February 2020

QIAamp® MinElute® Virus Spin Handbook

For simultaneous purification of viral RNA and DNA from plasma, serum, and cell-free body fluids

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp MinElute Virus Spin Kit **Catalog no.** **Number of preps**	**(50)** **57704** **50**
QIAamp MinElute Columns	50
Collection Tubes (2 ml)	200
Buffer AL*	12 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer AVE† (tubes with purple caps)	5 x 2 ml
Protease Resuspension Buffer†	6 ml
Carrier RNA (tubes with red caps)	310 µg
QIAGEN® Protease‡	1 vial

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 6 for safety information

† Contains sodium azide as a preservative.

‡ Resuspension volume 1.4 ml. See "Preparation of QIAGEN Protease", page 16.

Storage

QIAamp MinElute columns should be stored at 2–8°C upon arrival.

All buffers can be stored at room temperature (15–25°C).

Lyophilized carrier RNA can be stored at room temperature (15–25°C) until the expiration date on the kit box. Carrier RNA can only be dissolved in Buffer AVE; dissolved carrier RNA should be immediately added to Buffer AL as described on page 17. This solution should be prepared fresh, and is stable at 2–8°C for up to 48 hours. Unused portions of carrier RNA dissolved in Buffer AVE should be frozen in aliquots at –20°C.

Lyophilized QIAGEN Protease can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance.

QIAGEN Protease reconstituted in Buffer AVE or Protease Resuspension Buffer is stable for up to 1 year when stored at 2–8°C, but only until the kit expiration date. Keeping the QIAGEN Protease stock solution at room temperature for prolonged periods of time should be avoided.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAamp MinElute Virus Spin Kits is tested against predetermined specifications to ensure consistent product quality.

Intended Use

The QIAamp MinElute Virus Spin Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

All due care and attention should be exercised in the handling of the 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 waste containing Buffer AL or Buffer AW1.

Buffer AL and Buffer AW1 contain guanidine hydrochloride, which 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*.

* 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.

Introduction

The QIAamp MinElute Virus Spin Kit uses well-established technology for simultaneous purification of viral DNA and RNA. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 150 µl. The procedure is suitable for use with plasma, serum, and other cell-free body fluids. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once (see page 16). Viral nucleic acids are eluted in Buffer AVE, ready for use in amplification reactions or storage at –20°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

Principle and procedure

The QIAamp MinElute Virus Spin procedure comprises 4 steps (lyse, bind, wash, elute) and is carried out using QIAamp MinElute columns in a standard microcentrifuge or fully automated on the QIAcube Connect (see "Automated purification of nucleic acids on QIAcube Instruments", page 12). The procedure is designed to ensure that there is no sample-to-sample cross-contamination and allows safe handling of potentially infectious samples. The simple QIAamp MinElute Spin procedure, which is highly suited for simultaneous processing of multiple samples, yields pure nucleic acid in less than 1 hour. The QIAamp MinElute Virus Spin Kit can be used for isolation of viral RNA and DNA from a broad range of RNA and DNA viruses. However, performance cannot be guaranteed for every virus species and must be validated by the customer.

Sample volumes using the QIAamp MinElute Virus Spin Kit

Each QIAamp MinElute column can bind nucleic acids that are longer than 200 bases, but yield depends on sample volume and virus titer. The spin procedure is optimized for use with a starting volume of 200 µl.

Lysis with QIAGEN Protease

Samples are lysed under highly denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Protease and Buffer AL, which together ensure inactivation of RNases.

Adsorption to the QIAamp MinElute membrane

Binding conditions are adjusted by adding ethanol to allow optimal binding of the viral RNA and DNA to the membrane. Lysates are then transferred onto a QIAamp MinElute column and viral nucleic acids are adsorbed onto the silica-gel membrane as the lysate is drawn through by centrifugation. Salt and pH conditions ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp MinElute membrane.

QIAamp MinElute columns fit into most standard microcentrifuge tubes. Due to the volume of filtrate, 2 ml collection tubes (provided) are required to support the QIAamp MinElute column during loading and wash steps.

