Third Edition April 2010

QIAamp® Virus BioRobot® 9604 Kit Handbook

For simultaneous purification of viral RNA and DNA from plasma, serum, cell-culture supernatants, and cell-free body fluids using the BioRobot 9604 workstation

QIAGEN

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Contents

Kit Contents

QIAamp Virus BioRobot 9604 Kit	**(12)**
Catalog no.	**965662**
Number of preps	**12 x 96**
QIAamp® 96 Plates	12
S-Blocks*	26
Tape Pad	1
AirPore Tape	1 x 25 sheets
Caps for Elution Microtubes	3 x 50
Elution Microtubes CL	12 x 96
2 ml Tubes	50
15 ml Tubes	50
Caps for 2 ml Tubes (colorless)	50
500 ml Bottle for Buffer AW2	1
Carrier RNA (red caps)	12 x 1350 µg
QIAGEN Protease	6 vials†
Protease Solvent‡	6 x 10.2 ml
Buffer AL	1 x 330 ml
Buffer AW1§ (concentrate, green label)	6 x 175 ml
Buffer AW2‡ (concentrate, red label)	4 x 274 ml
2 ml Tubes containing Buffer AVE‡ (purple caps)	60 x 2 ml
Handbook	1

* Reusable; see page 16 for cleaning instructions.
† Resuspension volume 10.2 ml.
‡ Contains sodium azide as a preservative.
§ Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 6 for safety information.

Storage

QIAamp 96 plates and all buffers and reagents can be stored dry at the temperature indicated on the kit label. The expiration date for the kit is printed on the kit label and is valid only when the kit is stored at the indicated temperature.

Carrier RNA is provided lyophilized and can only be dissolved in Buffer AVE. Mixed carrier RNA and Buffer AVE should then be immediately added to Buffer AL as described in "Preparation of reagents" on page 13. This solution should be prepared fresh, and is stable at room temperature for up to 48 hours.

QIAGEN Protease is provided lyophilized. Reconstituted QIAGEN Protease is stable for 2 months when stored at 2–8°C. 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.

Product Use Limitations

The QIAamp Virus BioRobot 9604 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.

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).

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 components.

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

Buffers AW1 and AL 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 Virus BioRobot 9604 Kit:

Buffers AW1 and AL

Contain 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 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 feedingstuff; 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 this container or label.

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 this 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).

Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of QIAamp Virus BioRobot 9604 Kits is monitored routinely on a lot-to-lot basis. All components are tested separately to ensure highest performance and reliability.

Introduction

The QIAamp Virus BioRobot 9604 Kit uses well-established technology for simultaneous purification of viral DNA and RNA. The kit combines the selective binding properties of a silica-gel membrane with a high-throughput 96-well format, and is designed for automated processing of 200 µl samples on the BioRobot 9604. 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 13). 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. The automated process, including bar code reading and complete process documentation, requires less than 2 hours to process 96 samples with about 5 minutes hands-on time. Turnaround time between consecutive runs is approximately 10 minutes.

The QIAamp Virus BioRobot 9604 Kit can be used for isolation of nucleic acids from a broad range of RNA and DNA viruses. However, performance cannot be guaranteed for every virus species.

The QIAamp principle and procedure

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. Lysates are transferred to a second S-block and binding conditions are adjusted by adding ethanol. Lysates are then transferred to a QIAamp 96 plate and viral nucleic acids are adsorbed onto the silica-gel membrane as the lysate is drawn through by vacuum pressure. Nucleic acids bound to the membrane are efficiently washed in three steps, using vacuum pressure and centrifugation to draw wash buffers through the 96-well plate. Two different wash buffers are used, which considerably improves the purity of the eluted nucleic acids. Highly pure viral RNA and DNA is eluted in a single step in Buffer AVE, equilibrated to room temperature. The volume of eluate recovered depends on the nature of the sample, but is at least 50 µl.

