

# QIAamp<sup>®</sup> Viral RNA Mini Kit Handbook

For purification of viral RNA from

Plasma

Serum

Cell-free body fluids

Cell-culture supernatants

January 1999



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## Kit Contents

QIAamp Viral RNA Mini Kits			
Cat. No.	52904	52906	52908
Preparations per kit	50	250	1000
QIAamp Spin Columns	50	250	1000
Collection Tubes (2-ml)	200	1000	4000
Buffer AVL*	31 ml	5 x 31 ml	3 x 190 ml
Buffer AW1* (concentrate)	19 ml	95 ml	2 x 175 ml
Buffer AW2† (concentrate)	13 ml	66 ml	2 x 127 ml
Buffer AVE†	3 x 2 ml	8 x 2 ml	32 x 2 ml
Carrier RNA (poly A)	310 µg	5 x 310 µg	3 x 1900 µg
Handbook	1	1	1

\* Contains chaotropic salt which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting reagents which contain bleach.

† Contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead and copper drain pipes. Take appropriate safety measures, and wear gloves when handling. Dispose of azide containing solutions according to your institutions waste-disposal guidelines.

## Storage Conditions

QIAamp® spin columns should be stored dry at room temperature (15–25°C); storage at higher temperatures should be avoided. All solutions should be stored at room temperature unless otherwise stated. QIAamp spin columns, all buffers and reagents can be stored for up to 1 year under the above conditions without showing any reduction in performance.

Lyophilized Carrier RNA is stable for up to 1 year when stored at room temperature. Carrier RNA dissolved in Buffer AVL however must be stored at 2–8°C and is then stable for up to 6 months. If Buffer AVL/Carrier RNA solution is stored at room temperature, it will be stable for no more than 2 weeks. Buffer AVL/Carrier RNA solution develops a precipitate when stored at 2–8°C that 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 cause degradation of the carrier RNA, leading to reduced recovery of viral RNA and eventually false negative RT-PCR results, particularly when low-titer samples are used.**

## Reagents and Equipment to be Supplied by User

- Ethanol (96–100%)
- Microcentrifuge tubes (1.5-ml)
- Sterile, RNase-free pipet tips with aerosol barrier
- Disposable gloves
- Microcentrifuge (with rotor for 2-ml tubes)
- Vacuum manifold (QIAvac 6S [see ordering information, page 33] or equivalent)
- Luer adapters (see ordering information, page 33)
- VacConnectors (see ordering information, page 33)
- Vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g. KMS Neuberger Laboport type N 840.3 FT 18)
- Vacuum regulator (see ordering information, page 33)

## Product Use Limitations

QIAamp Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of QIAamp Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. QIAamp Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

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.

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor.

## Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding QIAamp Viral RNA Mini Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the [QIAGEN Technical Service Departments](#) or contact your local distributor listed on the last page.

# The QIAamp Principle and Procedure

Please take a few moments to read this handbook carefully before beginning your preparation. The “Important Notes Before Starting” on page 14, and the comments within the QIAamp Viral RNA Mini protocols, beginning on page 18, are particularly valuable.

QIAamp Viral RNA Mini Kits represent a well established general-purpose technology for viral RNA preparation. The kit combines the selective binding properties of a silica-gel–based membrane with the speed of microspin or vacuum technology and is ideally suited for simultaneous processing of multiple samples. The sample is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the sample is loaded onto the QIAamp spin column. The RNA binds to the membrane, and contaminants are efficiently washed away in two steps using two different wash buffers. High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors. The special QIAamp membrane guarantees extremely high recovery of pure, intact RNA in just twenty minutes without the use of phenol/chloroform extraction or alcohol precipitation. All buffers and reagents are guaranteed to be RNase-free.

QIAamp Viral RNA Mini Kits provide the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. Viral RNA can be purified from plasma (untreated or treated with anticoagulants other than heparin), serum, and other cell-free body fluids. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced viral titers and should be avoided for optimal sensitivity. Cryoprecipitates accumulate when samples are subjected to repeated freeze–thawing cycles. This may lead to clogging of the QIAamp membrane when using the vacuum protocol.

QIAamp Viral RNA Mini Kits are general purpose kits which can be used for isolation of viral RNA from a wide variety of viruses including HIV, HAV, HCV, HDV, and enteroviruses, but performance can not be guaranteed for every virus.

## Cellular DNA contamination

QIAamp Kits are not designed to separate viral RNA from cellular DNA, and both will be purified in parallel if present in the sample. To avoid copurification of cellular DNA, use of cell-free body fluids for preparation of viral RNA is recommended. Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, and most swabs, should first be filtered, or centrifuged for 10 minutes at 1500 x g and the supernatant used. If RNA and DNA have been isolated in parallel, the eluate can be DNase digested using RNase-free DNase, followed by heat treatment (15 min, 70°C) to inactivate the DNase.

## 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 "Handling RNA" in the Appendix (page 29) of this handbook before starting.