Removal of residual contaminants

Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away during 3 wash steps. In a single step, highly pure viral RNA and DNA are eluted in Buffer AVE, equilibrated to room temperature.

Elution of pure nucleic acids

Elution is performed using Buffer AVE. The QIAamp MinElute columns allow minimal elution volumes of only 20 µl. Low elution volume leads to highly concentrated nucleic acid eluates.

For downstream applications that require small starting volumes (e.g., some PCR and RT-PCR assays) a more concentrated eluate may increase assay sensitivity.

For downstream applications that require a larger starting volume, the elution volume can be increased up to 150 µl. However, an increase in elution volume will decrease the concentration of nucleic acids in the eluate.

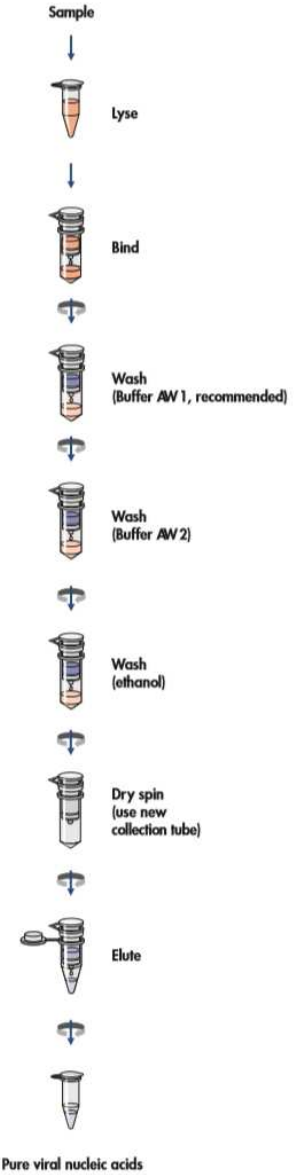
The eluate volume recovered can be up to 5 µl less than the volume of elution buffer applied to the column; for example, an elution buffer volume of 20 µl results in >15 µl final eluate. The volume of eluate recovered depends on the nature of the sample.

Eluted DNA can be collected in standard 1.5 ml microcentrifuge tubes (not provided). If the purified viral RNA and DNA is to be stored for up to 24 hours, storage at 2–8°C is recommended. For periods of storage longer than 24 hours, storage at –20°C is recommended.

Yield and size of viral nucleic acids

Yields of viral nucleic acid isolated from biological samples are normally below 1 µg and are therefore difficult to determine with a spectrophotometer. Quantitative amplification methods are recommended for determination of yields. When quantifying nucleic acids isolated using the QIAamp MinElute Virus Spin protocol, remember that there will be considerably more carrier RNA in the sample than viral RNA. The size distribution of viral nucleic acid purified with this procedure can be checked by agarose gel electrophoresis and hybridization to a virus-specific labeled probe followed by autoradiography (Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual* , 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

The QIAamp MinElute Virus Spin Procedure



Fully automatable on the QIAcube

Carrier RNA

Carrier RNA serves two purposes. Firstly, 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 and detergent in Buffer AL. If carrier RNA is not added to Buffer AL this may lead to reduced viral RNA or DNA recovery.

The amount of lyophilized carrier RNA (provided) is sufficient for the volume of Buffer AL supplied with the kit. The concentration of carrier RNA has been adjusted so that the QIAamp MinElute Virus Spin protocol can be used as a generic purification system compatible with many different amplification systems and is suitable for a wide range of RNA and DNA viruses.

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. Calculations of how much eluate to add to downstream amplifications should therefore be based on 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 AL.

Addition of internal controls

Using the QIAamp MinElute Virus Spin protocol in combination 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 together with the carrier RNA to the lysis buffer. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides, as smaller molecules are not efficiently recovered. Refer to the manufacturer's instructions in order to determine the optimal concentration. Using a concentration other than that recommended may reduce amplification efficiency.

Automated purification of nucleic acids on QIAcube Instruments

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

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.

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.