Sample volumes using the QIAamp 96 Virus Purification Protocol

The QIAamp 96 Virus Purification Protocol is optimized for use with 200 µl samples. The BioRobot 9604 is capable of removing this volume from tubes of various sizes, such as CryoTube® or BD VACUTAINER® tubes. Special sample carrier racks are available upon request. The BioRobot 9604 is equipped with a liquid-level detection system to facilitate aspiration of 200 µl aliquots directly from primary sample tubes.

Carrier RNA

Carrier RNA serves two purposes. Firstly, it enhances binding of viral nucleic acids to the QIAamp membrane, especially if there are very few target molecules in the specimen. 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 protease, chaotropic salts, and detergent in Buffer AL. Thus, not adding carrier RNA to Buffer AL may lead to reduced recovery of viral RNA or DNA.

Lyophilized carrier RNA is provided in sufficient quantity for the volume of Buffer AL supplied with the kit. The concentration of carrier RNA provided ensures that the QIAamp Virus BioRobot 9604 Protocol can be used as a generic purification system for a wide range of viruses that is compatible with many different amplification systems.

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

Addition of internal controls

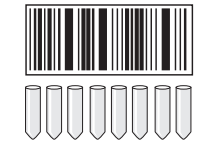
When using the QIAamp Virus BioRobot 9604 Kit together with amplification systems, it may be necessary to introduce an internal control into the purification procedure. If this is necessary, choose to run the QIAamp 96 Virus IC protocol in the QIAsoft Operating System (provided in the Viral Nucleic Acid Purification package). This protocol can be used with a number of amplification systems. It allows addition of a fixed volume of internal control RNA together with the carrier RNA to the lysis buffer. Different amplification systems may require the use of variable amounts of internal control. Refer to the manufacturer's instructions in order to determine the optimal concentration. Using a concentration other than that recommended may reduce amplification efficiency. Note that for optimal purification, internal control molecules should be more than 200 nucleotides long, as smaller molecules are not recovered efficiently.

Yield and size of viral nucleic acids

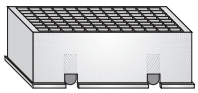
Each well of the QIAamp 96 plate can bind nucleic acids that are greater than 200 bases in length, but yield depends on sample volume and virus titer. Yields of viral nucleic acid isolated from biological samples are normally below 1 µg and are therefore difficult to determine with a spectrophotometer. Quantitative amplification methods are recommended for determination of yields. When quantifying nucleic acids isolated using the QIAamp 96 Virus Purification Protocol, remember that there will be much more carrier RNA in the sample than viral RNA.

The size distribution of viral nucleic acid purified with this procedure can be checked by agarose gel electrophoresis and hybridization to a virus-specific labeled probe followed by autoradiography (Sambrook, J., Russell, D.W., eds. [2001] *Molecular Cloning: a Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

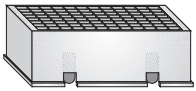
QIAamp 96 Virus Purification Protocol



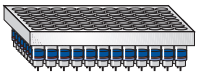
Scan bar codes



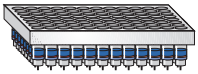
Aliquot QIAGEN Protease,
transfer samples,
and add Buffer AL
Lysis in thermoblock



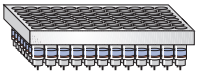
Add ethanol
and transfer lysates



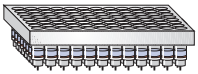
Transfer lysates



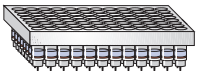
Wash with Buffer AW1



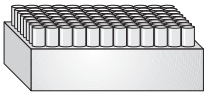
Wash with Buffer AW2



Second wash with
Buffer AW2



Add elution buffer



Purified viral nucleic acid

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.

- BioRobot 9604, configuration B
- Centrifuge 4-16 or 4-16K with Plate Rotor 2 x 96 (see page 27 for ordering information)
- Conductive QIAGEN 1.1 ml disposable filter tips for use with the BioRobot 9604
- Disposable gloves
- Ethanol (96–100%)*
- Distilled water
- If less than 96 samples will be processed per run, it is necessary to order extra Elution Microtubes CL (see Ordering Information, page 28)

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

Important Notes

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 25 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 DEPC (diethyl pyrocarbonate: an RNase-degrading chemical), 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 aliquots at –20°C or –80°C 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 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 reagents

QIAGEN Protease

Add 10.2 ml protease solvent to each vial of lyophilized QIAGEN Protease. After the protease has been reconstituted, pipet 8 x 1.25 ml into 2 ml tubes (provided). Close the caps and transfer four tubes into positions A1–A4 of the thermoblock on the BioRobot 9604. The remaining four tubes should be stored at 4°C until further use. Storage at –20°C will prolong the life of QIAGEN Protease, but repeated freezing and thawing should be avoided.

