

Third Edition

May 2010

QIAamp® Virus BioRobot® MDx Kit Handbook

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



Sample & Assay Technologies

Trademarks: QIAGEN®; QIAamp®; artus®; BioRobot® [QIAGEN Group]; PAXgene™ [PreAnalytiX GmbH]; BD Vacutainer® [Becton, Dickinson and Company]; Corex® [Corning, Inc.]; CryoTube® [Nalge Nunc International].

The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.

© 2002–2010 QIAGEN, all rights reserved.



QIAGEN is a member of the Forest Stewardship Council (FSC). For the production of printed materials, including handbooks, QIAGEN has a policy to select suppliers that comply with FSC standards for printing processes and well-managed forests.

Contents

Kit Contents	4
Storage	5
Product Use Limitations	5
Product Warranty and Satisfaction Guarantee	5
Safety Information	6
Quality Control	7
Technical Assistance	7
Introduction	8
Principle and procedure	8
Sample volumes using the QIAamp Virus BioRobot MDx protocol	8
Carrier RNA	9
Yield and size of viral nucleic acids	9
Equipment and Reagents to Be Supplied by User	11
Important Notes	12
Preparation of RNA	12
Sample storage	12
Preparation of reagents	12
Preparation of plasticware	17
Partially using a QIAamp 96 plate	19
Storage of purified nucleic acids	19
Protocol	
■ Purification of Viral RNA and DNA	20
Troubleshooting Guide	24
Appendix A: General Considerations	30
Appendix B: Calculating the Amount of Internal Control	32
Ordering Information	35

Kit Contents

QIAamp Virus BioRobot MDx Kit	(12)
Catalog no.	965652
Number of preps	12 x 96
QIAamp 96 Plates	12
S-Blocks	12
Caps for Elution Microtubes	3 x 50
Elution Microtubes CL	12 x 96
Disposable Troughs	3 x 10
Carrier RNA (red caps)	12 x 1350 µg
QIAGEN® Protease	12 vials*
Protease Resuspension Buffer	12 x 6 ml
Buffer AL [†]	12 x 33 ml
Buffer AW2 [‡] (concentrate)	4 x 127 ml
2 ml Tubes containing Buffer AVE [‡] (purple caps)	108 x 2 ml
Top Elute Fluid (orange caps)	48 x 1.48 ml
Tape Pad	1 x 25
Q-Card [§]	1
Handbook	1

* Resuspension volume 6 ml.

† Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

‡ Contains sodium azide as a preservative.

§ Do not discard the Q-Card. The information encoded in the bar code on the Q-Card is needed to start a run on the BioRobot MDx.

Storage

QIAamp 96 plates, lyophilized carrier RNA, QIAGEN Protease, and all buffers can be stored dry at room temperature (15–25°C). The kit expiration date for storage at room temperature is printed on the Q-Card.

Lyophilized QIAGEN Protease can be stored at room temperature (15–25°C) until the expiration date stated on the Q-Card in the kit, without decrease in performance. For longer storage or if ambient temperatures frequently exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

Reconstituted QIAGEN Protease is stable for 12 months when stored at 2–8°C. QIAGEN Protease stock solution should not be stored at room temperature for prolonged periods.

Product Use Limitations

The QIAamp Virus BioRobot MDx 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).

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 the sample-preparation waste.

Buffer AL contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer 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 MDx Kit:

Buffer AL

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 feedingstuffs; 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 container or label.

Quality Control

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

Technical Assistance

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

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

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Introduction

The QIAamp Virus BioRobot MDx Kit uses well-established technology for simultaneous purification of viral DNA and RNA from a broad range of viruses. The kit combines the selective binding properties of a silica-based membrane with a high-throughput 96-well format, and is designed for fully automated, simultaneous processing of ninety-six 263 µl samples on the BioRobot MDx. The procedure is suitable for use with plasma, serum, other cell-free body fluids, or cell-culture supernatants. Samples for processing can be either fresh or frozen, provided they have not been frozen and thawed more than once (see "Sample storage", page 12). 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 fully automated process, including bar code reading, load check, and complete process documentation, requires less than 2.5 hours to process 96 samples, with no hands-on time. Turnaround time between consecutive runs is about 10 minutes.