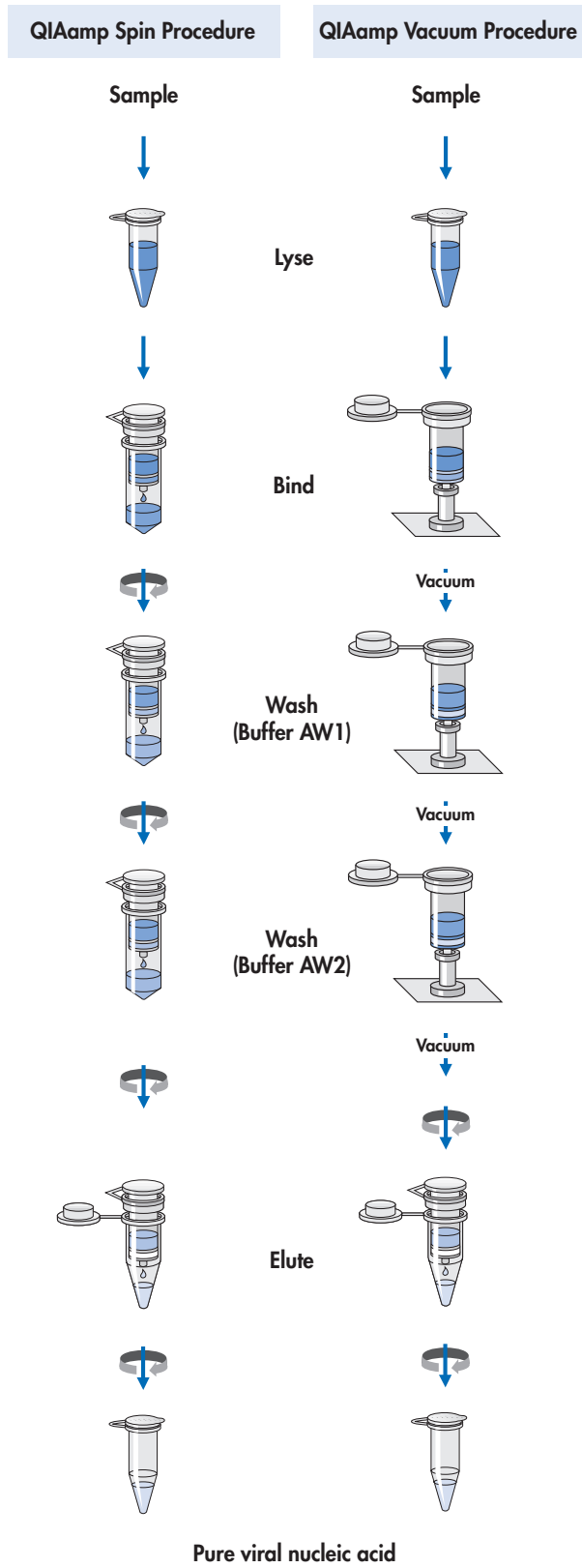
PCR should always be carried out using good laboratory practices. Accordingly, a PCR laboratory should always be divided into three areas: an area for preparation of reagents, an area for preparation of samples, and an area for amplification and detection. Due to the high sensitivity of PCR, it is absolutely necessary that all reagents remain pure and uncontaminated, and should be monitored carefully and routinely. Contaminated reagents must be discarded.

## Sample volumes

QIAamp spin columns can bind RNA greater than 200 nucleotides in length. Actual yield will depend on sample size, sample storage, and virus titer. The procedure is optimized for use with 140- $\mu$ l samples, but samples up to 280  $\mu$ l can be used. Small samples should be adjusted to 140  $\mu$ l with phosphate-buffered saline (PBS) before loading, and samples with a low viral titer should be concentrated to 140  $\mu$ l before processing. For samples larger than 140  $\mu$ l, the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally, but the amounts of Buffers AW1 and AW2 used in the wash steps usually do not need to be increased. Follow the special "Protocol for Large Sample Volumes," page 23. If the initial sample volume is increased, application of the lysed sample to the QIAamp spin column will require multiple loading steps. There is no danger of overloading the QIAamp spin column, and the quality of the purified RNA will be unaffected. For volumes greater than 560  $\mu$ l, concentration of the sample is recommended. See "Protocol for Sample Concentration," page 24.

## Lysis

The sample is first lysed under the 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.



**Figure 1.** QIAamp Viral RNA Mini spin and vacuum procedures

## **QIAamp spin-column procedure**

The QIAamp Viral RNA Mini purification procedure is carried out in three steps using QIAamp spin columns in a standard microcentrifuge or on a vacuum manifold. Both spin and vacuum procedures are designed to ensure that there is no sample-to-sample cross-contamination and allow safe handling of potentially infectious samples.

QIAamp spin columns fit into most standard microcentrifuge tubes. In the spin protocol, due to the volume of filtrate, 2-ml collection tubes (provided) are required to support the QIAamp spin column during loading and wash steps. For the vacuum protocol, a vacuum manifold (QIAvac 6S or similar) and a vacuum pump capable of producing a vacuum of  $-800$  to  $-900$  mbar (e.g. KMS Neuberger Laboport type N 840.3 FT 18) are required.

Eluted RNA can be collected in standard 1.5-ml microcentrifuge tubes (not provided). These tubes must be RNase-free to avoid degradation of viral RNA by RNases.

### **Adsorption to the QIAamp membrane**

The buffering conditions of the lysate must be adjusted to provide optimum binding conditions for the viral RNA before loading the sample onto the QIAamp spin column. Viral RNA is adsorbed onto the QIAamp silica-gel membrane during two brief centrifugation steps or by vacuum. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit downstream enzymatic reactions, are not retained on the QIAamp membrane. If the initial sample volume is larger than 140  $\mu$ l, it will be necessary to load the lysate onto the QIAamp spin column in several steps.

### **Removal of residual contaminants**

Viral RNA, bound to the QIAamp membrane, is washed free of contaminants during two short centrifugation or vacuum steps. The use of two different wash buffers, AW1 and AW2, has significantly improved the purity of the eluted RNA. Wash conditions ensure complete removal of any 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 and 280 nm but has no effect on downstream applications, such as RT-PCR. Should you wish to determine the purity of the eluted RNA, elution with RNase-free water instead of Buffer AVE is recommended.