Note: After the run, there may be some QIAGEN Protease left in the 2 ml tubes. Collect all remainders for use during the next run. Store at 4°C for up to 2 months. Use only the tubes provided with the kit.

Buffer AL*

Buffer AL is supplied as a single reagent in a stock bottle and should be stored at 15–25°C.

For runs of fewer than 96 samples: The QIAsoft Operating System indicates how much Buffer AL is required during the BioRobot setup process before a purification run is started. Transfer the given volume of Buffer AL into a 50 ml tube (not provided) and add reconstituted carrier RNA as described in "Addition of carrier RNA to Buffer AL" (see below). Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. Slowly pour or pipet equal volumes of Buffer AL-carrier RNA mix into each of four 15 ml tubes (provided). Buffer AL is viscous, so 5–10% of the mix may be lost during this transfer, but this is accounted for in the procedure. It is important to ensure that equal volumes are delivered to all four 15 ml tubes. Remove any large bubbles with a pipet tip and place the filled 15 ml tubes into reagent slots B1 to B4.

For runs of 96 samples: Pipet 26 ml Buffer AL into a 50 ml tube (not provided) and add reconstituted carrier RNA as described in "Addition of carrier RNA to Buffer AL" (see below). Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. Slowly pour or pipet 6 ml Buffer AL-carrier RNA mix into each of four 15 ml tubes (provided). Buffer AL is viscous and this may mean that 5–10% of the mix is lost during transfer, but this is accounted for in the procedure. It is important to ensure that equal volumes are delivered to all four 15 ml tubes. Remove any large bubbles with a pipet tip and place the filled 15 ml tubes into reagent slots B1 to B4.

Do not fill more than the required number of tubes. Use only the tubes provided with the kit.

Addition of carrier RNA to Buffer AL

Lyophilized carrier RNA is stable for up to 1 year when stored at room temperature (15–25°C). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AVE and then added to Buffer AL. After dissolving in Buffer AVE, carrier RNA is stable for up to 8 days at –20°C. Carrier RNA dissolved in Buffer AL is stable at room temperature for up to 48 hours.

For 96 preparations add 800 µl of Buffer AVE to one vial containing 1350 µg lyophilized carrier RNA. Use Buffer AVE from an unopened tube and discard the unused portion. One tube of Buffer AVE is provided for each tube of carrier RNA. Transfer 433.3 µl of carrier RNA in Buffer AVE to the tube containing 26 ml Buffer AL, to give a final concentration of 28 µg carrier RNA per milliliter of Buffer AL (5.6 µg carrier RNA per preparation). Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. Distribute 6 ml Buffer AL-carrier RNA into each of four 15 ml tubes for Buffer AL (provided), and remove any large air bubbles.

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

Note: If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of reconstituted carrier RNA to the tubes containing Buffer AL. For each microgram of carrier RNA required per preparation, add 77.4 µl carrier RNA reconstituted in Buffer AVE to the 50 ml tube containing 26 ml Buffer AL.

Always prepare a fresh aliquot of Buffer AL–carrier RNA solution for each run of 96 samples. Sufficient Buffer AL–carrier RNA is supplied with the kit for 12 runs of 96 samples each. If processing 96 samples divided over more than one run, QIAsoft calculates the required volume of Buffer AL-carrier RNA and informs you of how much to use at the appropriate time.

Buffer AW1 (green label)*

Add 230 ml of ethanol (96–100%) to a bottle containing 175 ml of Buffer AW1 concentrate, as described on the bottle. Store reconstituted Buffer AW1 at room temperature (15–25°C) between runs.