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. Binding conditions are adjusted by adding ethanol. Lysates are then transferred to a QIAamp 96 plate where viral nucleic acids are adsorbed onto the silica membrane as the lysate is drawn through under vacuum. Nucleic acids bound to the membrane are efficiently washed in two steps under vacuum. Two different wash buffers are used, which considerably improves the purity of the eluted nucleic acids. Highly pure viral nucleic acids are eluted under vacuum in a single step in Buffer AVE, equilibrated to room temperature. Recovery is enhanced by overlaying the elution buffer with Top Elute Fluid.

Sample volumes using the QIAamp Virus BioRobot MDx Protocol

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. The procedure is optimized for use with 263 µl samples. The BioRobot MDx is capable of removing this volume from tubes of various sizes, such as CryoTube® or BD Vacutainer® tubes containing a minimum sample volume of 500 µl. Special tube holders are available upon request. Elution is routinely performed in a single step using approximately 91 µl Buffer AVE. The volume of eluate recovered depends on the nature of the sample and ranges from 60 µl to 90 µl, with a mean value of 83 µl for plasma samples.

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 chaotropic salts and detergent in Buffer AL. Thus, not adding carrier RNA to Buffer AL may reduce the recovery of viral RNA or DNA.

Sufficient lyophilized carrier RNA is provided for the volume of Buffer AL supplied with the kit. The concentration of carrier RNA used in the QIAamp Virus BioRobot MDx procedure allows the QIAamp Virus BioRobot MDx Kit to be used as a generic purification system for a wide range of RNA and DNA viruses. The concentration of RNA in the final eluates is compatible with many amplification systems.

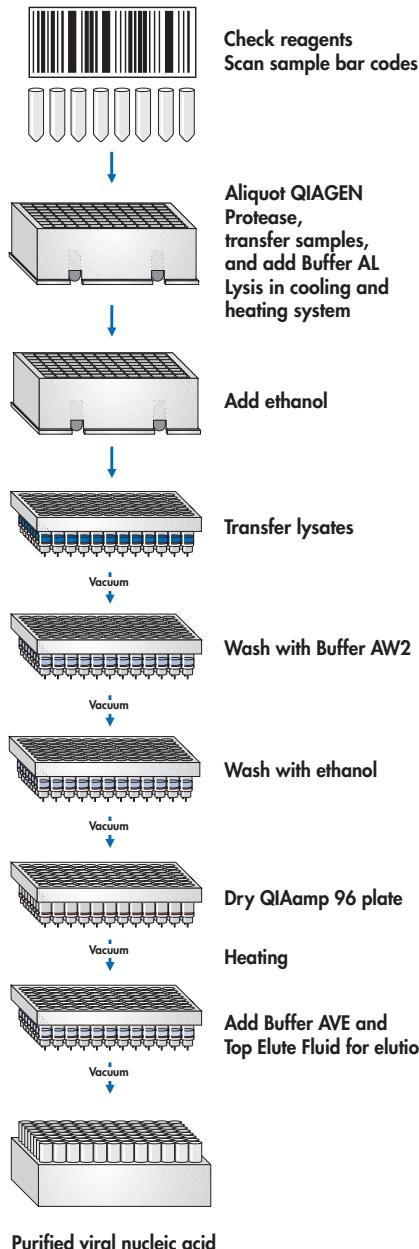
Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain viral nucleic acids and carrier RNA. The amount of carrier RNA present will greatly exceed the amount of viral nucleic acids. Calculations of how much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

Yield and size of viral nucleic acids

Yields of viral nucleic acids isolated from biological samples using the QIAamp Virus BioRobot MDx Kit 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 Virus BioRobot MDx Kit, remember that there will be much more carrier RNA than viral RNA in the sample.

