January 2021

QIAamp® 96 Viral RNA Handbook

For high-throughput purification of viral RNA from respiratory swab samples

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp 96 Viral RNA Kit	**(10)**
Catalog no.	**52962**
Number of preps	**960**
QIAamp 96 plates	10
Buffer AVL*	4 x 155 ml
Buffer AW1* (concentrate)	2 x 190 ml
Buffer AW2† (concentrate)	2 x 127 ml
Buffer AVE†	1 x 125 ml
Carrier RNA (poly A)	4 x 1550 µg
TopElute Fluid	1 x 60 ml
Tape Pad	1
AirPore Tape Sheet	2
Quick-Start Protocol	1

* Contains chaotropic salt. Not compatible with disinfectants containing bleach. See “Safety Information” on page 5.

† Contains sodium azide as preservative.

Shipping and Storage

The QIAamp 96 Viral RNA Kit, including all reagents and buffers, is shipped at room temperature (15–25°C). It should be stored immediately upon receipt in a dry place at room temperature. When stored correctly, the QIAamp 96 Viral RNA Kit is good until the expiration date printed on the kit box lid. Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality (see expiration date for maximum storage time).

Intended Use

The QIAamp 96 Viral RNA Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The QIAamp 96 Viral RNA Kit is for the extraction of total RNA from human respiratory swab samples and can be used with a centrifuge or be automated on the QIAcube® HT or other liquid handling instruments (please refer to the product page for more information).

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

Buffers AVL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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.

If liquid containing potentially infectious agents is spilt on the QIAcube HT instrument, refer to the instrument user manual for decontamination instructions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAamp 96 Viral RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp 96 Viral RNA Kit replaces time-consuming and tedious viral RNA purification by enabling simultaneous purification of viral RNA from up to 96 samples in one plate. The QIAamp 96 Viral RNA Kit can be used to isolate viral RNA from a wide variety of viruses. RNA purification of SARS-CoV-2 viruses from swab material has been specifically tested and verified during kit development. Updates will be released as performance data for other virus types are generated.

Warnings and precautions

RNA is extremely sensitive to RNases and should always be prepared with due care. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Please read "Appendix A: General Remarks on Handling RNA" (page 24) before starting. PCR should always be carried out using good laboratory practices. Accordingly, a PCR laboratory should always be divided into 3 areas: reagent preparation, sample preparation, and amplification and detection. The high sensitivity of PCR necessitates that all reagents remain pure and uncontaminated; they should be monitored carefully and routinely. Contaminated reagents must be discarded.

Handling of QIAamp 96 RNA plates

The sensitivity of nucleic acid amplification technologies necessitates the following precautions when handling QIAamp 96 Plates, to avoid cross-contamination between sample preps:

- Carefully apply the sample or solution to the QIAamp 96 plate well. Pipet the sample into the center of the respective well.
- Change pipette tips between all liquid transfer steps. The use of aerosol-barrier tips is recommended.
- Avoid touching the QIAamp membrane with the pipette tip.
- Wear gloves throughout the procedure. In case of contact between gloves and sample, change gloves immediately

Principle and procedure

The QIAamp 96 Viral RNA Kit uses well-established technology for RNA preparation in a 96-well format. The kit combines the selective binding properties of silica-based membrane with the speed of centrifuge processing.

The QIAamp 96 Viral RNA purification procedure (Figure 1) eliminates risk of cross-contamination and allows safe handling of potentially infectious samples. Samples are lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to bind RNA to the membrane of the QIAamp 96 plates. Contaminants are efficiently removed using 2 wash buffers and a final wash with ethanol. High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage.

The purified RNA is free of proteins, nucleases, and other contaminants or inhibitors. The special QIAamp membrane guarantees extremely high recovery of pure, intact RNA without the use of phenol/chloroform extraction or alcohol precipitation.