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

For easy identification, the Buffer AW1 bottle has a green label. It should be connected to the green adapter on the BioRobot 9604.

Sufficient Buffer AW1 is supplied for 12 runs of 96 samples. One bottle of reconstituted Buffer AW1 contains enough wash buffer for two runs of 96 samples each. Approximately 100 ml Buffers AW1 and AW2 is used to purge the system tubing during each run, and this volume remains constant regardless of the number of samples to be purified. Therefore processing 96 samples divided over more than one run (e.g., two 48-sample runs) will require more buffer than one 96-sample run. If runs of fewer than 96 samples are often performed, additional Buffer AW1 must be purchased (see ordering information, page 27).

Buffer AW2 (red label)†

Add 640 ml of ethanol (96–100%) to a bottle containing 274 ml of Buffer AW2 concentrate, as described on the bottle. For a single run of 96 samples, pour 500 ml reconstituted Buffer AW2 into the empty 500 ml bottle (provided). Residual Buffer AW2 after a run should be kept for the next run, and should be stored at room temperature (15–25°C).

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

For easy identification, the Buffer AW2 bottle has a red label. It should be connected to the red adapter on the BioRobot 9604.

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

† Contains sodium azide as a preservative.

Sufficient Buffer AW2 is supplied for 12 runs of 96 samples. Approximately 100 ml Buffers AW1 and AW2 is used to purge the system tubing during each run, and this volume remains constant regardless of the number of samples to be purified. Therefore processing 96 samples divided over more than one run (e.g., two 48-sample runs) will require more buffer than one 96-sample run. If runs of fewer than 96 samples are often performed, additional Buffer AW2 must be purchased (see ordering information, page 27).

Buffer AVE (purple cap)*

For one run of 96 samples, 4 tubes of Buffer AVE (2 ml each) are required. If you intend to purify RNA, always wear a fresh pair of gloves when handling Buffer AVE.

Note: Buffer AVE is RNase-free upon delivery. Take great care to avoid contamination with RNases during handling.

Place the four Buffer AVE tubes in positions A5 to A8 on the thermoblock of the BioRobot 9604.

Note: Even if fewer than 96 samples are prepared at a time, it is still necessary to attach all 4 Buffer AVE tubes containing 2 ml of buffer each. If runs of fewer than 96 samples are performed, additional Buffer AVE must be purchased (see ordering information, page 27).

Ethanol

Before starting a run, fill the empty 500 ml bottle for ethanol (supplied with the BioRobot 9604) with ethanol.

S-Blocks

Twenty-six S-Blocks are supplied and two are used per run of 96 samples. One S-Block is placed in the 96-well thermostat system for lysis. The other is used during adjustment of binding conditions and should be placed onto the MP-slot extension adapter in MP-slot 3. Discard both S-Blocks after use. Two S-Blocks are left over, and these are intended for repeated use to collect flow-through from the QIAamp 96 plate during centrifugation.

Reuse of S-Blocks

The two S-Blocks intended for repeated use should be thoroughly cleaned before using again, in order to avoid cross-contamination. Rinse them thoroughly in tap water, incubate for 1 minute at room temperature in 0.4 M HCl,† empty, and wash thoroughly with distilled water before reusing. Used S-Blocks can also be autoclaved after washing. Additional S-Blocks can be ordered separately (see ordering information, page 28).

* Contains sodium azide as a preservative.

† 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.

Adhesive tape

Two kinds of adhesive tape are provided with this kit. AirPore tape is used during the final centrifugation step only and is permeable to vapors but not to liquids. The other tape pad is used to seal the unused wells of the QIAamp 96 plate if fewer than 96 samples are to be processed in a run. This tape should not be used during centrifugation steps.

Partially using a QIAamp 96 plate

The QIAamp 96 plate can be used for runs of 24 samples or more (sample number must be a multiple of 4), although we recommend using a minimum of 32 samples per run.

If only part of a QIAamp 96 plate is used (e.g., the first 48 wells), seal the unused wells with the tape pad and leave them sealed throughout the purification procedure. After use, keep the unused wells sealed, and store the QIAamp 96 plate in the blister pack in which it was supplied.