## Determination of yield

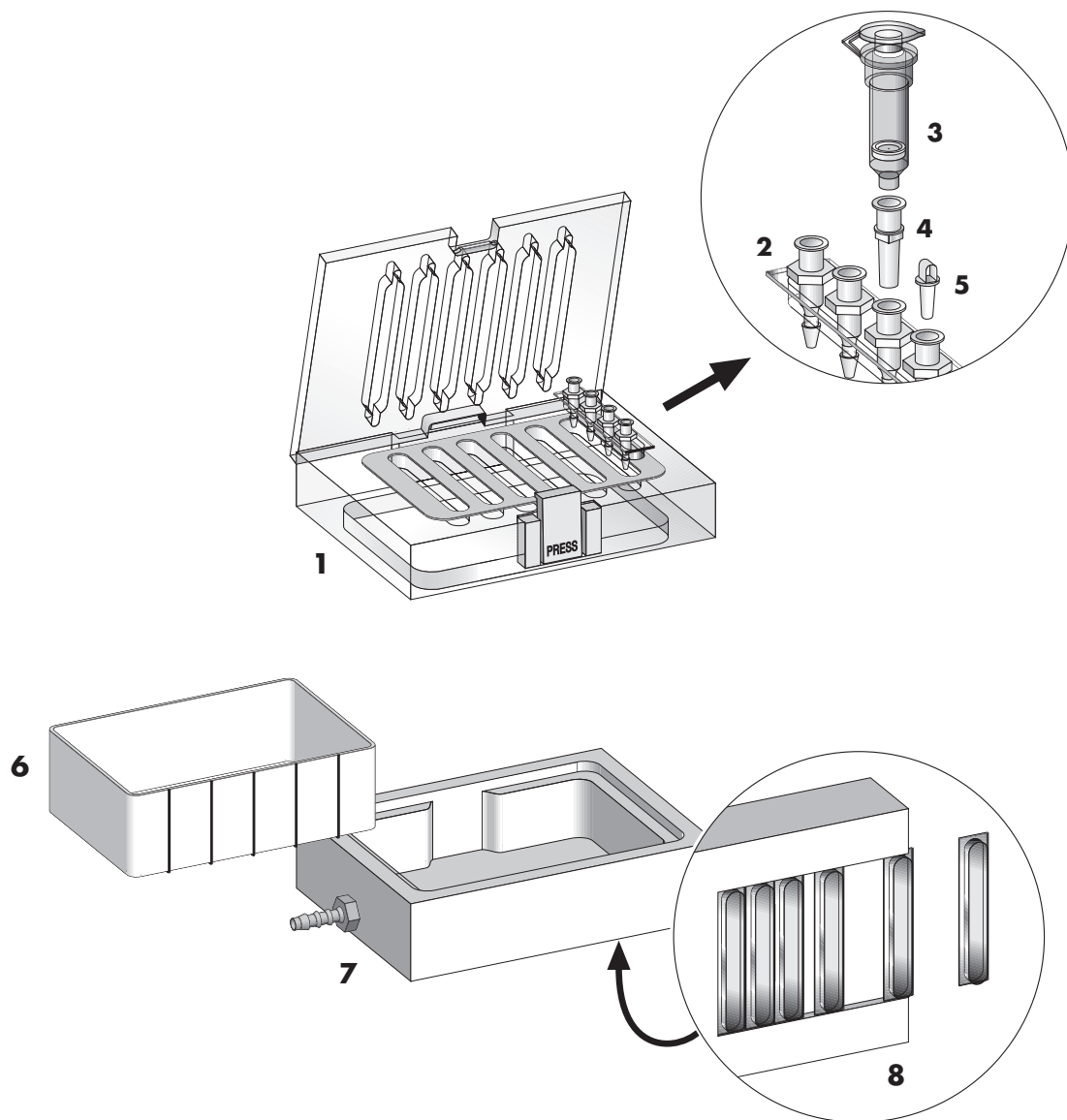
Yields of viral RNA isolated from biological samples are normally less than 1 µg and therefore difficult to determine photometrically. Keep in mind that the carrier RNA (5.6 µg per 140-µl sample) will account for most of the RNA present. Quantitative RT-PCR is recommended for determination of viral RNA yield.

## Determination of viral RNA length

The size distribution of viral RNA purified using QIAamp spin columns can be checked by denaturing agarose gel electrophoresis followed by hybridization with a virus-specific labeled probe and autoradiography (1).

1. Sambrook, J., Fritsch, E.F., Maniatis, T., eds. (1989) *Molecular cloning: a laboratory manual*, 2nd ed. New York: Cold Spring Harbor Laboratory Press.

# QIAvac 6S Vacuum Manifold



**Figure 2.** Exploded diagram of QIAvac 6S manifold and components

1. QIAvac top plate with slots for QIAvac Luer Adapters
2. QIAvac Luer Adapter\*
3. QIAamp mini spin column
4. VacConnector\*
5. Plug to seal unused luer connectors\*
6. Waste tray
7. QIAvac base, which can hold a waste tray or a microtube rack
8. Blanks to seal unused slots

\* Not included — must be purchased separately

## QIAvac 6S handling guidelines

The QIAvac 6S vacuum manifold facilitates RNA purification with QIAamp Viral RNA Mini Kits. In combination with QIAvac Luer Adapters and VacConnectors, it allows easy processing of QIAamp mini spin columns. The following recommendations should be followed when handling the QIAvac 6S vacuum manifold:

- Always store the QIAvac 6S vacuum manifold clean and dry. To clean, simply rinse all components with water, and dry with paper towels. Do not air-dry, as the screws may rust. Do not use abrasives or solvents.
- Always place the QIAvac 6S vacuum manifold on a secure bench top or work area. If dropped, the manifold may crack.
- The components of QIAvac manifolds are not resistant to ethanol, methanol, or other organic solvents (Table 1). Do not bring the vacuum manifold into contact with solvents. If solvents are spilled on the unit, rinse thoroughly with distilled water. Do not incubate acrylic components in alcohol-containing reagents for long periods.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 6S manifold. The spring lock on the top plate and the self-sealing gasket provide an airtight seal when vacuum is applied to the assembled unit. To maximize gasket lifetime, rinse the gasket free of salts and buffers after each use, and dry with paper towels before storage.