- Ethanol (96–100%)*
- 1.5 ml microcentrifuge tubes (e.g., Sarstedt Safety-Cap, cat. no. 72.690 or Eppendorf®
- Safe-Lock, cat. no. 0030 120.086)† for elution
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
- Heating block for lysis of samples at 56°C
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- Vortexer
- For samples <200 µl: 0.9% NaCl solution

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
†This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Handling of QIAamp MinElute columns

Because of the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp MinElute columns in order to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp MinElute column. Pipet the sample into the QIAamp MinElute column without wetting the rim of the column.
- Change pipet tips between all liquid transfers. The use of aerosol-barrier pipet tips is recommended.
- Avoid touching the QIAamp MinElute membrane with the pipet tip.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lid.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Centrifugation

QIAamp MinElute columns will fit into most standard 1.5–2 ml microcentrifuge tubes. Additional 2 ml collection tubes are available separately.

Centrifugation of QIAamp MinElute columns is performed at 6000 x *g* (8000 rpm) to reduce centrifuge noise. Centrifuging QIAamp MinElute columns at full speed will not affect DNA or RNA yield. Centrifugation at lower speeds is also acceptable, provided that nearly all of each solution is transferred through the QIAamp MinElute membrane.

For the dry spin at the end of the washing procedure and for elution, centrifugation should be carried out at full speed.

All centrifugation steps should be carried out at room temperature.

Processing QIAamp MinElute columns in a microcentrifuge

- Close the QIAamp MinElute column before placing it in the microcentrifuge. Centrifuge as described.
- Remove the QIAamp MinElute column and collection tube from the microcentrifuge. Place the QIAamp MinElute column in a new collection tube. Discard the filtrate and the collection tube. Please note that the filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp MinElute column at a time, and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, we recommend filling a rack with collection tubes so that the QIAamp MinElute columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the QIAamp MinElute columns can be placed directly in the microcentrifuge.

Preparation of RNA

When preparing viral RNA, work quickly during the manual steps of the procedure. If you have not previously worked with RNA, read the Appendix on page 26 before starting.

Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase inhibitors, it will not actively inhibit RNases introduced by inappropriate handling. Extreme care should be taken to avoid contamination with RNases when handling Buffer AVE.

Sample storage

After collection and centrifugation, plasma or serum can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C or –80°C in aliquots is recommended. Frozen plasma or serum samples must not be thawed more than once. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitates formed during freeze–thawing will clog the QIAamp MinElute membrane. If cryoprecipitates are visible, they can be pelleted by centrifugation at 6800 x *g* for 3 minutes. The cleared supernatant should be removed and processed immediately without disturbing the pellet. This step will not reduce viral titers.

Preparation of QIAGEN Protease

This kit provides two alternative buffers for dissolving QIAGEN Protease — Buffer AVE (recommended) or Protease Resuspension Buffer.

Dissolving the protease in Buffer AVE provides a generic and efficient working solution for all starting materials.

As an alternative, dissolving QIAGEN Protease in Protease Resuspension Buffer provides efficient viral lysis for most sample types. For some starting materials, such as EDTA plasma, performance is slightly enhanced. However, Protease Resuspension Buffer is not compatible with samples or internal controls that contain phosphate (e.g., viral transport medium, cell culture supernatants, or phosphate-buffered saline). If the sample or internal control contains phosphate, it is highly recommended to resuspend QIAGEN Protease in Buffer AVE.

Add 1.4 ml of Buffer AVE or Protease Resuspension Buffer to the vial of lyophilized QIAGEN Protease, and mix carefully to avoid foaming. Make sure that the QIAGEN Protease is completely dissolved. Label the resuspended QIAGEN Protease to indicate which buffer was used for resuspension.

Note: Do not add QIAGEN Protease directly to Buffer AL.

QIAGEN Protease reconstituted in Buffer AVE or Protease Resuspension Buffer is stable for 12 months when stored at 2–8°C, but only until the kit expiration date. Keeping the QIAGEN Protease stock solution at room temperature for prolonged periods of time should be avoided. Storage at –20°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and freezing at –20°C is recommended. Label the aliquots and indicate which buffer was used for resuspension.