When reusing partially used plates, label used wells with a waterproof marker pen, and remove the adhesive tape covering the unused wells. Cover the used wells with adhesive tape before beginning the purification procedure.

Centrifugation

Centrifugation of QIAamp 96 plates is performed at 6000 rpm (5788 x *g*). The speed limit of the centrifuge is programmed so that the given g-force will not be exceeded. All centrifugation steps are carried out at room temperature. Use AirPore tape to seal the QIAamp 96 plate only during the final centrifugation step to elute viral nucleic acids.

Only use the 96-well centrifuge adapter for the elution step (the final centrifugation). Use of the adapter during earlier centrifugation steps can lead to the centrifuge and/or the rotor being damaged as the adapter will cause the QIAamp 96 plate to protrude over the top of the rotor buckets, where it can foul the swing mechanism.

Note: If a Centrifuge 4-16K is used, set the temperature to 40°C for all centrifugation steps. It is important that the QIAamp 96 plates are not cooled during centrifugation to ensure that all ethanol evaporates.

Abbreviated instructions for using the Centrifuge 4-16

1. **Switch on the centrifuge by pressing the main switch on the back.**
2. **Select the rotor selection list in the display field by turning the knob. After pressing the knob, turn it again to select the rotor/bucket combination "09100/09158" for the Plate Rotor 2 x 96. Confirm entry by pressing the knob.**

   Entering the rotor number automatically sets the time and speed limits for centrifugation for that particular rotor, thus eliminating the danger of the centrifuge running too fast.

3. **Select "Speed" by turning the knob. Press the knob and by turning the knob again, set the speed to "6000". Confirm entry by pressing the knob.**

   The corresponding relative centrifugal force (RCF) is calculated from the rotor number and speed and appears automatically in the RCF field. It is also possible to enter the RCF value "5788 x *g*" manually in the RCF field after selecting "RCF" in the same way.

4. **Select "Time" by turning the knob. Press once and by turning the knob again, set the time required. Confirm entry by pressing the knob.**

5. **Open the lid, place the 96-well plates with the metal carriers in the buckets, then close the lid.**

   The start and lid keys light up.

6. **Push "Start" to start the centrifuge.**

   When the centrifuge is running the lid key will not be lit. Each run can be interrupted by pushing Stop.

7. **At the end of the run, the lid key will light up. Open the centrifuge lid by pressing the lid key. Remove the plates.**

   All preset parameters remain after a run has finished.

Protocol: Purification of Viral RNA and DNA

Important point before starting

- Before beginning the procedure, read "Important Notes" on pages 13–17.

Things to do before starting

- Equilibrate up to 96 plasma/serum samples to room temperature (15–25°C).
- Orient samples in the sample identification system (SIS) racks so that the bar codes face the bar code reader. Bar code labels should be stuck to the sample tubes such that the bar code lines are horizontal.
- Check that Buffers AW1, AW2, and the Buffer AL–carrier RNA mixture have been prepared according to the instructions on pages 14–15.

Procedure

1. **Make sure that the BioRobot 9604 is switched on.**

   The power switch is located on the lower right of the rear BioRobot panel.
2. **Make sure that the high-speed pipetting system is switched on.**
3. **Switch on the computer and monitor.**
4. **Launch QIAsoft 3.0, if necessary.**

   QIAsoft 3.0 can be started from the Windows Start menu, where it is located in Programs→BioRobot 9604.

   The computer controlling the BioRobot is normally set to launch QIAsoft 3.0 upon startup, but this setting may have been changed.
5. **Start the Execute environment by pressing "Start" in the QIAsoft Main Menu, if necessary.**

   By default, QIAsoft 3.0 is configured to start the Execute environment automatically, but this setting may have been changed.
6. **Select the QIAamp Virus BioRobot 9604 or the QIAamp 96 Virus IC Protocol (which is in the Viral Nucleic Acid Purification package) using the protocol button in the Execute environment toolbar.**
7. **Click "RUN" to start the QIAamp 96 Virus Purification or the QIAamp 96 Virus IC Protocol.**

QIAsoft 3.0 will now walk you through the remaining steps required to set up the BioRobot 9604 for the QIAamp 96 Virus Purification Protocol. Follow the steps detailed in each protocol message before continuing.