**Table 1. Chemical resistance properties of QIAvac manifolds**

<b>Resistant to:</b>	<b>Not resistant to:</b>	
Chlorine bleach (12%)	Acetic acid	Concentrated alcohols
Hydrochloric acid	Acetone	Ether
Sodium chloride	Benzene	Phenol
Sodium hydroxide	Chloroform	Toluene
Urea	Chromic acid	

## Important Notes Before Starting

If preparing RNA for the first time please read "Handling RNA" in the Appendix of this handbook (page 29). All steps of the QIAamp Viral RNA Mini protocols should be performed quickly and at room temperature.

After collection and centrifugation, plasma (untreated or treated with anticoagulants other than heparin) or serum can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C to –80°C in aliquots is recommended. Frozen plasma or serum samples must not be thawed more than once. Repeated freezing and thawing leads to denaturation and precipitation of proteins, causing reduced viral titers and subsequently reduced yields of the isolated viral RNA. In addition, cryoprecipitates formed by freeze–thawing will cause clogging of the QIAamp membrane. If cryoprecipitates are visible, they can be pelleted by briefly centrifuging at 6800 x g for 3 minutes. The cleared supernatant should be removed, without disturbing the pellet, and processed immediately. This step will not reduce viral titers.

The QIAamp procedure is not designed to separate RNA from DNA. To avoid cellular DNA contamination follow the guidelines in "Cellular DNA contamination" on page 7 of this handbook.

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

## Preparation of reagents

### Addition of Carrier RNA to Buffer AVL\*

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

Add 1 ml of Buffer AVL to one tube of lyophilized Carrier RNA. Dissolve Carrier RNA thoroughly. Transfer to the Buffer AVL bottle, and mix thoroughly before using Buffer AVL for the first time.

**Note:** If less Carrier RNA has been shown to be better for your particular amplification system, add 1 ml of Buffer AVL to one tube of lyophilized Carrier RNA, as described above, and transfer only the required aliquot to the Buffer AVL bottle. For example, if 1 µg Carrier RNA per ml of Buffer AVL has been shown to provide optimal RT-PCR efficiency, transfer 100 µl of reconstituted Carrier RNA to the Buffer AVL bottle. Discard the unused portion of the reconstituted Carrier RNA.

\* Contains chaotropic salt which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach.

Lyophilized Carrier RNA is stable for up to 1 year when stored at room temperature (15–25°C). Carrier RNA dissolved in Buffer AVL, however, should be stored at 2–8°C and will be stable for up to 6 months. If Buffer AVL/Carrier RNA is stored at room temperature, it will be stable for no more than 2 weeks. When stored at 2–8°C, the Buffer AVL/Carrier RNA solution forms a precipitate; this precipitate must be redissolved by warming at 80°C and the solution cooled to room temperature 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 cause degradation of Carrier RNA, leading to reduced recovery of viral RNA and eventually false negative RT-PCR results. This is particularly the case with low-titer samples.

### Buffer AW1\*

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 and in Table 2.

Buffer AW1 is stable for 1 year when stored closed at room temperature.

**Table 2. Preparation of Buffer AW1**

Kit Cat. No.	No. of preps	AW1 concentrate	Ethanol	Final volume
51104	50	19 ml	25 ml	44 ml
51106	250	95 ml	125 ml	220 ml
51108	1000	175 ml	230 ml	405 ml

### Buffer AW2†

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle and in Table 3.

Buffer AW2 is stable for 1 year when stored closed at room temperature.

**Table 3. Preparation of Buffer AW2**

Kit Cat. No.	No. of preps	AW2 concentrate	Ethanol	Final volume
51104	50	13 ml	30 ml	43 ml
51106	250	66 ml	160 ml	226 ml
51108	1000	127 ml	300 ml	427 ml

\* Contains chaotropic salt which is an irritant. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach.

† Contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead and copper drain pipes. Take appropriate safety measures, and wear gloves when handling. Dispose of azide containing solutions according to your institutions waste-disposal guidelines.

## Handling of QIAamp spin columns

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

- Carefully apply the sample or solution to the QIAamp spin column. Pipet the sample into the QIAamp spin column without wetting the rim of the column.
- Change pipet tips between all liquid transfer steps. The use of aerosol-barrier tips is recommended.
- Avoid touching the QIAamp membrane with the pipet tip.
- After all pulse-vortexing steps, briefly centrifuge 1.5-ml microcentrifuge tubes to remove drops from the inside of the lid.
- Wear gloves throughout the procedure. In case of contact between gloves and sample, change gloves immediately.

### Spin protocol

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

## Vacuum protocol

- Insert new VacConnectors into the luer connectors on the Luer Adapters in the manifold (see Figure 2, page 12). Remove the QIAamp spin columns from the blister pack and attach spin column to the VacConnector. The collection tube can be saved for the dry spin in step 10 of the protocol. Used VacConnectors should be discarded.
- The vacuum pressure is the pressure difference between the inside of the manifold and the atmosphere (standard atmospheric pressure 1013 millibar) and can be measured using a vacuum regulator (see ordering information on page 33). The vacuum protocol requires a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g. KMS Neuberger Laboport type N 840.3 FT 18). Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce DNA yield and purity.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure.
- Leave the lid of the QIAamp spin column open while applying vacuum.

## Centrifugation

QIAamp spin columns will fit into most standard 1.5- or 2-ml microcentrifuge tubes. Additional 2-ml collection tubes are available separately.

