolved	Be sure to completely dissolve the saliva-derived pellet in Buffer RLT (see step 4 of the second protocol).
b)	Too much sample	Be sure to purify RNA from 200 µl saliva per RNeasy MinElute spin column.
c)	RNA still bound to spin column membrane	Repeat RNA elution, but incubate the RNeasy MinElute spin column on the benchtop for 10 min with RNase-free water before centrifuging.
d)	Ethanol carryover After washing with 80% ethanol, be sure to dry the RNeasy	MinElute spin column membrane by centrifuging at full speed for 5 min (see step 13 of the second protocol). After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
e)	80% ethanol not made with RNase-free water	RNases can be introduced if the water used RNase-free water to dilute the ethanol is not RNase-free. Prepare 80% ethanol using ethanol (96–100%) and the RNase-free water supplied with the kit, as described in "Things to do before starting" (page 12).
f)	Insufficient incubation time after mixing saliva with RNAprotect Saliva Reagent	After mixing the saliva sample with RNAprotect Saliva Reagent, store the sample for at least 24 hours before starting the RNA purification procedure.
Low or no recovery of RNA		
a)	RNase-free water incorrectly dispensed	Pipet RNase-free water to the center of the RNeasy MinElute spin column membrane to ensure that the membrane is completely covered.
b)	Ethanol carryover	After washing with 80% ethanol, be sure to dry the RNeasy MinElute spin column membrane by centrifuging at full speed for 5 min (see step 13 of the second protocol). After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
c)	80% ethanol not made with RNase-free water	RNases can be introduced if the water used to dilute the ethanol is not RNase-free. Prepare 80% ethanol using ethanol (96–100%) and the RNase-free water supplied with the kit, as described in "Things to do before starting" (page 12).
DNA contamination in downstream experiment		
	No DNase treatment	Be sure to perform the on-column DNase digestion as described in steps 8–9 of the second protocol.

		Comments and suggestions
RNA does not perform well in downstream experiments		
a)	Ethanol carryover	After washing with 80% ethanol, be sure to dry the RNeasy MinElute spin column membrane by centrifuging at full speed for 5 min (see step 13 of the second protocol). After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
b)	Salt carryover during elution	Ensure that Buffer RPE is at room temperature and ethanol is added as described on the bottle.
c)	Reverse transcription with too small an amount of RNA	Most reverse transcriptases are intended for use with approximately 1 µg RNA. When performing reverse transcription with very small amounts of RNA, we recommend using the QIAGEN Sensiscript RT Kit, which is specially designed for highly sensitive reverse transcription using <50 ng RNA. For one-step RT-PCR and quantitative, real/=time RT-PCR, we recommend the QIAGEN OneStep Ahead RT-PCR Kit and QuantiNova RT-PCR Kits, respectively. These kits contain a specially formulated blend of Omniscript and Sensiscript Reverse Transcriptases for amplification of a wide range of RNA amounts, from as little as 1 pg per reaction.
d)	Too much sample	Be sure to purify RNA from 200 µl saliva per RNeasy MinElute spin column.
e)	Insufficient incubation time after mixing saliva with RNAprotect Saliva Reagent	After mixing the saliva sample with RNAprotect Saliva Reagent, store the sample for at least 24 hours before starting the RNA purification procedure.

Appendix A: General Remarks on Handling RNA

Handling RNA

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

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 21), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant) and allow to dry.

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

Disposable plasticware

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

Solutions

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

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

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

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

Storage of RNA

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

Quantification of RNA

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

Spectrophotometric quantification of RNA

Using the QIAxpert UV/VIS Spectrophotometer for microvolume analysis

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

Using a standard spectrophotometer

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

this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 24), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 21). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

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

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

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

Purity of RNA

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

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

For standard photometric measurements, the ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution. For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 23).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. RNeasy Kits will, however, remove the vast majority of cellular DNA. gDNA Eliminator Solution helps to further reduce genomic DNA contamination; however, trace amounts of genomic DNA may still remain, depending on the amount and nature of the sample. For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

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

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

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

DNA cannot be avoided, we recommend using the QuantiTect Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see ordering information, page 27).

Integrity of RNA

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

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

Ordering Information

Product	Contents	Cat. no.
RNeasy Protect Saliva Mini Kit (50)	RNAprotect Saliva Reagent (50 ml) and RNeasy Micro Kit (50)	74324
Accessories		
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201
Buffer RLT (220 ml)	220 ml Buffer RLT	79216
RNase-Free DNase Set (50)	1500 units RNase-Free DNase I, RNase-Free Buffer RDD and RNase-Free Water	79254
RNeasy Micro Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free DNase I, Carrier RNA, RNase-Free Reagents and Buffers	74004
Related products for RNA stabilization and purification		
RNAprotect Tissue Reagent — for immediate stabilization of RNA in tissues		
RNAprotect Tissue Reagent (50 ml)	50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Reagent (250 ml)	250 ml RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples	76106

Product	Contents	Cat. no.
RNAprotect Tissue Tubes — for collection, RNA stabilization, transport, and storage of tissues		
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
RNeasy Protect Mini Kit — for immediate stabilization of the gene expression profile in animal 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
RNeasy Protect Mini Kit (250)	RNAprotect Tissue Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74126
RNAprotect Bacteria Reagent — for in vivo stabilization of the gene expression profile in bacteria		
RNAprotect Bacteria Reagent	RNAprotect Bacteria Reagent (2 x 10 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

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† 762174‡
Related products for downstream applications		
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 faster one-step RT-PCR with high sensitivity, specificity and fidelity		
QIAGEN OneStep Ahead RT-PCR Kit	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

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Product	Contents	Cat. no.
QuantiNova Reverse Transcription Kit — for fast cDNA synthesis and reproducible real-time two-step RT-PCR		
QuantiNova Rev. 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
QuantiNova SYBR® Green PCR Kit— for unparalleled result using SYBER Green based qPCR		
QuantiNova SYBR® Green PCR Kit (500)	For 500 x 20 µl reactions: 3 x 1.7 ml 2x QuantiNova SYBR® Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 1 ml QN ROX Reference Dye, 1.9 ml Water	208054
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

Product	Contents	Cat. no.
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
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 20µl reactions: 500µl QuantiNova Multiplex PCR Master Mix, 500µl 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

Product	Contents	Cat. no.
QuantiNova Multiplex RT-PCR Kit (2500)	For 2500 x 20 µl reactions: 10 x 1.3 ml 4x QuantiNova Multiplex RT-PCR Master Mix, 5 x 100 µl QuantiNova Multiplex RT-Mix, 3 x 100 µl QuantiNova IC RNA, 3 x 500 µl QuantiNova Yellow Template Dilution Buffer, 3 x 1 ml QN ROX Reference Dye, 20 x 1.9 ml RNase-Free Water	

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

† Canada and USA only.

‡ Rest of the world; kit not available in all countries.

§ Recommended for all other cyclers.

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
April 2021	Updated branding of RNA protection products. Added information to a note about stabilization that clarifies the storage procedure.

Notes

The PAXgene Blood RNA Kit (cat. nos. 762164 and 762174) is for in vitro diagnostic use. Not available in all countries.

Purchase of QIAGEN products for PCR containing HotStarTaq DNA Polymerase is accompanied by a limited license to use them in the polymerase chain reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.

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Patents of third parties in certain countries may cover the process of multiplex PCR or of certain applications.