Addition of carrier RNA to Buffer AL*

Add 310 µl Buffer AVE to the tube containing 310 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 –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AVE and then added to Buffer AL.

Calculate the volume of Buffer AL–carrier RNA mix needed per batch of samples by selecting the number of samples to be **simultaneously** processed from

Table 1. For larger numbers of samples, volumes can be calculated using the following sample calculation:

n x 0.22 ml = y ml
y ml x 28 µl/ml = z µl

where: **n** = number of samples to be processed simultaneously
y = calculated volume of Buffer AL
z = volume of carrier RNA–Buffer AVE to add to Buffer AL

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 6 for safety information.

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

Table 1 Volumes of Buffer AL and carrier RNA–Buffer AVE mix required for the QIAamp MinElute Virus Spin procedure

No. samples	Vol. Buffer AL (ml)	Vol. carrier RNA–AVE (µl)	No. samples	Vol. Buffer AL (ml)	Vol. carrier RNA–AVE (µl)
1	0.22	6.2	13	2.86	80.1
2	0.44	12.3	14	3.08	86.3
3	0.66	18.5	15	3.30	92.4
4	0.88	24.6	16	3.52	98.6
5	1.10	30.8	17	3.74	104.7
6	1.32	37.0.	18	3.96	110.9
7	1.54	43.1	19	4.18	117.0
8	1.76	49.3	20	4.40	123.2
9	1.98	55.4	21	4.62	129.4
10	2.20	61.6	22	4.84	135.5
11	2.42	67.8	23	5.06	141.7
12	2.64	73.9	24	5.28	147.8

Note: The sample-preparation 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 AL. For each microgram of carrier RNA required per preparation, add 5 µl Buffer AVE-dissolved carrier RNA per milliliter of Buffer AL. (Use of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

Buffer AW1*

Add 25 ml of ethanol (96–100%) to a bottle containing 19 ml of Buffer AW1 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been

*Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 10 for safety information.

added. Store reconstituted Buffer AW1 at room temperature (15–25°C). Reconstituted Buffer AW1 is stable for up to 1 year when stored at room temperature, but only until the kit expiration date.

Note: Always mix reconstituted Buffer AW1 by shaking before starting the procedure.

Buffer AW2*

Add 30 ml of ethanol (96–100%) to a bottle containing 13 ml of Buffer AW2 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted Buffer AW2 at room temperature (15–25°C). Reconstituted Buffer AW2 is stable for up to 1 year when stored at room temperature, but only until the kit expiration date.

Note: Always mix reconstituted Buffer AW2 by shaking before starting the procedure.

Elution of nucleic acids

Elution buffer should be equilibrated to room temperature before it is applied to the column. Yields will be increased if the QIAamp MinElute column is incubated with the elution buffer at room temperature for 5 minutes before centrifugation.

* Contains sodium azide as a preservative.

Protocol: Purification of Viral Nucleic Acids from Plasma or Serum

This protocol is for purification of viral nucleic acids from 200 µl of plasma or serum using the QIAamp MinElute Virus Spin Kit and a microcentrifuge. For automated purification using the QIAamp MinElute Virus Spin Kit with the QIAcube Connect, refer to the *QIAcube User Manual* and the relevant protocol sheet.

Important point before starting

- All centrifugation steps are carried out at room temperature (15–25°C). Things to do before starting
- Equilibrate samples to room temperature.
- Equilibrate Buffer AVE to room temperature for elution in step 14.
- Prepare a 56°C heating block for use in steps 4 and 13.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions on page 16–19.
- Add carrier RNA reconstituted in Buffer AVE to Buffer AL according to instructions on page 17.

Procedure

1. Pipet 25 µl QIAGEN Protease into a 1.5 ml microcentrifuge tube (not provided).

   Note: Read "Preparation of QIAGEN Protease", page 16, for information about resuspending QIAGEN Protease in Buffer AVE (recommended) or Protease Resuspension Buffer.

2. Add 200 µl of plasma or serum into the microcentrifuge tube.

   If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of protease and sample up to a total of 225 µl.