Centrifugation of QIAamp spin columns is performed at 6000  $\times$  *g* (8000 rpm) in order to limit centrifuge noise. Centrifugation at full speed will not affect RNA yield. Centrifugation at lower speeds for lysate loading and the first wash step is also acceptable, provided that the complete solution is transferred through the membrane. At the second wash step centrifugation at full speed is strongly recommended.

All centrifugation steps are carried out at room temperature.

## QIAamp Viral RNA Mini Spin Protocol

**Notes:** Please read “Important Notes Before Starting” on pages 14–17.

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AVE to room temperature for elution in step 10.
- Check that Buffer AW1, Buffer AW2, and Carrier RNA have been prepared according to the instructions on pages 14–15.
- Redissolve precipitate in Buffer AVL/Carrier RNA by heating, if necessary, and cool to room temperature before use.
- All centrifugation steps are carried out at room temperature.

**1. Pipet 560 µl of prepared Buffer AVL containing Carrier RNA into a 1.5-ml microcentrifuge tube.**

If the sample volume is larger than 140 µl, increase the amount of Buffer AVL/Carrier RNA proportionally (e.g., a 280-µl sample will require 1120 µl Buffer AVL/Carrier RNA).

**2. Add 140 µl plasma, serum, urine, cell-culture supernatant, or cell-free body fluid to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.**

To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer AVL to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.

**3. Incubate at room temperature (15–25°C) for 10 min.**

Viral particle lysis is complete after lysis for 10 min at room temperature. Longer incubation times have no effect on the yield or quality of the purified RNA. Potentially infectious agents and RNases are inactivated in Buffer AVL.

**4. Briefly centrifuge the 1.5-ml microcentrifuge tube to remove drops from the inside of the lid.**

**5. Add 560 µl of ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the 1.5-ml microcentrifuge tube to remove drops from inside the lid.**

Only ethanol should be used since other alcohols may result in reduced RNA yield and purity. If the sample volume is greater than 140 µl, increase the amount of ethanol proportionally (e.g., a 280-µl sample will require 1120 µl of ethanol). In order to ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.

**6. Carefully apply 630 µl of the solution from step 5 to the QIAamp spin column (in a 2-ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp spin column into a clean 2-ml collection tube, and discard the tube containing the filtrate.**

Close each spin column in order to avoid cross-contamination during centrifugation. Centrifugation is performed at 6000 x g (8000 rpm) in order to limit microcentrifuge noise. Centrifugation at full speed will not affect the yield or purity of the viral RNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.

**7. Carefully open the QIAamp spin column, and repeat step 6.**

If the sample volume was greater than 140 µl, repeat this step until all of the lysate has been loaded onto the spin column.

**8. Carefully open the QIAamp spin column, and add 500 µl of Buffer AW1. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp spin column in a clean 2-ml collection tube (provided), and discard the tube containing the filtrate.**

It is not necessary to increase the volume of Buffer AW1 even if the original sample volume was larger than 140 µl.

**9. Carefully open the QIAamp spin column, and add 500 µl of Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Continue directly with step 10, or to eliminate any chance of possible Buffer AW2 carryover, perform step 9a, and then continue with step 10.**

**Note:** Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in flow-through, containing Buffer AW2, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column. In these cases, the optional step 9a should be performed.

**9a. (Optional): Place the QIAamp spin column in a new 2-ml collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

**10. Place the QIAamp spin column in a clean 1.5-ml microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QIAamp spin column and add 60 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6000 x g (8000 rpm) for 1 min.**

A single elution with 60 µl of Buffer AVE is sufficient to elute at least 90% of the viral RNA from the QIAamp spin column. Performing a double elution using 2 x 40 µl of Buffer AVE will increase yield by up to 10%. Elution with volumes of less than 30 µl will lead to reduced yields and will not increase the final concentration of RNA in the eluate.

Viral RNA is stable for up to one year when stored at -20°C or -70°C.

## QIAamp Viral RNA Mini Vacuum Protocol

**Notes:** Please read “Important Notes Before Starting” on pages 14–17.

- Equilibrate samples to room temperature (15–25°C).
  - Equilibrate Buffer AVE to room temperature for elution in step 11.
  - Check that Buffers AW1 and AW2, and Carrier RNA have been prepared according to instructions (see pages 14–15).
  - Redissolve precipitate in Buffer AVL/Carrier RNA by heating, if necessary, and cool to room temperature before use.
  - Connect the QIAvac 6S vacuum manifold (see Figure 2, page 12) to the vacuum pump and open the QIAvac 6S lid. Place QIAvac Luer Adapters (or blanks to seal unused slots) into the slots of the QIAvac top plate and close the QIAvac 6S lid. Place the waste tray inside the QIAvac 6S base and place the top plate squarely over the base. Insert a VacConnector into the luer connectors of the Luer Adapter(s) in the vacuum manifold. Seal unused luer connectors with the plugs provided in the QIAvac Luer Adapter Set.
  - Switch off the vacuum between protocol steps to ensure that a consistent, even vacuum is applied during each step.
  - All centrifugation steps are carried out at room temperature.
- 1. Pipet 560 µl of prepared Buffer AVL containing Carrier RNA into a 1.5-ml microcentrifuge tube.**

If the sample volume is larger than 140 µl, increase the amount of Buffer AVL/Carrier RNA proportionally (e.g., a 280-µl sample will require 1120 µl Buffer AVL/Carrier RNA).
  - 2. Add 140 µl plasma, serum, urine, cell-culture supernatant, or cell-free body fluid to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.**

To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer AVL to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.
  - 3. Incubate at room temperature (15–25°C) for 10 min.**

Viral particle lysis is complete after lysis for 10 min at room temperature. Longer incubation times have no effect on the yield or quality of the purified RNA. Potentially infectious agents and RNases are inactivated in Buffer AVL.
  - 4. Briefly centrifuge the 1.5-ml microcentrifuge tube to remove drops from the inside of the lid.**

- 5. Add 560  $\mu$ l of ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the 1.5-ml microcentrifuge tube to remove drops from inside the lid. Insert a QIAamp spin column into the VacConnector on the QIAvac 6S vacuum manifold.**

Only ethanol should be used since other alcohols may result in reduced yield and purity of the RNA. If the sample volume is greater than 140  $\mu$ l, increase the amount of ethanol proportionally (e.g., a 280- $\mu$ l sample will require 1120  $\mu$ l of ethanol). In order to ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.