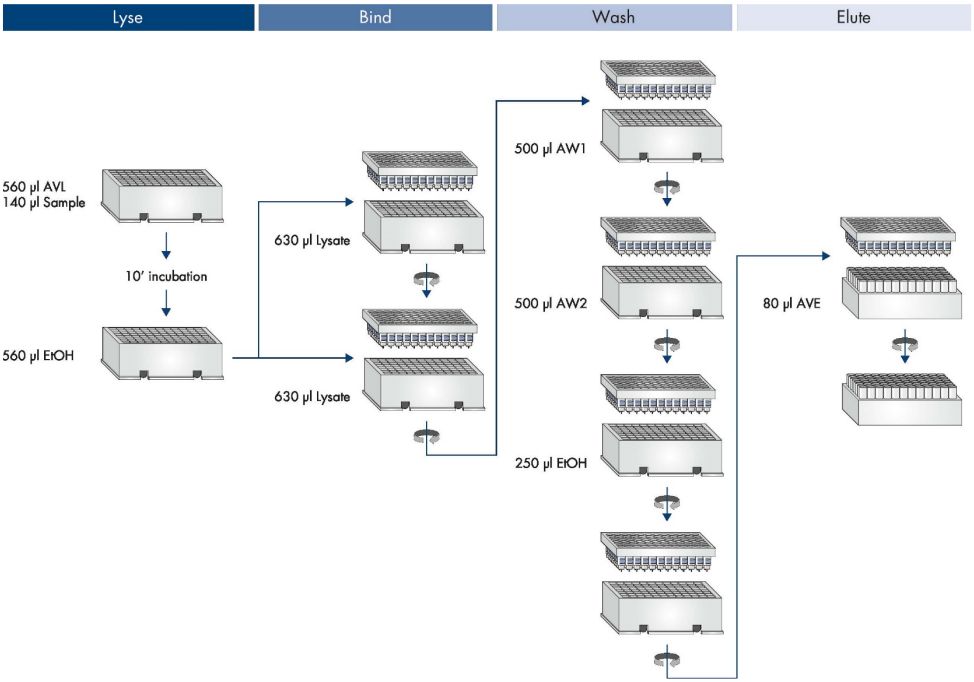

Figure 1. The QIAamp 96 Viral RNA Kit workflow.

Adsorption to the QIAamp membrane

The buffering conditions of the lysate must be adjusted to provide optimum binding conditions for the viral RNA, which is subsequently adsorbed onto the QIAamp silica membrane. Salt and pH conditions in the lysate ensure that protein and other contaminants, which could inhibit downstream enzymatic reactions, are not co-purified.

Removal of residual contaminants

Viral RNA that is bound to the QIAamp membrane is cleared of contaminants through 3 short washing steps. The use of Buffer AW1, Buffer AW2, and 96–100% ethanol significantly improves the purity of the eluted RNA. Optimized wash conditions completely remove residual contaminants without affecting RNA binding.

Elution with Buffer AVE

Buffer AVE is RNase-free water that contains 0.04% sodium azide to prevent microbial growth and subsequent contamination with RNases. Sodium azide affects spectrophotometric absorbance readings between 220 nm and 280 nm but has no effect on downstream applications such as RT-PCR. If determination of RNA purity in eluate by spectrophotometric analysis is required, we recommend elution with RNase-free water instead of Buffer AVE.

Sample volumes

QIAamp 96 plates can bind RNA greater than 200 nucleotides in length. Actual yield depends on sample size, sample storage, and virus titer. The procedure is optimized for sample volumes of 140 µl samples. If the sample volume is less than 140 µl, it should be adjusted to 140 µl with phosphate-buffered saline (PBS) before loading.

Lysis

Samples are first lysed under highly denaturing conditions provided by Buffer AVL to inactivate RNases and to ensure isolation of intact viral RNA. Carrier RNA added to Buffer AVL improves the binding of viral RNA to the QIAamp membrane, especially in the case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity.

Carrier RNA

Carrier RNA serves 2 purposes: First, it enhances binding of viral nucleic acids to the QIAamp membrane, especially if there are very few target molecules in the sample. Secondly, the addition of large amounts of carrier RNA reduces the chance of viral RNA degradation in the rare event that RNase molecules escape denaturation by the chaotropic salts in Buffer AVL. Not adding carrier RNA to Buffer AVL may lead to reduced viral RNA recovery.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer AVL supplied with the kit. Carrier RNA concentration has been adjusted so that the QIAamp 96 Viral RNA Kit can be used as a generic purification system compatible with many different amplification systems.

