Third Edition April 2010

QIAamp® MinElute® Virus Vacuum Handbook

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

QIAGEN®

Sample & Assay Technologies

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Contents

Kit Contents

QIAamp MinElute Virus Vacuum Kit	**(50)**
Catalog no.	**57714**
Number of preps	**50**
QIAamp MinElute Columns	50
Extension Tubes (3 ml)	50
Collection Tubes (1.5 ml)	50
Buffer AL*	33 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer AVE† (tubes with purple caps)	7 x 2 ml
Protease Resuspension Buffer†	6 ml
Carrier RNA (tubes with red caps)	310 µg
QIAGEN® Protease‡	1 vial
Handbook	1

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 7 for safety information.

† Contains sodium azide as a preservative.

‡ Resuspension volume 4.4 ml. See "Preparation of QIAGEN Protease", page 20.

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 20. 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 Vacuum Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

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

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

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 or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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 (see back cover).

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 the QIAamp MinElute Virus Vacuum Kit 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 local distributors (see back cover).

Safety Information

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

CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffer AL or Buffer AW1.

Buffers AL and 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.

The following risk and safety phrases apply to components of the QIAamp MinElute Virus Vacuum Kit:

Buffers AL and AW1

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

QIAGEN Protease

Contains subtilisin: sensitizer, irritant. Risk and safety phrases:* R37/38-41-42, S22-24-26-36/37/39-46

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R37/38: Irritating to respiratory system and skin; R41: Risk of serious damage to eyes; R42: May cause sensitization by inhalation; S13: Keep away from food, drink and animal feeding stuffs; S22: Do not breathe dust; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed, seek medical advice immediately and show the container or label.

Introduction

The QIAamp MinElute Virus Vacuum 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, or other cell-free body fluids. Samples can be either fresh or frozen, provided they have not been frozen and thawed more than once (see page 19). 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 Vacuum procedure comprises 4 steps (lyse, bind, wash, elute) and is carried out using QIAamp MinElute columns on a vacuum manifold. 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 Vacuum procedure, which is highly suited for simultaneous processing of multiple samples, yields pure nucleic acid in less than 1 hour. The QIAamp MinElute Virus Vacuum 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.

Automated viral nucleic purification on the QIAcube™ using the QIAamp MinElute Virus Spin Kit

Purification of viral nucleic acids using the QIAamp MinElute procedure can now be fully automated on the new QIAcube using the QIAamp MinElute Virus Spin Kit (cat. no. 57704). The kit uses the same chemistries as the QIAamp MinElute Virus Vacuum Kit but with smaller sample volumes. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. The QIAcube performs the same steps as the manual spin procedure (lyse, bind, wash, and elute) enabling you to continue using QIAamp MinElute Virus chemistries for purification of high-quality viral nucleic acids.

The QIAcube is 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/MyQIAcube .

Sample volumes using the QIAamp MinElute Virus Vacuum 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 vacuum procedure is optimized for use with a starting volume of 500 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 vacuum pressure. 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.

A vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413) and a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., Vacuum Pump, cat. no. 84010 [US and Canada], 84000 [Japan], or 84020 [rest of world]) are required for the protocol. A Vacuum Regulator (e.g., cat. no. 19530) should be used for easy monitoring of vacuum pressures and convenient vacuum release.

Removal of residual contaminants

Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away during a sequence of 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. 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 is collected in 1.5 ml microcentrifuge tubes (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 Vacuum protocol, remember that there will be much more carrier RNA in the sample than viral RNA.

The size distribution of viral nucleic acid purified using 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).