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

QIAamp Virus BioRobot MDx Procedure



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 MDx (cat. no. 900600)
- QIAGEN conducting disposable filter-tips (1100 µl) for use with BioRobot systems (cat. no. 9012598)
- Ethanol (96–100%)*
- Deionized water

For runs of fewer than 96 samples

- 50 ml tubes

* 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 Appendix A 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 reduced viral titers and therefore reduced yields of viral nucleic acids.

Freezing of samples can lead to denaturation and precipitation of proteins that may clog the QIAamp 96 plate membrane. If cryoprecipitates are visible, they can be pelleted by centrifugation at 6800 $\times g$ for 3 minutes. The cleared supernatant should be transferred to a new sample tube without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Preparation of reagents

Important: Sufficient reagents are supplied to isolate DNA from 12 \times 96 plasma or serum samples. If fewer than 96 samples have to be processed in each run, additional reagents must be purchased (see ordering information, page 35).

QIAGEN Protease

For runs of 96 samples: One vial of lyophilized QIAGEN Protease and Protease Resuspension Buffer is supplied for each run of 96 samples. Add one bottle (6 ml) of Protease Resuspension Buffer to a vial of lyophilized QIAGEN Protease and mix carefully to avoid foaming. Make sure that the Protease Resuspension Buffer is transferred completely and the QIAGEN Protease is fully dissolved.

For runs of fewer than 96 samples: For runs of 32–88 samples, each vial of lyophilized QIAGEN Protease and Protease Resuspension Buffer needs to be reused 2–3 times. Add one bottle (6 ml) Protease Resuspension Buffer to a vial of lyophilized QIAGEN Protease and mix carefully to avoid foaming. Make sure that the Protease Resuspension Buffer is transferred completely and the QIAGEN Protease is fully dissolved.

After the run, seal the vial and store the unused portion of the reconstituted QIAGEN Protease at 2–8°C for use in subsequent runs. Table 1 shows the minimum volume of reconstituted QIAGEN Protease needed to process 32–88 samples.

Note: In most cases, QIAGEN Protease should be resuspended in Protease Resuspension Buffer for purification of viral nucleic acids using the QIAamp Virus BioRobot MDx Kit. Use of Protease Solvent (included in QIAamp DNA Blood Kits) can lead to reduced sensitivity when using EDTA plasma. However, Protease Resuspension Buffer is not compatible with phosphate-buffered solutions such as PBS. If phosphate cannot be avoided, resuspend QIAGEN Protease in Protease Solvent (see page 35 for ordering information). If both Protease Resuspension Buffer and Protease Solvent are used in the same laboratory, it is recommended to label the resuspended QIAGEN Protease to indicate which buffer was used for resuspension.

Table 1. Minimum Volume of QIAGEN Protease Required for the Given Number of Samples

No. of samples	32	40	48	56	64	72	80	88
Volume of reconstituted QIAGEN Protease (ml)	3.2	3.5	3.9	4.2	4.6	4.9	5.3	5.6

Addition of carrier RNA to Buffer AL*

Lyophilized carrier RNA is stable 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 either added immediately to Buffer AL or stored at –20°C. Once added to Buffer AL, carrier RNA is stable at 2–8°C for up to 48 hours.

To dissolve carrier RNA, add 450 µl of Buffer AVE to one vial containing 1350 µg lyophilized carrier RNA and mix. 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. Always prepare a fresh aliquot of Buffer AL–carrier RNA solution for each run. Sufficient Buffer AL–carrier RNA is supplied with the kit for 12 runs of 96 samples each.

* Contains sodium azide as a preservative.

For runs of 96 samples: Transfer 230 µl of carrier RNA reconstituted in Buffer AVE to one bottle containing 33 ml Buffer AL to give a final concentration of 5.6 µg carrier RNA per preparation. Gently mix by inverting the bottle 10 times. To avoid foaming, do not vortex.