Different amplification systems vary in efficiency, depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain both viral nucleic acids and carrier RNA, and amounts of carrier RNA will greatly exceed amounts of viral nucleic acids. Therefore, calculations of how much eluate to add to downstream amplifications should consider the amount of carrier RNA added. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer AVL.

Addition of internal controls

Combining the QIAamp 96 Viral RNA protocols with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control RNA or DNA should be added with the carrier RNA to the lysis buffer. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides.

Refer to the manufacturer's instructions to determine the optimal concentration. Using a concentration other than what is recommended may reduce amplification efficiency.

Determination of yield and length of viral RNA

Yield of viral RNA isolated from biological samples is normally less than 1 µg and therefore difficult to determine photometrically. Carrier RNA (5.6 µg per 140 µl sample) will account for most of the RNA present. We recommend quantitative RT-PCR for determining viral RNA yield.

The size distribution of viral RNA purified using QIAamp 96 plates can be checked by denaturing agarose gel electrophoresis, followed by hybridization with a virus-specific labeled probe and autoradiography (Sambrook, J. and Russell, D. W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). Keep in mind that the broad size distribution of RNA smear, seen on a denaturing agarose gel, will reflect the size distribution of the spiked-in synthetic carrier 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.

- Pipettes and disposable pipette tips with aerosol barriers
- Ethanol (96–100%)*

Important: Alternative materials may also be used. For requirements and examples, see "Alternative centrifuges" (page 13) and "Alternative consumables" (page 13). For the latest updates on compatible instruments and consumables, visit the QIAamp 96 Viral RNA product page at **www.qiagen.com**.

- S-Blocks (24) (cat. no. 19585)
- Elution Microtubes CL (24 x 96) (cat. no. 19588)
- Plate Rotor 2 x 96 (cat. no. 81031)
- Centrifuge 4-16S or 4-16KS (cat. nos. vary; see **www.qiagen.com**)

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

Alternative centrifuges

The following centrifuges may also be compatible with "Protocol: Manual Processing with a Centrifuge". QIAGEN has not performed experimental testing of compatibility with these instruments; final confirmation of compatibility needs to be performed by the user:*

- Rotanta 460 (Hettich cat. no. 5650) with Swing-out Rotor (Hettich cat. no. 5622)
- Rotanta R (Hettich cat. no. 5660) with Swing-out Rotor (Hettich cat. no. 5622)
- Similar centrifuges with swing-out rotor, minimum 5788 x *g* capacity, and the ability to accommodate plate assemblies with a height of minimum 75 mm or more when using QIAGEN S-Blocks and Elution Microtubes CL.†

Alternative consumables

The following consumables may also be compatible with the protocol for processing with a centrifuge. QIAGEN has not extensively tested these consumables; final confirmation of compatibility needs to be performed by the user:*

- S-Block alternatives
  - Deepwell Plate 2 ml (e.g., Eppendorf cat. no. 0030501306)
  - BioBlock™ Deep Well Plate (Simport cat. no. T110-10)
  - Other 96-well plates with 2 ml capacity in SBS-format; blocks need to withstand a force of 5788 x *g*
- Elution microtube rack alternatives
  - Deepwell Plate 96/500 µl (Eppendorf cat. no. 0030501101)
  - Other elution microtube racks in SBS format and with 500 µl minimum capacity; blocks need to withstand a force of 5788 x *g*

* This is not a complete list of suppliers and does not include many alternative vendors.

† Required height compatibility might be higher, depending on centrifuge-specific assemblies, such as holders, that need to be incorporated

Important Notes

If preparing RNA for the first time, see "Appendix A: General Remarks on Handling RNA", page 24. All steps of the QIAamp 96 Viral RNA protocol should be performed quickly and at room temperature (15–25°C).

The QIAamp 96 Viral RNA procedure isolates all RNA molecules larger than 200 nucleotides. Smaller RNA molecules will not bind quantitatively under the conditions used.