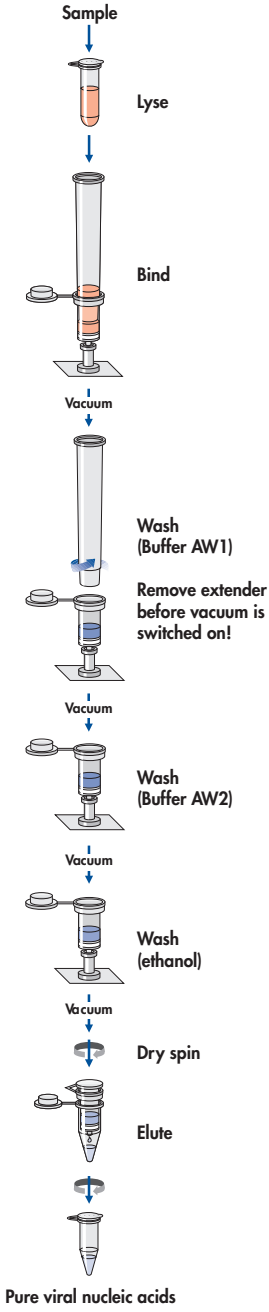
Carrier RNA

Carrier RNA serves two purposes. Firstly, it enhances binding of viral nucleic acids to the QIAamp MinElute 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 Vacuum 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.

The QIAamp MinElute Virus Vacuum Procedure



Addition of internal controls

Using the QIAamp MinElute Virus Vacuum protocols 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.

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 material safety data sheets (MSDSs), available from the product supplier.

- Ethanol (96–100%)*
- 2 ml microcentrifuge tubes
- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Heating block for lysis of samples at 56°C
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- Vortexer
- QIAvac 24 Plus vacuum manifold (cat. no. 19413) or equivalent
- VacConnectors (cat. no. 19407)
- Vacuum Regulator (cat. 19530) for easy monitoring of vacuum pressures and easy releasing of vacuum
- Vacuum Pump (cat. no. 84010 [USA and Canada], 84000 [Japan], or 84020 [rest of world]) or equivalent pump capable of producing a vacuum of –800 to –900 mbar
- Optional: VacValves (cat. no. 19408)
- Optional: QIAvac Connecting System (cat. no. 19419)

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

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.

The QIAvac 24 Plus

The QIAvac 24 Plus is designed for fast and efficient vacuum processing of up to 24 QIAGEN spin columns in parallel. Samples and wash solutions are drawn through the column membranes by vacuum instead of centrifugation, providing greater speed and reduced hands-on time in purification procedures.

In combination with the QIAvac Connecting System (optional), the QIAvac 24 Plus can be used as a flow-through system. The sample flow-through is collected in a separate waste bottle.

For maintenance of the QIAvac 24 Plus, please refer to the handling guidelines in the *QIAvac 24 Plus Handbook*.

Processing QIAamp MinElute columns on the QIAvac 24 Plus

QIAamp MinElute spin columns are processed on the QIAvac 24 Plus using disposable VacConnectors and reusable VacValves. VacValves (optional) are inserted directly into the luer slots of the QIAvac 24 Plus manifold and ensure a steady flow rate, facilitating parallel processing of samples of different natures (e.g., serum and plasma), volumes, or viscosities. They should be used if sample flow rates differ significantly in order to ensure consistent vacuum. VacConnectors are disposable connectors that fit between QIAamp MinElute columns and VacValves or between the QIAamp MinElute columns and the luer slots of the QIAvac 24 Plus. They prevent direct contact between the spin column and VacValve during purification, thereby avoiding any cross-contamination between samples. VacConnectors are discarded after a single use.

Handling guidelines for the QIAvac 24 Plus

- Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the QIAvac 24 Plus manifold may crack.
- Always store the QIAvac 24 Plus clean and dry. For cleaning procedures see the *QIAvac 24 Plus Handbook*.
- The components of the QIAvac 24 Plus are not resistant to certain solvents (Table 1). If these solvents are spilled on the unit, rinse it thoroughly with water.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 Plus manifold.
- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- Contact QIAGEN Technical Services or your local distributor for information concerning spare or replacement parts.
- The vacuum pressure is the pressure differential between the inside of the vacuum manifold and the atmosphere (standard atmospheric pressure 1013 millibar or 760 mm Hg) and can be measured using the QIAvac Connecting System or a vacuum regulator (see Figure 1). The vacuum protocol requires a vacuum pump capable of producing a vacuum or –800 to –900 mbar (e.g., QIAGEN, Vacuum Pump). Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce DNA yield and purity and increase the frequency of clogged membranes.