For runs of fewer than 96 samples: The QIAsoft MDx Operating System indicates during the BioRobot setup process how much Buffer AL reconstituted with carrier RNA is required before a purification run is started. Transfer the volume of Buffer AL given in Table 2 into a 50 ml tube (not provided) and add the appropriate volume of carrier RNA dissolved in Buffer AVE. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. Buffer AL is viscous, and a portion of the mix may therefore be lost during transfer, but this is accounted for in the procedure.

Approximately 2.7 ml Buffer AL (dead volume) is left in the disposable trough at the end of each run. Therefore, processing 96 samples over more than one run (e.g., two runs of 48 samples each) will require more buffer than a single run of 96 samples. If runs with fewer than 96 samples are frequently performed, additional Buffer AL should be purchased (see page 35 for ordering information).

Table 2. Volumes of Buffer AL and Carrier RNA Dissolved in Buffer AVE Required for the Given Number of Samples

No. of samples	32	40	48	56	64	72	80	88
Volume of Buffer AL (ml)	13.8	16.2	18.7	21.1	23.6	26.1	28.5	31.0
Volume of dissolved carrier RNA (µl)	96.0	113.1	130.2	147.4	164.5	181.6	198.7	215.8

Note: Using the volumes listed in Table 2 gives a final concentration of 5.6 µg carrier RNA per preparation. In some amplification systems, using less carrier RNA may improve results. For each microgram of carrier RNA required per preparation, add 40.75 µl carrier RNA reconstituted in Buffer AVE to the bottle containing 33 ml Buffer AL, or add 1.235 µl carrier RNA solution per milliliter of Buffer AL if performing less than 96 preparations.

Addition of internal controls

Using the QIAamp Virus BioRobot MDx procedure in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal controls should be added together with carrier RNA to the lysis buffer. For optimal purification, internal-control molecules should be more than 200 nucleotides long, as smaller molecules are not recovered efficiently. Refer to Appendix B, page 32, to determine the optimal concentration of internal control to add to Buffer AL. Using a concentration other than that recommended may reduce amplification efficiency.

Buffer AW2*

Add 300 ml of ethanol (96–100%) to the bottle containing 127 ml of Buffer AW2 concentrate, as described on the bottle. Tick the check box on the label of the bottle to show that ethanol has been added. Sufficient Buffer AW2 is supplied for 12 runs of 96 samples. One bottle of reconstituted Buffer AW2 contains enough wash buffer for 3 runs of 96 samples each. Buffer AW2 left over after a run should be stored at room temperature (15–25°C) for the next run.

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

For easy identification on the BioRobot MDx, the Buffer AW2 bottle has a bar code on its label. The open bottle should be placed in the reagent carousel with the bar code facing the outside.

Approximately 30 ml of Buffer AW2 is used to flush 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 runs of 48 samples each) will require more buffer than one 96-sample run. If runs of fewer than 96 samples are often performed, additional Buffer AW2 should be purchased (see page 35 for ordering information).

* Contains sodium azide as a preservative.

Ethanol

Before starting a run, fill the 500 ml bottle for ethanol (supplied with the BioRobot MDx) with ethanol (96–100%).

Add 500 µl of reconstituted Buffer AW2 to a bottle containing 500 ml of ethanol (1:1000 dilution), to allow the BioRobot MDx to detect the fill level in the ethanol bottle by conductivity measurements. One bottle of ethanol contains enough ethanol for 2 runs of 96 samples each. Ethanol left over after a run should be stored at room temperature (15–25°C) for the next run.

For easy identification on the BioRobot MDx, the ethanol bottle has a bar code on its label. The open bottle should be placed in the reagent carousel with the bar code facing the outside.

Buffer AVE (purple cap)

For one run of 96 samples, 8 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. Buffer AVE is stable when stored at room temperature (15–25°C). Buffer AVE left over after a run should be discarded.

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

If 48–96 samples are prepared at a time, it is still necessary to place all eight tubes of Buffer AVE containing 2 ml of Buffer AVE each onto the worktable. If runs of fewer than 96 samples are performed, additional Buffer AVE must be purchased. If less than 48 samples are run only 4 tubes of Buffer AVE should be used.