The collection tube from the blister pack can be saved for the centrifugation in step 10.

- 6. Carefully apply 630  $\mu$ l of the solution from step 5 to the QIAamp spin column without wetting the rim. Switch on the vacuum pump. Be sure to leave the lid of the QIAamp spin column open while applying vacuum. After all lysates have been drawn through the spin column, switch off the vacuum pump.**

If at this stage all of the solution has not passed through the membrane, place the QIAamp spin column into a clean 2-ml collection tube (provided), close the cap, and centrifuge at 6000  $\times$  g (8000 rpm) for 3 min or until it has completely passed through. Place the QIAamp spin column into another clean 2-ml collection tube, and discard the tube containing the filtrate. Continue with step 7 of the spin protocol on page 19.

Centrifugation is performed at 6000  $\times$  g (8000 rpm) in order to limit centrifuge noise. Centrifugation at full speed will not affect the yield or purity of viral RNA.

- 7. Repeat step 6.**

If the sample volume was higher than 140  $\mu$ l, repeat this step until all of the lysate has been drawn through the spin column.

- 8. Carefully add 750  $\mu$ l of Buffer AW1 to the QIAamp spin column, and switch on the vacuum pump. Do not wet the rim of the spin column. After all of Buffer AW1 has been drawn through the spin column, switch off the vacuum pump.**
- 9. Carefully add 750  $\mu$ l of Buffer AW2 to the QIAamp spin column, and switch on the vacuum pump. Do not wet the rim of the spin column. After all of Buffer AW2 has been drawn through the spin column, switch off the vacuum pump.**
- 10. Close the lid of the QIAamp spin column, remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp spin column into a clean 2-ml collection tube, and centrifuge at full speed for 1 min to dry the membrane completely.**

- 11. Place the QIAamp spin column into a clean 1.5-ml microcentrifuge tube (not provided). Discard the collection tube containing the filtrate. Carefully open the QIAamp spin column. Add 60  $\mu$ l of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6000 x g (8000 rpm) for 1 min.**

A single elution with 60  $\mu$ l of Buffer AVE is sufficient to elute at least 90% of the viral RNA from the QIAamp spin column. Performing a double elution using 2 x 40  $\mu$ l of Buffer AVE will increase yield by up to 10%. Elution with volumes of less than 30  $\mu$ l will lead to reduced yields and will not increase the final concentration of RNA in the eluate.

Viral RNA is stable for up to one year when stored at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$ .

## Protocol for Large Sample Volumes

Samples of up to 560  $\mu\text{l}$  can be processed with the QIAamp Viral RNA Mini Kit using this protocol.

1. Pipet sample into a 5-ml microcentrifuge tube. Use up to 560  $\mu\text{l}$  of fresh plasma, serum, cell-culture supernatant, or cell-free body fluids. Up to 560  $\mu\text{l}$  of frozen samples (thawed only once) can also be used. Sample volume should always be a multiple of 140  $\mu\text{l}$ .
2. Add 560  $\mu\text{l}$  Buffer AVL/Carrier RNA (prepared as described on page 14) per 140  $\mu\text{l}$  of sample. Mix thoroughly by vortexing.
3. Incubate at room temperature (15–25°C) for 10 min.
4. Add 560  $\mu\text{l}$  of ethanol (96–100%) per 140  $\mu\text{l}$  of initial sample volume, and mix again by vortexing.
5. Apply 630  $\mu\text{l}$  of the lysate to the QIAamp spin column. Centrifuge 1 min at 6000  $\times g$  (8000 rpm), discard the filtrate.
6. Repeat step 5 until the entire lysate has been loaded onto the column. A maximum of 8  $\times$  630  $\mu\text{l}$  can be loaded onto the QIAamp spin column.
7. Place QIAamp spin column in a clean 2-ml collection tube.
8. Follow the QIAamp Viral RNA Mini Spin Protocol from step 8 (page 14).

## Protocol for Sample Concentration

Plasma, serum, urine, cerebrospinal fluid, bone marrow, and other body fluids often have very low viral titers. In these cases, concentrating samples of up to 3.5 ml to a final volume of 140  $\mu$ l is recommended.

1. Use centrifugal microconcentrators such as Centricon<sup>®</sup>-100 (Amicon: 2 ml, Cat. No. 4211), Microsep 100 (Filtron: 3.5 ml, Cat. No. OD100C40), Ultrafree<sup>®</sup>-CL (Millipore: 2 ml, Cat. No. UFC4 THK 25), or equivalent from other suppliers.
2. Apply up to 3.5 ml of sample to the microconcentrator following the manufacturer's instructions.
3. Centrifuge according to manufacturer's instructions to a final volume of 140  $\mu$ l.  
Some samples, plasma in particular, may be difficult to concentrate to 140  $\mu$ l due to high viscosity. Centrifugation for up to 6 hours may be necessary.
4. Pipet 140  $\mu$ l of concentrated sample into a 1.5-ml microcentrifuge tube, and follow the QIAamp Viral RNA Mini Spin Protocol on page 18.