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 14. Repeat the purification procedure with new samples.
b) Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was stored for more than 8 days at –20°C. Alternatively, Buffer AL-carrier RNA mixture was stored for more than 48 hours at room temperature. Prepare a new tube of carrier RNA dissolved in Buffer AVE and mix with Buffer AL. Repeat the purification procedure with new samples.
c) Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided (see page 13). Always use fresh samples or samples thawed only once.
d) Low concentration of virus in the samples	Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples.
e) Insufficient sample lysis in Buffer AL	QIAGEN Protease was subjected to elevated temperature for prolonged time. Repeat the procedure using new samples and fresh QIAGEN Protease.
	Alternatively, the 96-well thermostat system was not switched on or the temperature was set too low. Ensure the 96-well thermostat system is switched on and the temperature is set to 60°C. Repeat the procedure with new samples.

	Comments and suggestions
f) Insufficient protein denaturation in Buffer AL	Tubes of QIAGEN Protease were placed in the wrong positions on the thermoblock. Repeat the procedure with new samples, and ensure that 4 tubes of QIAGEN Protease are placed in positions A1–A4 of the thermoblock.
g) Buffer AL–carrier RNA mixture prepared incorrectly	Check Buffer AL for foam after addition of carrier RNA. Remove any large bubbles present on the surface using a pipet.
h) 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.
i) 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.
j) Isopropanol used instead of ethanol	We recommend the use of ethanol, as use of isopropanol causes reduced yields.
k) 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 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.
l) RNase contamination in Buffer AVE	Replace Buffer AVE with RNase-free water. Repeat the purification procedure with new samples.

	Comments and suggestions
m) Buffer AW1 or AW2 prepared incorrectly, or with 70% ethanol	Check that Buffer AW1 and AW2 concentrates were diluted with correct volumes of 96–100% ethanol. Repeat the purification procedure with new samples. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
n) Buffer AW1 or AW2 bottles attached incorrectly to the BioRobot 9604	Ensure that Buffer AW1 is connected to the green adapter and Buffer AW2 to the red adapter on the BioRobot 9604. Repeat the purification procedure with new samples.
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) 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 14).
c) 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.
d) Performance of purified nucleic acids in downstream assays varies according to their original positions on the QIAamp 96 plate	Salt and ethanol components of Buffers AW1 and AW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
e) A new combination of reverse transcriptase and *Taq* DNA polymerase was used	If enzymes are changed it may be necessary to adjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.

	Comments and suggestions
f) Elution microtubes autoclaved before elution	Do not autoclave elution microtubes. Autoclaving may leach chemicals from the tubes, which may inhibit enzymatic reactions. Repeat the purification procedure with a new set of elution microtubes.
General handling	
a) Some bar codes not identified	Sample tubes were not positioned correctly in the sample identification system racks. Turn the tubes so that the bar codes face the bar code reader on the left of the BioRobot. Scan the sample tubes again and continue with the run once all samples have been correctly identified. Bar code labels should be stuck to the sample tubes such that the bar code lines are horizontal. If some bar code labels were incorrectly oriented, remove the unidentified tubes from the sample identification system rack and enter their identification codes into the report file either manually or using the hand-held bar code scanner. Put the sample tubes back in the sample identification system rack and continue with the protocol. Check that the type of bar code used can be read by the QIAsoft Operating System (refer to the BioRobot manual for a list of bar code systems that the software can interpret). Remove the unidentified tubes from the sample identification system rack and manually enter their identification codes into the report file. Replace the sample tubes in the sample identification system rack and continue with the protocol.