3. Add 200 µl Buffer AL (containing 28 µg/ml of carrier RNA). Close the cap and mix by pulse-vortexing for 15 s. In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

4. Incubate at 56°C for 15 min in a heating block.
5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
6. Add 250 µl of ethanol (96–100%) to the sample, close the cap and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

   Note: If ambient temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.

7. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
8. Carefully apply all of the lysate from step 7 onto the QIAamp MinElute column without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

   If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp MinElute column is empty.

9. **Recommended**: Carefully open the QIAamp MinElute column, and add 500 µl of Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

   Note: This step increases kit performance when processing inhibitory samples.

10. Carefully open the QIAamp MinElute column, and add 500 µl of Buffer AW2 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

11. Carefully open the QIAamp MinElute column and add 500 µl of ethanol (96–100%) without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Discard the collection tube containing the filtrate.

    Ethanol carryover into the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, contacting the QIAamp MinElute column. Removing the QIAamp MinElute column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp MinElute column.

12. Place the QIAamp MinElute column in a clean 2 ml collection tube. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min to dry the membrane completely.

13. **Recommended**: Place the QIAamp MinElute column into a new 2 ml collection tube (not provided), open the lid, and incubate the assembly at 56°C for 3 min to dry the membrane completely.

    This step serves to evaporate any remaining liquid.

14. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube with the filtrate. Carefully open the lid of the QIAamp MinElute column, and apply 20–150 µl of Buffer AVE or RNase-free water to the center of the membrane. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

    Important: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

    Elution volume is flexible and can be adapted according to the requirements of the downstream application. Remember that the recovered eluate volume will be approximately 5 µl less than the elution buffer volume applied onto the column. Incubating the QIAamp MinElute column loaded with Buffer AVE or water for 5 min at room temperature before centrifugation generally increases DNA and RNA yield.

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 protocol in this handbook or molecular biology applications (see back cover for contact information).

		Comments and suggestions
Little or no nucleic acid in the eluate		
a)	Carrier RNA not added to Buffer AL	Reconstitute carrier RNA in Buffer AVE and mix with Buffer AL as described on page 17. Repeat the purification procedure with new samples.
b)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –20°C or underwent multiple freeze-thaw cycles. Alternatively, Buffer AL–carrier RNA mixture was stored for more than 48 hours at 2–8°C. Prepare a new tube of carrier RNA dissolved in Buffer AVE and mix with Buffer AL. Repeat the purification procedure with new samples.
c)	Buffer AL–carrier RNA mixture mixed insufficiently	Mix Buffer AL with carrier RNA by gently inverting the tube of Buffer AL–carrier RNA at least 10 times.
d)	Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
e)	RNA degraded	Check the integrity of the RNA in the original samples. Often RNA is degraded by RNases in the starting material (plasma, serum, body fluids). Ensure that the samples are processed quickly following collection or removal from storage. Check for RNase contamination of buffers and water and ensure that no RNase is introduced during the procedure. Use Buffer AVE or RNase-free water for elution.
f)	RNase contamination in Buffer AVE	If tubes containing Buffer AVE are accessed repeatedly, be careful to not introduce RNases. In case of RNase contamination, replace the open vial of Buffer AVE with a new vial. Repeat the purification procedure with new samples.
g)	Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with the correct volume of 96–100% ethanol. Repeat the purification procedure with new samples.