Table 1. Chemical resistance properties of QIAvac 24 Plus

Resistant to:		
Acetic acid	Chaotropic salts	Chlorine bleach
Chromic acid	Concentrated alcohols	Hydrochloric acid
SDS	Sodium chloride	Sodium hydroxide
Tween®20	Urea	
Not resistant to:		
Benzene	Chloroform	Ethers
Phenol	Toluene	

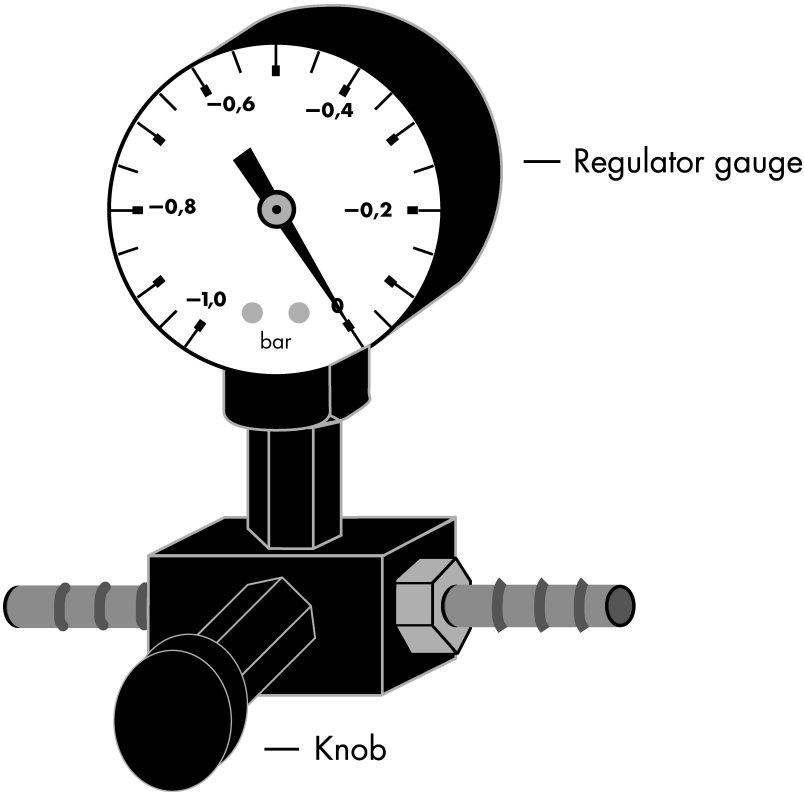


Figure 1. Schematic diagram of the Vacuum Regulator.

Setup of the QIAvac 24 Plus vacuum manifold

1. **Connect the QIAvac 24 Plus to a vacuum source. If using the QIAvac Connecting System, connect the system to the manifold and vacuum source as described in Appendix A of the QIAvac 24 Plus Handbook.**
2. **Insert a VacValve into each luer slot of the QIAvac 24 Plus that is to be used (see Figure 2). Close unused luer slots with luer plugs or close the inserted VacValve.**

   VacValves should be used if flow rates of samples differ significantly to ensure consistent vacuum.

3. **Insert a VacConnector into each VacValve (see Figure 2).**

   Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.

4. **Place the QIAamp MinElute columns into the VacConnectors on the manifold (see Figure 2).**

5. **Insert an Extension Tube into each QIAamp MinElute column (see Figure 3).**

6. **For nucleic acid purification, follow the instructions in the vacuum protocol. Discard the VacConnectors appropriately after use.**

   Leave the lid of the QIAamp MinElute column open while applying vacuum.

   Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during processing. For faster vacuum release, a vacuum regulator should be used (see Figure 1).