Starting material

Swabs

The standard starting material for QIAamp 96 Viral RNA are respiratory swab samples. QIAGEN has tested compatibility to this sample material. Other sample materials may be compatible with the kit. Please refer to the product page for up-to-date information on sample materials that have been tested by QIAGEN.

Handling of starting material

Swabs should be placed immediately into a sterile transport tube containing 2–3 ml of either viral transport medium (VTM) or sterile saline. If particles are visible, the swabs should first be liquefied or centrifuged for 10 min at 1500 x *g*, to avoid clogging during isolation procedure.

Protocol: Preparation of Reagents

This procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing Buffer AVL.

Note: The usage of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.

Important points before starting

- Buffer AVL–carrier RNA should be freshly prepared and is stable at 2–8°C for up to 48 h. This solution develops a precipitate when stored at 2–8°C and must be redissolved by warming at 80°C before use.
- Do not warm Buffer AVL–carrier RNA solution more than 6 times. Do not incubate at 80°C for more than 5 min. Frequent warming and extended incubation will degrade carrier RNA, which might lead to reduced recovery of viral RNA and, eventually, false-negative RT-PCR results. This is particularly the case with low-titer samples.
- Buffer AW1* is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle.
- Buffer AW2† is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle.

Things to do before starting

- Equilibrate all buffers to room temperature before use.

* Contains chaotropic salt. Not compatible with disinfectants containing bleach. See "Safety Information" on page 5.
† Contains sodium azide as a preservative.

Procedure: Preparation of carrier RNA–Buffer AVL solution

1. Add 1550 µl Buffer AVE to the tube containing 1550 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl.
   Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –30 to –15°C.

   Note: Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

2. Check Buffer AVL* for precipitate, and if necessary incubate at 80°C until the precipitate is dissolved.

   2a. Calculate the volume of Buffer AVE–carrier RNA mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 1.

Table 1. Volumes of Buffer AVL and Buffer AVE–carrier RNA mix required for the QIAamp 96 Viral RNA procedure

No. of samples	Buffer AVL [ml]	Buffer AVE–carrier RNA [µl]
8	5	50
16	10	100
24	15	150
32	20	200
40	24	240
48	29	290
56	34	340
64	39	390
72	44	440
80	48	480
88	53	530
96	58	580

Note: Buffer AVL values and Buffer AVE–carrier RNA values are rounded up to simplify pipetting.

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See page 5 for safety information.

2b. For larger numbers of samples, calculate volumes using the following sample calculation:

$$\boldsymbol{n} \; x \; 0.6 \; ml = \boldsymbol{y} \; ml$$

$$\boldsymbol{y} \; ml \; x \; 10 \; \mu l/ml = \boldsymbol{z} \; \mu l$$

Where: $\boldsymbol{n}$ = number of samples to be processed simultaneously

$\boldsymbol{y}$ = calculated volume of Buffer AVL

$\boldsymbol{z}$ = volume of carrier RNA–Buffer AVE to add to Buffer AVL

3. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Protocol: Manual Processing with a Centrifuge

Important points before starting

- Centrifugation of QIAamp 96 plates is usually performed at 5788 x *g* (6000 rpm when processed with Centrifuge 4-16 or 1-16KS). The speed limit of the centrifuge is programmed so that the required *g*-force will not be exceeded. If an alternative centrifuge with lower *g*-force is used, centrifugation times need to be adapted respectively. Centrifugation times for processing with a lower *g*-force than 5788 x *g*, but with at least 3486 x *g*, are given in the protocol in brackets.
- All centrifugation steps are carried out at room temperature. Use an AirPore tape sheet to seal the QIAamp 96 plate during all centrifugation steps except step 11.
- **Important**: Do not cool the centrifuge. For the final dry spin of the plate, it is of highest importance that the centrifuge heats up during the centrifugation, to evaporate the residual washing buffer.
- **Important**: Do not centrifuge the Sigma® plate carriers without the QIAamp 96 plates and S-Blocks inside the bucket. If unsupported, the carriers will collapse under high *g*-forces. Remove the carriers during test runs.
- Standard microtiter plates may be centrifuged in the same carriers (the silver brackets inside the buckets) if the *g*-force does not exceed 500 x *g*.