# Protocol for Isolation of Cellular, Bacterial, or Viral DNA from Urine

Buffer AVL, used in the QIAamp Viral RNA Mini procedure, inactivates the numerous unidentified PCR inhibitors found in urine. Therefore, for isolation of cellular, bacterial, or viral DNA from urine for use in PCR the QIAamp Viral RNA Mini Spin Protocol (page 18) is recommended.

Urine often contains very low numbers of cells, bacteria, or viruses. In these cases, we recommend concentrating samples of up to 3.5 ml to a final volume of 140  $\mu$ l, as described in the "Protocol for Sample Concentration" on page 24, before processing.

For isolation of DNA from Gram-positive bacteria, please contact QIAGEN Technical Services.

# Troubleshooting Guide

## Comments and Suggestions

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### Little or no RNA in the eluate

- |    |  |   |
|----|--|---|
| a) | Carrier RNA not added to Buffer AVL            | Dissolve carrier RNA in Buffer AVL as described on page 14, and repeat the purification procedure with a new sample.  |
| b) | Degraded carrier RNA                           | Buffer AVL/Carrier RNA was warmed more than 6 times or incubated for more than 5 min at 80°C. Prepare a new bottle of Buffer AVL/Carrier RNA according to the instructions on page 14, and repeat the purification procedure with a new sample.   |
| c) | Sample frozen and thawed more than once        | Repeated freezing and thawing should be avoided. 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 micro-concentrator. Repeat the RNA purification procedure with a new sample. See "Protocol for Sample Concentration" on page 24.  |
| 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 5)        | 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 5.   |
| i) | Isopropanol used instead of ethanol            | We strictly recommend the use of ethanol as isopropanol causes reduced yields.  |
| j) | 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.  |

## Comments and Suggestions

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- |    |   |  |
|----|---|--|
| l) | Buffer AW1 or AW2 prepared incorrectly      | Check that Buffer AW1 and AW2 concentrates were diluted with correct volumes of pure (96–100%) ethanol. Repeat the purification procedure with a new sample. |
| m) | Buffer AW1 or AW2 prepared with 70% ethanol | Check that Buffer AW1 and AW2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.                           |
| n) | Buffers AW1 and AW2 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 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 added to Buffer AVL.   |
| d) | Too much Carrier RNA in the eluate  | Determine the maximum amount of Carrier RNA suitable for your RT-PCR. Adjust the concentration of Carrier RNA added to Buffer AVL accordingly.  |
| 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) | Buffers AW1 and AW2 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 <i>Taq</i> DNA polymerase used | If enzymes are changed, it may be necessary to readjust the amount of Carrier RNA added to Buffer AVL.  |

## Comments and Suggestions

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### DNA contamination

DNA and RNA present in the sample

To avoid copurification of DNA, use of cell-free body fluids for preparation of viral RNA is recommended. Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, and most swabs, should be made cell-free by centrifugation or filtration. If using centrifugation, pellet the cells for 10 min at 1500 x g and use supernatant for isolation of viral RNA. If DNA-free RNA is required, digest either the sample or the eluate with RNase-free DNase. DNase in the eluate must be inactivated by heat treatment (15 min, 70°C).

### General handling

- a) Lysate not completely passed through the membrane
- Using spin protocol: Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane.
- Using vacuum protocol: Insufficient vacuum was applied or the lid of the spin column was closed during the vacuum step. Increase the vacuum, and open the lid while applying the vacuum. If the vacuum pressure cannot be increased, place the QIAamp spin column in a clean 2-ml collection tube, close the cap, and centrifuge at 6000 x g (8000 rpm) for 3 min or until the lysate has completely passed through the membrane. Place the QIAamp spin column into another clean 2-ml collection tube, and discard the tube containing the filtrate. Continue with step 7 of the spin protocol on page 19.
- b) Clogged membrane
- Cryoprecipitates have formed in plasma due to repeated freezing and thawing. Do not use plasma that has been frozen and thawed more than once.
- c) Cross-contamination between samples
- To avoid cross-contamination when handling QIAamp spin columns follow the guidelines in "Handling of QIAamp spin columns" on page 16. Repeat the purification procedure with new samples.
- d) Vacuum pressure too high/too low
- Using a vacuum pressure that is too high may damage the QIAamp membrane. Using a vacuum pressure which is too low may cause reduced DNA yield and purity. Use a vacuum regulator (see ordering information on page 33) to adjust the pressure to -800 to -900 mbar for all vacuum steps.

# 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. Great 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 whenever possible. During the procedure, work quickly to avoid degradation of RNA by endogenous or residual RNases.

### 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.

### Non-disposable plasticware

Non-disposable 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 RNase-free water (see "Solutions", page 30). 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 inactivate ribonucleases. Alternatively, glassware can be treated with DEPC\* (diethyl pyrocarbonate). Rinse the

\* DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.

glassware with 0.1% DEPC (0.1% 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% H<sub>2</sub>O<sub>2</sub>. 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 the presence of Tris buffers and decomposes rapidly into ethanol and CO<sub>2</sub>. 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 carboxymethylation. Carboxymethylated 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. Shake vigorously to bring the DEPC into solution, or let the solution bake 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 Viral RNA buffers are not rendered RNase-free by DEPC treatment and are therefore free of any DEPC contamination.

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

† DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.