	Comments and suggestions
b) Unexpected results	The QIAamp 96 plate was wrongly oriented on the elution microtube rack, so that the samples in the top left of the plate were eluted into the tubes in the bottom right of the elution microtube rack, and vice versa. Using the 96-well centrifuge adapter prevents placing the QIAamp 96 plate in the wrong orientation.
c) Blocked wells in the QIAamp 96 plate	Cryoprecipitates may have formed in plasma due to repeated freezing and thawing. These can clog the QIAamp 96 plate. Do not use plasma that has been frozen and thawed more than once.
d) Overflowing wells in the QIAamp 96 plate	Insufficient vacuum. Ensure that the lid of the top plate of the vacuum manifold is properly closed. If fewer than 96 samples are purified at a time, ensure that unused wells in the QIAamp 96 plate are sealed with adhesive tape.

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 even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the 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. Keep isolated RNA on ice when aliquots are pipetted for downstream applications.

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. The BioRobot 9604 procedure uses sterile, conductive Disposable Filter Tips (1100 µl); see page 26 for ordering information.

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 26). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* 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.

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 4 or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Oven-baking will both inactivate ribonucleases and ensure that no other nucleic acids (such as plasmid DNA) remain on the surface of the glassware. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Cover the glassware with 0.1% DEPC in water overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to remove residual DEPC.

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

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.

Solutions

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

Note: Buffers in the QIAamp Virus BioRobot 9604 Kit 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.

Ordering Information

Product	Contents	Cat. no.
QIAamp Virus BioRobot 9604 Kit (12)	12 QIAamp 96 Plates, RNase-free Buffers, QIAGEN Protease, AirPore Tape Sheets, Tape Pad, S-Blocks, Elution Microtubes CL (maximum elution volume 0.4 ml), Carrier RNA, Caps	965662
BioRobot 9604*		Inquire
Disposable Filter Tips, 1100 µl (960)	Conducting disposable filter-tips, pack of 960	9012598
QIAGEN 96-Well Centrifugation System		
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81300†
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (120 V, 60 Hz)	81310‡
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (220 V, 50 Hz)	81320¶
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81400†
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (120 V, 60 Hz)	81410‡
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81420¶
Plate Rotor 2 x 96**	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031

* The BioRobot 9604 is not available in all countries. Please inquire.
† For Japan.
‡ For US.
¶ For rest of world.
** The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-16 and 4-16K from QIAGEN and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.

Ordering Information

Product	Contents	Cat. no.
Accessories		
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Buffer AL (216 ml)	216 ml for 1000 preps	19075
QIAGEN Protease (7.5 AU)	7.5 Anson units per vial (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson units per vial (lyophilized)	19157
S-Blocks (24)	96-well blocks with 2.2 ml wells for use with QIAamp 96 and QIAamp 96 BioRobot Kits, 24 blocks per case	19585
Elution Microtubes CL	Polypropylene tubes (maximum elution volume 0.4 ml), 960 in racks of 96	19588
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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www.qiagen.com

Australia ▪ Orders 1-800-243-800 ▪ Fax 03-9840-9888 ▪ Technical 1-800-243-066

Austria ▪ Orders 0800-28-10-10 ▪ Fax 0800/28-10-19 ▪ Technical 0800-28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

China ▪ Orders 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

France ▪ Orders 01-60-920-926 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930 ▪ Offers 01-60-920-928

Germany ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

Hong Kong ▪ Orders 800 933 965 ▪ Fax 800 930 439 ▪ Technical 800 930 425

Ireland ▪ Orders 1800 555 049 ▪ Fax 1800 555 048 ▪ Technical 1800 555 061

Italy ▪ Orders 800-789-544 ▪ Fax 02-334304-826 ▪ Technical 800-787980

Japan ▪ Telephone 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 080-000-7146 ▪ Fax 02-2626-5703 ▪ Technical 080-000-7145

Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

Mexico ▪ Orders 01-800-7742-639 ▪ Fax 01-800-1122-330 ▪ Technical 01-800-7742-639

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Singapore ▪ Orders 1800-742-4362 ▪ Fax 65-6854-8184 ▪ Technical 1800-742-4368

Spain ▪ Orders 91-630-7050 ▪ Fax 91-630-5145 ▪ Technical 91-630-7050

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

USA ▪ Orders 800-426-8157 ▪ Fax 800-718-2056 ▪ Technical 800-DNA-PREP (800-362-7737)