		Comments and suggestions
h)	Buffer AW1 or Buffer AW2 prepared with low-percentage ethanol	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with new samples.
i)	Protease Resuspension Buffer used with incompatible starting materials	Protease Resuspension Buffer is not compatible with samples or internal controls that contain phosphate (e.g., viral transport medium, cell culture supernatants, or phosphate-buffered saline). If the sample or internal control contains phosphate, it is highly recommended to resuspend QIAGEN Protease in Buffer AVE. See "Preparation of QIAGEN Protease", page 16.
RNA or DNA does not perform well in downstream enzymatic reactions		
a)	Little or no nucleic acid in the eluate	See "Little or no nucleic acid in the eluate for possible reasons. Increase the amount of eluate added to the reaction, if possible.
b)	Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided (see page 16). Always use fresh samples or samples thawed only once.
c)	Low concentration of virus in the samples.	Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples
d)	Insufficient sample lysis in Buffer AL	QIAGEN Protease was subjected to elevated temperature for a prolonged time. Repeat the procedure using new samples and fresh QIAGEN Protease.
e)	Too much or too little carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the concentration of carrier RNA added to Buffer AL accordingly (see "Addition of carrier RNA to Buffer AL", page 17).
f)	Reduced sensitivity.	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. The elution volume can be adapted proportionally
g)	Performance of purified nucleic acids in downstream assays varies with aging of reconstituted wash buffers.	Salt and ethanol components of Buffer AW1 or Buffer AW2 may have separated out after being left for a long period between preparations. Always mix buffers thoroughly before each preparation
h)	A new combination of reverse transcriptase and *Taq* DNA polymerase was used	If enzymes are changed it may be necessary to readjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.

		Comments and suggestions
General handling		
a)	Clogged QIAamp MinElute column	Cryoprecipitates may have formed plasma due to repeated freezing and thawing. These can block the QIAamp MinElute column. Do not use plasma that has been frozen and thawed more than once. If cryoprecipitates have formed clear sample by centrifugation as described in "Sample storage" on page 16 before starting the sample preparation.
b)	Variable elution volumes	This is normal when different sample types have been processed.

Appendix

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 only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed.

Disposable plasticware

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

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by

* 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.

RNase-free water* (see "Solutions", page 27). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* 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 detergent, thoroughly rinsed and oven-baked at >240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Oven baking will both inactivate ribonucleases and ensure that no other nucleic acids (such as plasmid DNA) remain on the surface of the glassware. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Cover the glassware with 0.1% DEPC in water overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to remove residual DEPC.

Note: Corex® tubes should be rendered RNase-free by treatment with DEPC and not by baking. This will reduce the failure rate of this type of tube during centrifugation.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* rinsed with water, dried with ethanol,*† and then filled with a solution of 3% hydrogen peroxide.* After 10 minutes at room temperature, the electrophoresis tanks should be rinsed thoroughly with RNase-free water.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in

* 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.

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

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.

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. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Add 0.1 ml DEPC to 100 ml of the solution to be treated, and shake vigorously to bring the DEPC into solution or let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. It may be desirable to test water sources for the presence of contaminating RNases since many sources of distilled water are free of RNase activity.

Note: QIAamp MinElute Virus Spin Kit buffers are not rendered RNase-free by DEPC treatment and are therefore free of any DEPC contamination.

Ordering Information

Product	Contents	Cat. no.
QIAamp MinElute Virus Spin Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Filter-Tips, 1000 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	990352
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	990332
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters; for use with the QIAcube instruments	990394
Related products		
QIAamp MinElute Virus Vacuum Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	57714
QIAamp DSP Virus Kit	For 50 minipreps: QIAamp MinElute Columns, Buffers, Reagents, Tubes, Column Extenders, VacConnectors	60704

Product	Contents	Cat. no.
EZ1® DSP Virus Kit (48)	For 48 viral nucleic acid preps Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, Carrier RNA:	62724
EZ1 DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol	9017707
QIAamp Virus BioRobot® MDx Kit (12)	For 12 x 96 preps: 12 QIAamp 96 Plates, RNase-free Buffers, QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Carrier RNA	965652
QIAamp Media MDx Kit (12)	For 12 x 96 preps: 12 QIAamp 96 Plates, Buffers, Proteinase K, S-Blocks, Disposable Troughs, Racks with Elution Microtubes CL (0.4 ml), Carrier RNA, Top Elute Fluid, Caps, Tape Pad	965752
Accessories		
QIAGEN Protease (7.5 AU)	7.5 Anson Units (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson Units (lyophilized)	19157
Buffer AL (216 ml) 216 ml	Lysis Buffer AL	19075
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Collection Tubes (2 ml)	1000 collection tubes (2 ml)	19201

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

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
February 2020	Updated text, ordering information and intended use for QIAcube Connect.