   Note: Each VacValve can be closed individually when the sample is completely drawn through the spin column, allowing parallel processing of samples of different volumes or viscosities.

7. **After processing samples, clean the QIAvac 24 Plus (see "Cleaning and Decontaminating the QIAvac 24 Plus" in the *QIAvac 24 Plus Handbook*).**

   Note: Buffers AL and AW1 used in QIAamp MinElute procedure are not compatible with disinfecting agents containing bleach. See page 7 for safety information.

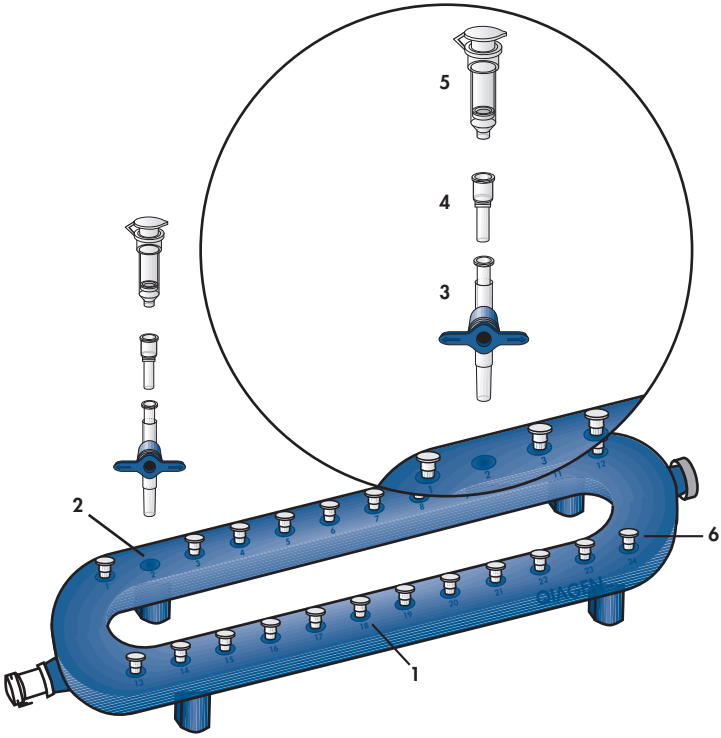


Figure 2. Setting up the QIAvac 24 Plus with QIAamp MinElute columns using VacValves and VacConnectors.

1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus
3. VacValve*
4. VacConnector*
5. QIAamp column
6. Luer slot closed with luer plug

* Must be purchased separately.

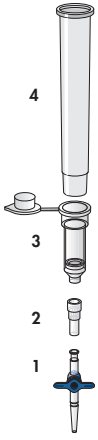


Figure 3. Assembly of components of the QIAamp MinElute Vacuum Kit.

1. VacValve†
2. VacConnector†
3. QIAamp MinElute column
4. Extension tube

† Must be purchased separately.

Centrifugation

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 (15–25°C).

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. Discard the filtrate and the used 2 ml collection tube. Place the QIAamp MinElute column in the 1.5 ml collection tube.
- Open only one QIAamp MinElute column at a time, and take care to avoid generating aerosols.

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 30 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 4.4 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 2. For larger numbers of samples, volumes can be calculated using the following sample calculation:

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 7 for safety information.

n x 0.55 ml = **y** ml

y ml x 11.2 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

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

Table 2. Volumes of Buffer AL and carrier RNA–Buffer AVE mix required for the QIAamp MinElute Virus Vacuum procedure

No. samples	Vol. Buffer AL (ml)	Vol. Carrier RNA–AVE (µl)	No. samples	Vol. Buffer AL (ml)	Vol. Carrier RNA–AVE (µl)
1	0.55	6.2	13	7.15	80.1
2	1.10	12.3	14	7.70	86.2
3	1.65	18.5	15	8.25	92.4
4	2.20	24.6	16	8.80	98.6
5	2.75	30.8	17	9.35	104.7
6	3.30	37.0	18	9.90	110.9
7	3.85	43.1	19	10.45	117.0
8	4.40	49.3	20	11.00	123.2
9	4.95	55.0	21	11.55	129.4
10	5.50	61.6	22	12.10	135.5
11	6.05	67.8	23	12.65	141.7
12	6.60	73.9	24	13.20	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 2 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 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 viral 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 chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 7 for safety information.