Top Elute Fluid (orange cap)

For one run of 96 samples, four tubes of Top Elute Fluid (1.48 ml each) are required. Top Elute Fluid is stable when stored at room temperature (15–25°C). Top Elute Fluid left over after a run should be discarded.

Even if fewer than 96 samples are prepared at a time, it is still necessary to place all four opened tubes containing 1.48 ml of Top Elute Fluid each onto the worktable. If runs of fewer than 96 samples are performed, it will be necessary to purchase additional Top Elute Fluid (see page 36 for ordering information).

Preparation of plasticware

S-Blocks

Twelve S-Blocks are supplied with the kit. One S-Block is used in each run of 96 samples. When loading the S-Block onto the BioRobot MDx worktable, make sure that position A1 is located at the upper left corner. Discard the S-Block after use.

Elution Microtubes CL

Twelve racks of Elution Microtubes CL are supplied with the kit. For each run, one rack of elution microtubes is required. Make sure that the bar code of the elution microtube rack faces the right side. Use the caps for elution microtubes to seal the elution microtubes. These caps are optimized for use at low temperatures (e.g., -20°C or -80°C).

QIAamp 96 plates

Twelve QIAamp 96 plates are supplied with the kit. When loading the BioRobot MDx worktable, make sure that position A1 is located at the upper-left corner. Discard the QIAamp 96 plate after use.

Setting up the worktable

The QIAsoft MDx software guides you through worktable setup. For a summary of worktable setup, see Tables 3 and 4 and Figure 1.

Table 3. Positions of Accessories

Item	Position	Holder/adapter
Elution Microtubes CL	Reagent holder for microtubes and trough	Blue elution microtube adapter
Disposable trough	MP Slot 2	Reagent holder for 2 troughs (EtOH and Al)
QIAamp 96 plate	QIAplate Slot	Silver multiwell-plate holder
Channeling adapter	Channeling Adapter Slot	Black multiwell-plate holder
S-Block	Cooling and heating system	Silver heat-transfer adapter
Disposable tips	Varies with sample number	Red tip-tray holder

Table 4. Positions of Buffers and Reagents

Item	Position	Volume
Buffer AL*	Disposable trough in reagent holder for 2 troughs (position AL)	Varies with sample number (see next page)
QIAGEN Protease	Reagent holder for 1 bottle (QP)	Varies with sample number (see next page)
Buffer AVE*	Reagent holder for microtubes and trough (position AVE)	Four to eight 2 ml microcentrifuge tubes (purple cap)
Top Elute Fluid (TOPE)*†	Reagent holder for microtubes and trough (position TOPE)	Four 1.5 ml microcentrifuge tubes (orange cap)
Buffer AW2*	Reagent carousel	Minimum of 191 ml (reconstituted)
Ethanol	Reagent carousel	Minimum of 350 ml
System liquid	Reagent carousel	Minimum of 700 ml deionized water
Samples	Sample tracking system	Minimum of 500 µl sample in each tube. Always use 12-tube holders.

* Remember that buffer consumption is calculated assuming that 96 samples are processed in each run. If runs of fewer than 96 samples are performed, additional Top Elute Fluid, carrier RNA, and Buffers AL, AW2, and AVE must be purchased.

† Presence of TOPE is not monitored in the load check.