Things to do before starting

- Set up the centrifuge by referring to the user manual.
- Make sure that the correct rotor is defined and the centrifugation speed is adjusted to achieve a force of 5788 x *g* (6000 rpm if using Sigma centrifuge 4-16S or 4-16KS).

Procedure

1. Pipet 560 µl Buffer AVL–carrier RNA into each well of an S-Block.

2. Add 140 µl samples to the S-Block by touching the insides of the wells. Mix thoroughly by pipetting up and down 8 times.
   Note: To ensure efficient lysis, mix the sample thoroughly with Buffer AVL to yield a homogeneous solution. Samples that have only been thawed once can also be used.
3. Seal the S-Block with Tape Pad and incubate at room temperature for 10 min.
   Note: Incubation times longer than 10 min have no effect on the yield or quality of the purified RNA.
4. Carefully remove the Tape Pad and add 560 µl ethanol (96–100%) to the sample. Mix thoroughly by pipetting up and down 8 times.
   Note: Use only ethanol, because other alcohols may result in reduced RNA yield and purity. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. To ensure efficient binding, mix the sample thoroughly with the ethanol to yield a homogeneous solution.
5. Place a QIAamp 96 Plate on a new S-Block. Pipet 630 µl of the solution from the previous step into the QIAamp 96 Plate. Seal the QIAamp 96 plate with an AirPore tape sheet.
6. Place the assembly from step 5 on a carrier into a rotor bucket. Centrifuge at 5788 x *g* for 4 min (7 min with at least 3486 x *g*). If solution has not completely passed through the membrane, centrifuge again.
7. Carefully remove the AirPore tape sheet and repeat steps 5 and 6 with the remaining lysate. The QIAamp 96 Plate should not be removed from the S-Block until all the lysate has gone through. Discard the S-Block that contains the flow-through afterwards.
8. Place the QIAamp 96 Plate on a new S-Block. Carefully remove the AirPore tape sheet, and add 500 µl Buffer AW1 to each well. Seal the QIAamp 96 plate with a new AirPore tape sheet. Load the S-Block and QIAamp 96Viral RNA plate onto the carrier, then place it in the rotor bucket. Centrifuge at 5788 x *g* for 4 min (7 min with at least 3486 x *g*).
9. Carefully remove the AirPore tape sheet and add 500 µl Buffer AW2. Seal the QIAamp 96 Plate with new AirPore tape sheet. Load the S-Block and QIAamp 96 Viral RNA Plate

onto the carrier, and then place it in the rotor bucket. Centrifuge at 5788 x *g* for 5 min (7 min with at least 3486 x *g*).

10. Remove the AirPore tape sheet and add 250 µl 96–100% ethanol. Seal the QIAamp 96 Plate with new AirPore tape sheet. Load the S-Block and the QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 5 min at 5788 x *g* (7 min with at least 3486 x *g*). Discard the S-Block with the flow-through.
11. Place the QIAamp 96 Plate on a new S-Block. Remove the AirPore tape sheet and load the S-Block and QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 10 min at 5788 x *g* (25 min with at least 3486 x *g*) without AirPore tape sheet to dry the membrane. Discard the S-Block.

    Note: It is important to set the temperature of the centrifuge to 40°C to avoid that the cooling of the centrifuge is activated. The centrifuge must heat-up during the dry spin in order to efficiently evaporate residual washing buffer. It is also important not to use an AirPore tape, in order to allow for air convection
12. Place the QIAamp 96 plate in a clean 96-well elution plate. Add 80 µl Buffer AVE equilibrated to room temperature, and incubate at room temperature for 1 min.
13. Load the S-Block and the QIAamp 96 Plate onto the carrier, and then place it in the rotor bucket. Centrifuge at 5788 x *g* for 4 min (7 min with at least 3486 x *g*). If storage is required, seal the wells of the microtubes.