† 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 500 µl of plasma or serum using the QIAamp MinElute Virus Vacuum Kit.

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 20.
- Prepare a 56°C heating block for use in steps 4 and 19.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions on pages 19–21.
- Add carrier RNA reconstituted in Buffer AVE to Buffer AL according to instructions on page 20.
- For processing using VacConnectors and VacValves, set up the QIAvac 24 Plus as described on pages 16–18.

Procedure

1. **Pipet 75 µl QIAGEN Protease into a 2 ml microcentrifuge tube (not provided).**

   Note: Read "Preparation of QIAGEN Protease", page 20, for information about resuspending QIAGEN Protease in Buffer AVE (recommended) or Protease Resuspension Buffer.

2. **Add 500 µl of plasma or serum into the 2 ml microcentrifuge tube.**

3. **Add 500 µl of Buffer AL (containing 11.2 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.**

5. **Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.**

6. **Add 600 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 ambeint temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.

7. **Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.**
8. **Insert the QIAamp MinElute column into the VacConnector on the QIAvac 24 Plus. Insert an extension tube into the open QIAamp MinElute column.**

   Note: Keep the collection tube for the dry spin in step 18.
9. **Make sure that the main vacuum valve (between the vacuum pump and the vacuum manifold) and the screw cap valve (on the end of the QIAvac 24 Plus vacuum manifold) are closed. Switch on the vacuum pump by pressing the power switch.**

   The vacuum is applied only to the connecting system (if used) and not to the vacuum manifold.

   Note: For fast and convenient release of the vacuum pressure, the QIAvac Connecting System or the Vacuum Regulator should be used, see "Ordering Information", page 32.
10. **Carefully apply all of the lysate from step 7 into the extension tube of the QIAamp MinElute column without wetting the rim. Avoid touching the QIAamp MinElute column membrane with the pipet tip.**
11. **Open the main vacuum valve. After all lysates have been drawn through the QIAamp MinElute column, close the main vacuum valve and open the screw cap valve to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.**

    After closing the main vacuum valve, the vacuum is applied only to the connecting system (if used) and not the vacuum manifold.

    If the lysates from individual samples have not completely passed through the membrane despite the VacValves of all other QIAamp MinElute columns being closed, place the QIAamp MinElute column into a clean 2 ml collection tube (not provided), close the cap, and centrifuge at full speed for 3 min or until it has completely passed through. Additional collection tubes can be purchased separately, see "Ordering Information", page 32.
12. **Apply 600 µl of Buffer AW1 to the QIAamp MinElute column without wetting the rim. Avoid touching the QIAamp MinElute column membrane with the pipet tip. Remove and discard the extension tube.**

    Note: To avoid cross contamination, take care not to take tubes across neighboring QIAamp MinElute columns during extension tube removal.
13. **Open the main vacuum valve. After all Buffer AW1 has been drawn through the QIAamp MinElute column, close the main vacuum valve and open the screw cap valve to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.**
14. **Apply 750 µl of Buffer AW2 to the QIAamp MinElute column without wetting the rim. Avoid touching the QIAamp MinElute column membrane with the pipet tip. Leave the lid of the column open.**

15. **Open the main vacuum valve. After all Buffer AW2 has been drawn through the QIAamp MinElute column, close the main vacuum valve and open the screw cap valve to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.**
16. **Apply 750 µl of ethanol (96–100%) to the QIAamp MinElute column without wetting the rim. Avoid touching the QIAamp MinElute column membrane with the pipet tip. Leave the lid of the column open.**
17. **Open the main vacuum valve. After all ethanol has been drawn through the QIAamp MinElute column, close the main vacuum valve and open the screw cap valve to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.**
18. **Close the lid of the QIAamp MinElute column. Remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp MinElute column in a clean 2 ml collection tube saved from step 8, and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min to dry the membrane completely.**
19. **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.
20. **Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (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 applications. Remember that the recovered eluate volume will differ by approximately 5 µl from 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 20. 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.