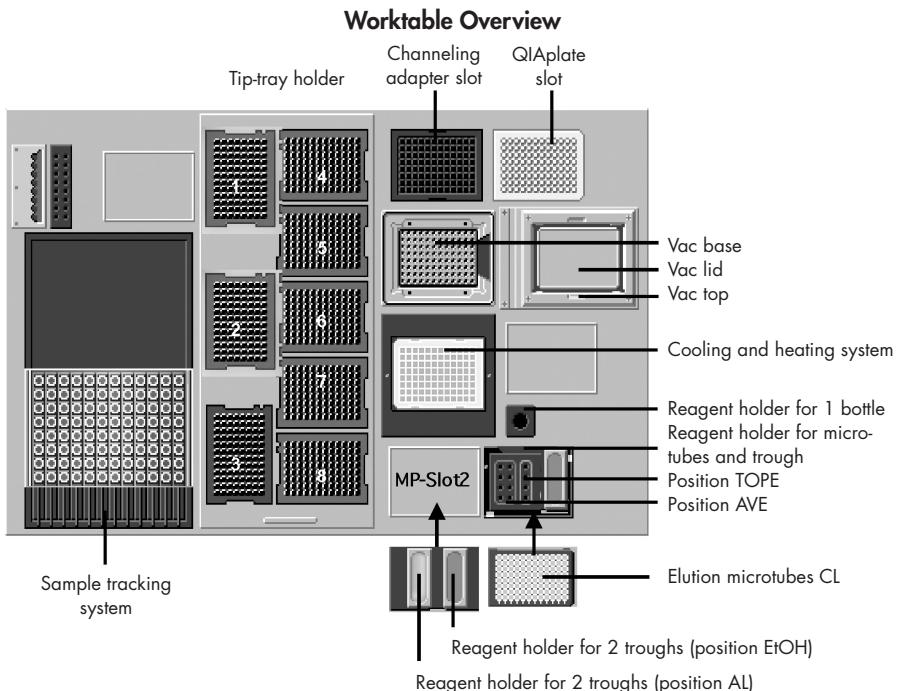


Figure 1 The BioRobot MDx worktable.

Partially using a QIAamp 96 plate

The QIAamp 96 plate can be used for runs of 32–96 samples (sample number must be a multiple of 8).

If only part of a QIAamp 96 plate is used (e.g., the first 48 wells), seal the unused wells with tape and leave them sealed throughout the purification procedure. Ensure that complete columns of eight samples are processed. 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.

Storage of purified nucleic acids

Viral nucleic acids are eluted with Buffer AVE into the Elution Microtubes CL. After purification, the eluates should be used directly in downstream applications or stored for up to 24 hours at 2–8°C. For longer storage, the purified nucleic acids should be stored at –20°C or –80°C.

Protocol: Purification of Viral RNA and DNA

Important points before starting

- Before beginning the procedure, read the Important Notes on pages 12–19.
- Ensure that you are familiar with operating the BioRobot MDx. Refer to the *BioRobot MDx User Manual* for operating instructions.

Things to do before starting

- Equilibrate the plasma/serum samples and the QIAGEN Protease to room temperature (15–25°C).
- Orient samples in the tube holders of the sample tracking system so that the bar codes face the bar code reader camera. Bar code labels should be stuck to the sample tubes so that the bar code lines are horizontal. Each tube should contain at least 500 µl of sample.
- Check that QIAGEN Protease, Buffer AW2, and the Buffer AL–carrier RNA mixture have been prepared according to the instructions on pages 12–15.

Procedure

1. Make sure that the BioRobot MDx is switched on.

The power switch is located on the lower right of the front BioRobot MDx panel.

2. Switch on the computer and monitor.

3. Launch the QIAsoft MDx Operating System.

The QIAsoft MDx software can be started from the desktop.

4. Login with your username and personal password.

The username and password for each user can be defined by the supervisor. For detailed information on how to assign a username and password to each user, refer to the *BioRobot MDx User Manual*.

5. Select the “QIAamp Virus MDx Protocol” (located in the “Viral Nucleic Acid Purification” package) using the protocol selection box in the “Execute” environment toolbar.

6. Click to start the QIAamp Virus MDx Protocol.

7. Enter the number and position of samples to be processed.

A multiple of 8 between 32 and 96 samples should be selected. Mark the corresponding position on the screen by selecting the appropriate columns and click the “OK” button to continue. If only part of the QIAamp 96 plate is used, ensure that the unused positions are sealed with tape.