Troubleshooting Guide

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

		Comments and suggestions
Little or no RNA in the eluate		
a)	Carrier RNA not added to Buffer AVL	Reconstitute carrier RNA in Buffer AVE and mix with Buffer AVL as described in **Table 1**, page 16. Repeat the purification procedure with new samples.
b)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –30 to –15°C, or it underwent multiple freeze–thaw cycles. Alternatively, Buffer AVL–carrier RNA mixture was stored for more than 48 h at 2–8°C. Prepare a new tube of carrier RNA dissolved in Buffer AVE, and mix with Buffer AVL. Repeat the purification procedure with new samples.
c)	Sample frozen and thawed more than once	Avoid repeated freezing and thawing. Always use fresh samples or samples thawed only once.
d)	Low concentration of virus in the sample	Concentrate the sample volume to 140 µl using a microconcentrator. Repeat the RNA purification procedure with a new sample.
e)	Inefficient protein denaturation in Buffer AVL	Precipitate formed in Buffer AVL–carrier RNA after storage at 2–8°C was not redissolved by heating before starting the procedure. Redissolve the precipitate and repeat the procedure with a new sample.
f)	Buffer AVL prepared incorrectly	Check Buffer AVL for precipitate. Dissolve precipitate by incubation at 80°C.
g)	No ethanol added to the lysate (step 4, page 19)	Repeat the purification procedure with a new sample.
h)	Low percentage ethanol used	Repeat the purification procedure with a new sample. Use 96–100% ethanol in step 4 of "Protocol: Manual Processing with a Centrifuge". Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
i)	Isopropanol used instead of ethanol	We recommend the use of ethanol, because isopropanol reduces yields.

		Comments and suggestions
i)	RNA degraded	Often RNA is degraded by RNases in the starting material (plasma, serum, body fluids). Ensure that the samples are processed quickly. If necessary, add RNase inhibitor to the sample. Check for RNase contamination of buffers and water, and ensure that no RNase is introduced during the procedure.
k)	RNase contamination in Buffer AVE	Discard contaminated Buffer AVE. Repeat the purification procedure with a new sample and a fresh tube of Buffer AVE. Additional Buffer AVE is available separately.
l)	Buffer AW1 or AW2 prepared incorrectly	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with correct volumes of pure (96–100%) ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample.
m)	Buffer AW1 or Buffer AW2 prepared with 70% ethanol	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.
n)	Wash buffers used in the wrong order	Ensure that Buffer AW1 and Buffer AW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
RNA does not perform well in subsequent enzymatic reactions		
a)	Little or no RNA in the eluate	Check "Little or no RNA in the eluate" (above) for possible reasons.
b)	Inefficient virus lysis in Buffer AVL	Precipitate has formed in Buffer AVL–carrier RNA due to temperature change before start of process. Repeat the procedure with new samples, and ensure that no precipitate has formed in Buffer AVL–carrier RNA at the beginning of the process.
c)	Buffer AVL prepared incorrectly	Ensure that carrier RNA has been reconstituted in Buffer AVE and added to Buffer AVL (see page 15).
d)	Too much carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your RT-PCR. Adjust accordingly the concentration of carrier RNA added to Buffer AVL.
e)	Reduced sensitivity	Determine the maximum volume of eluate suitable for your RT-PCR. Reduce the volume of eluate added to the RT-PCR.
f)	Wash buffers used in the wrong order	Ensure that Buffer AW1 and Buffer AW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
g)	New combination of reverse transcriptase and *Taq* DNA polymerase used	If enzymes are changed, it may be necessary to readjust the amount of carrier RNA solution added to Buffer AVL.

		Comments and suggestions
General handling		
a)	Clogged membrane	If respiratory samples are too viscous, the membranes may clog during the binding. Please use pretreatment describe in "Appendix B: Sample Pretreatment for Respiratory Samples" to liquefy the samples prior isolation.
b)	Cross-contamination between samples	To avoid cross-contamination when handling QIAamp plates, follow the guidelines in "Handling of QIAamp 96 RNA plates" on page 7. Repeat the purification procedure with new samples.