		Comments and suggestions
f)	RNase contamination be careful to 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 AW2 prepared incorrectly	Check that Buffers AW1 and AW2 concentrates were diluted with the correct volume of ethanol. Repeat the purification procedure with new samples.
h)	Buffer AW1 or AW2 prepared with 70% ethanol	Check that Buffer AW1 and 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 20.
RNA or DNA does not perform well in downstream enzymatic reactions		
a)	Little or no RNA 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 19). 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	Reconstituted QIAGEN Protease was subjected to elevated temperature for a prolonged time. Repeat the procedure using new samples and fresh QIAGEN Protease.

		Comments and suggestions
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 20).
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 Buffers AW1 and 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 Tay 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.
General handling		
a)	Clogged QIAamp MinElute column	Remove the QIAamp MinElute column from the vacuum manifold, place it in a 2 ml collection tube and centrifuge it at full speed until sample has completely passed the membrane. Cryoprecipitates may have formed in 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. In case cryoprecipitates are visible clear the sample by centrifugation as described in "Sample storage" on page 19 before starting the sample preparation.
b)	Variable elution volumes	This is normal when different sample types have been processed.

		Comments and suggestions
c)	Vacuum pressure of 800–900 mbar not reached	Gasket of QIAvac lid has worn out. Check the seal of the manifold visually and replace it if necessary. VacValves have worn out. Remove all VacValves and insert VacConnectors directly into the luer extensions. Insert QIAamp MinElute columns into VacConnectors, close the lid of the columns and switch on vacuum. Check if vacuum pressure is reached. Replace VacValves if necessary. Connection to vacuum pump is leaky. Close all luer extension with luer caps and switch on the vacuum pump. Check if vacuum pressure is stable after the pump is switched on (and the Vacuum Regulator valve is closed). Exchange the connections between pump and vacuum manifold if necessary. After all above checks have been made, replace the vacuum pump with a stronger one.

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 RNase-free water* (see "Solutions", page 31). 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

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

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 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. Carbethyoxylated 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 Vacuum Kit buffers are not rendered RNase-free by DEPC treatment and are therefore free of any DEPC contamination.

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

Ordering Information

Product	Contents	Cat. no.
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
Accessories		
QIAvac 24 Plus	Vacuum Manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
VacValves (24)	24 valves for use with the QIAvac 24 Plus	19408
VacConnectors (500)	500 disposable connectors for use with QIAamp spin columns on luer slots or VacValves	19407
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubing, Couplings, Valve, Gauge, 24 VacValves	19419
Vacuum Pump	Universal vacuum pump	Inquire
Vacuum Regulator	For use with QIAvac manifolds	19530
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
Extension Tubes (3 ml)	For use with QIAGEN Mini or MinElute columns on vacuum manifold: 100 per pack	19587

Ordering Information

Product	Contents	Cat. no.
Related products		
QIAamp DSP Virus Kit	For 50 minipreps: QIAamp MinElute Columns, Buffers, Reagents, Tubes, Column Extenders, VacConnectors	60704
QIAamp MinElute Virus Spin Kit (50)	For 50 manual or fully automated minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704
QIAcube (110 V)* QIAcube (230 V)†	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor‡	9001292* 9001293†
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 DSP 96 Virus MDx Kit	For 12 x 96 preps: 12 QIAamp 96 Plates, RNase-Free Buffers, QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Carrier RNA	61762

* US, Canada, and Japan.

† Rest of world.

‡ Agreements for comprehensive service coverage are available; please inquire.

Ordering Information

Product	Contents	Cat. no.
QIAamp MinElute Media Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	57414
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

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Notes

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