## Ordering Information

Product	Contents	Cat. No.
<b>QIAamp Viral RNA Mini Kits — for viral RNA purification from plasma, serum, and cell-free body fluids</b>		
QIAamp Viral RNA Mini Kit (50)	50 QIAamp Spin Columns, Carrier RNA , Buffers and Collection Tubes (2-ml)	52904
QIAamp Viral RNA Mini Kit (250)	250 QIAamp Spin Columns, Carrier RNA , Buffers and Collection Tubes (2-ml)	52906
<b>Related Products</b>		
<b>QIAamp 96 Viral RNA BioRobot Kit* — for automated, high-throughput viral RNA purification from plasma, serum, and cell-free body fluids</b>		
QIAamp 96 Viral RNA BioRobot™ Kit (12)	12 QIAamp 96 Plates, Carrier RNA, Buffers, AirPore™ Tape Sheets, Tape Pad, Square-Well Blocks, Racks with Collection Microtubes, Caps	965542
<b>QIAamp RNA Blood Mini Kit — for total RNA purification from blood and body fluids</b>		
QIAamp RNA Blood Mini Kit (20)	20 QIAamp Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5-ml and 2-ml), RNase-free Reagents and Buffers	52303
QIAamp RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5-ml and 2-ml), RNase-free Reagents and Buffers	52304
QIAamp RNA Blood Mini Kit (250)	250 QIAamp Mini Spin Columns, 250 QIAshredder Spin Columns, Collection Tubes (1.5-ml and 2-ml), RNase-free Reagents and Buffers	52306

\* For more information, please contact your local Technical Services Department or distributor listed on the last page of this handbook.

## Ordering Information

Product	Contents	Cat. No.
<b>QIAamp DNA Blood Mini Kits — for genomic DNA purification from blood and body fluids</b>		
QIAamp DNA Blood Mini Kit (50)	50 QIAamp Spin Columns, QIAGEN Protease, Reagents, Buffers and Collection Tubes (2-ml)	51104
QIAamp DNA Blood Mini Kit (250)	250 QIAamp Spin Columns, QIAGEN Protease, Reagents, Buffers and Collection Tubes (2-ml)	51106
<b>QIAamp 96 DNA Blood Kits* — for high-throughput genomic DNA purification from blood and body fluids</b>		
QIAamp 96 DNA Blood Kit (4)	4 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Plates, and Collection Vessels	51161
QIAamp 96 DNA Blood Kit (24)	24 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Plates, and Collection Vessels	51163
<b>QIAamp DNA Mini Kits — for genomic DNA purification from tissue, blood, and body fluids</b>		
QIAamp DNA Mini Kit (50)	50 QIAamp Spin Columns, Proteinase K, Reagents, Buffers and Collection Tubes (2-ml)	51304
QIAamp DNA Mini Kit (250)	250 QIAamp Spin Columns, Proteinase K, Reagents, Buffers and Collection Tubes (2-ml)	51306
<b>Accessories</b>		
Buffer AW1 (concentrate)	242 ml Wash Buffer 1 Concentrate for 1000 preparations	19081
Buffer AW2 (concentrate)	324 ml Wash Buffer 2 Concentrate for 1000 preparations	19072
Buffer AVL	5 x 31 ml Viral Lysis Buffer and 5 x 310 µg Carrier RNA for 250 preparations	19073
Buffer AL	216 ml for 1000 preparations	19075

\* Requires use of the QIAGEN 96-Well-Plate Centrifugation System. Please inquire.

## Ordering Information

Product	Contents	Cat. No.
Buffer ATL	200 ml Tissue Lysis Buffer for 1000 preparations	19076
Buffer AE	240 ml Elution Buffer for 1000 preparations	19077
Collection Tubes (2-ml)	1000 Collection Tubes (2-ml)	19201
QIAGEN Protease	125 mg (40–45 mAU/mg lyophilized)	19155
QIAGEN Protease	4 x 125 mg (40–45 mAU/mg lyophilized)	19157
QIAGEN Proteinase K (2)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10)	10 ml (>600 mAU/ml, solution)	19133
QIAvac 6S	Vacuum manifold for processing 1–6 QIAGEN 8-well strips: includes QIAvac 6S Top Plate with flip-up lid, Base, Waste Tray, Blanks, Strip Holder	19503
QIAvac Luer Adapter Set*	For processing 1–24 QIAGEN spin columns on QIAvac 6S: 6 adapters with 4 luer connectors each, 24 plugs	19541
VacConnectors (100)	100 disposable connectors for use with QIAamp spin columns on QIAvac Luer Adapters	19405
VacConnectors (500)	500 disposable connectors for use with QIAamp spin columns on QIAvac Luer Adapters	19407
Vacuum Regulator	For use with QIAvac manifolds	19530

\* Compatible only with QIAvac Top Plates containing flip-up lid.

## Notes



<b>Germany</b>	<b>QIAGEN GmbH</b>	Orders 02103-892-230	• Fax 02103-892-233	• Technical 02103-892-240
<b>USA</b>	<b>QIAGEN Inc.</b>	Orders 800-426-8157	• Fax 800-718-2056	• Technical 800-DNA-PREP (800-362-7737)
<b>Canada</b>	<b>QIAGEN Inc.</b>	Orders 800-572-9613	• Fax 905-501-0373	• Technical 800-DNA-PREP (800-362-7737)
<b>France</b>	<b>QIAGEN S.A.</b>	Orders 01-60-920-920	• Fax 01-60-920-925	• Technical 01-60-920-930
<b>Japan</b>	<b>QIAGEN K.K.</b>	Telephone 03-5805-7261	• Fax 03-5805-7263	• Technical 03-5805-7261
<b>Switzerland</b>	<b>QIAGEN AG</b>	Orders 061-319-30-30	• Fax 061-319-30-33	• Technical 061-319-30-31
<b>UK</b>	<b>QIAGEN Ltd.</b>	Orders 01293-422-911	• Fax 01293-422-922	• Technical 01293-422-999
<b>Australia</b>	<b>QIAGEN Pty Ltd</b>	Orders 03-9489-3666	• Fax 03-9489-3888	• Technical 03-9489-3666

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