Appendix A: General Remarks on Handling RNA

Ribonucleases (RNases) are very stable and active enzymes. RNases are very stable and therefore difficult to inactivate. Even minimal amounts are sufficient to destroy RNA. Make sure that every plasticware or glassware that comes into contact with the solution has undergone sufficient RNase decontamination. 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, take the following precautions 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.

RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipettes and electrophoresis tanks) can be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 25), 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, 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 h (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in "Solutions", page 25.

Solutions

Solutions should be purchased RNase-free or treated with 0.1% DEPC. We recommend purchasing RNase-free water, because if trace amounts of DEPC remain after autoclaving buffer, purine residues in RNA might be modified by carbethoxylation, and performance of enzymatic reactions such as PCR may be negatively affected. Therefore, residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for at least 15 min.

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. 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 h at 37°C. Autoclave for 15 min 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, use RNase-free water to dissolve Tris to make the appropriate buffer.

* 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: Sample Pretreatment for Respiratory Samples

This protocol is intended for swabs from viscous respiratory samples. Nonviscous respiratory samples do not require pretreatment and can be used directly as starting material.

Procedure

1. Liquefy the sample according to either step 1a, 1b, or 1c:
   1a. Add 1 volume of Sputasol solution to 1 volume of sample, and shake well. Place in a 37°C water bath, and incubate with periodic shaking until the sample is completely liquefied.
   1b. Mix 1 volume of sample with 1 volume of NAC buffer (10 g N-acetylcysteine per liter of 0.9% NaCl solution).
   Note: If the sample is very viscous or solid (e.g., when working with lower respiratory samples), try to disrupt it mechanically by pipetting up and down. Incubate at room temperature for 30 min with constant shaking.
   Note: For easier pipetting, it may be necessary to cut off the end of the pipette tip. If the sample is solid, the incubation time needs to be increased to completely liquefy the sample.
   1c. Mix 1 volume of sample with 1 volume of 1 x PBS, or Buffer AE (cat. no. 19077). Add freshly prepared DTT to a final concentration of 0.15% (w/v). Incubate the sample at 37°C until the sample is completely liquefied.
2. Centrifuge the liquefied sample to pellet debris, and transfer the clear supernatant to a clean tube.
3. Use 140 µl lysate as starting material and proceed directly with “Protocol: Preparation of Reagents” on page 15.

Ordering Information

Product	Contents	Cat. no.
QIAamp 96 Viral RNA Kit	For 960 preps: QIAamp 96 plates, Buffers, RNase-Free Water	52962
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
Buffer AVL	155 ml Viral Lysis Buffer, 1550 µg Carrier RNA	19073
Carrier RNA(poly A)	Carrier RNA(poly A) 12x1350µg-15-25°C,KG	1017647
Buffer AW1	242 ml Wash Buffer (1) concentrate	19081
Buffer AW2	324 ml Wash Buffer (2) concentrate	19072
RNase-Free Water	12 x 1.9 ml RNase-free water prepared without the use of diethylpyrocarbonate (DEPC)	129112

Product	Contents	Cat. no.
For centrifugation-based processing		
Centrifuge 4-16KS	Refrigerated universal laboratory centrifuge with brushless motor	Varies*
Centrifuge 4-16S	Universal laboratory centrifuge with brushless motor	Varies*
Plate Rotor 2 x 96	Plate rotor for QIAGEN 96-well plates to be used with Centrifuge 4-16S or 4-16KS	81031

* QIAGEN offers region-specific configurations. Please see **www.qiagen.com** or contact Technical Service for more information.

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Document Revision History

Date	Changes
06/2020	Initial revision
01/2021	Corrected occurrences of “Buffer AVE” to “Buffer AVL” and occurrences of “Buffer AVL–carrier” to “Buffer AVE–carrier” in the “Procedure: Preparation of carrier RNA–Buffer AVL solution” section. Updated "Protocol: Manual Processing with a Centrifuge": added text with regards to centrifugation times. Corrected the content (number of preps) of the QIAamp 96 Viral RNA Kit in the “Ordering Information” section.

Notes

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