Note: Buffer consumption is calculated on processing runs of 96 samples. If runs of fewer than 96 samples are performed, it will be necessary to purchase additional Top Elute Fluid, carrier RNA, and Buffers AL, AW2, and AVE.

8. Scan the bar code of the kit found on the Q-Card.

The QIAsoft MDx software will prompt you to enter the kit bar code printed on the Q-Card supplied with the kit. Use the hand-held bar code reader to scan in the bar code from the Q-Card. The bar code contains information about the material number, lot number, and expiration date of the reagents. This information will be logged in a report file generated for every processed run.

9. Choose to process samples with unlocked or locked worktable hood.

For additional safety, the worktable hood can be locked by the software after the load check has been completed successfully. Locking prevents adulteration or removal of samples, components, or reagents needed for completion of the run. The operator is protected from potentially infectious material and from contact with moving parts of the BioRobot MDx during the run.

10. Choose whether to use sample bar codes.

The QIAsoft MDx software provides complete data tracking including scanning and processing of sample bar codes. If samples are not labeled with bar codes, the feature can be switched off.

11. Follow the instructions for setting up the worktable.

The QIAsoft MDx software will now take you through the remaining steps required to set up the BioRobot MDx for the QIAamp Virus BioRobot MDx Protocol.

A choice box will appear in which you can decide if you wish to be guided by a wizard in setting up the worktable. The wizard will take you through the remaining steps required to set up the BioRobot MDx. Follow the steps detailed in each protocol message before continuing. The wizard allows you to go backwards and forwards between the next 19 steps and describes which reagent or labware must be placed at which position. We strongly recommend using the wizard for starting a run and suggest that only very experienced operators should load the BioRobot MDx without using the wizard.

After the worktable hood is closed, the BioRobot MDx checks if every component needed for the protocol is in place and if sample bar codes can be read. If a setup error is detected, the instrument pauses and a display message prompts you to recheck that item thoroughly. Follow the steps detailed in each protocol message before continuing. Once the protocol has passed the load check it will complete the purification process without interruption.

Generation of a report file

After the run, a report file is generated to summarize all the data relevant to the run. The file is automatically saved in rich text format (*.rtf) under the default directory **C:\Program Files\QIAssoft MDx\UserData\ReportData**. The identification bar code of the processed elution microtube rack is used as a file name.

Operator:

Date/Time:

Protocol name:

Run Time:

Number of processed samples:

Q-card bar code:

- Catalog number:
- Lot number
- Expiration date:

BioRobot MDx:

QIAsoft Version:

Robot Configuration:

Message Log File:

Report log file:

Manual entered sample bar codes: Number of bar codes entered manually

TempPressureControl: PASSED/FAILED

Maintenance: The protocol was executed with no/1-n open maintenance action(s)

General Results:

<i>Result table</i>	<i>Position</i>	<i>Sample bar codes</i>	<i>Sample result</i>
1	A1	4581565415	4581565415
2	B1	358252511	358252511
3	C1	1156615656	1156615656
4	D1	415615615	415615615
5	E1	555488466	555488466
6	F1	5464815156	5464815156 (invalid)*
7	G1	1988911118	1988911118
.....	1515115616	1515115616
89	A12	1151511661	1151511661
90	B12	2312313232	2312313232
91	C12	8748866361	8748866361
92	D12	13548483648	†
93	E12	13355656545	13355656545
94	F12	56165156561	56165156561
95	G12	23156165156	23156165156
96	H12	8941878191	8941878191

All samples where bar codes have been copied from the “sample bar codes” to the “sample result” column have been processed successfully.

* If a position is flagged with the comment “invalid”, a safety check during the process has identified a problem with this sample (e.g., cryoprecipitates or clots). For more detailed information, refer to the Troubleshooting Guide on pages 24–29.

† If positions in the “sample result” column do not contain any information, a non-recoverable process error occurred during the sample preparation. For more detailed information, refer to the *BioRobot MDx User Manual*.

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 pages 13–14. Repeat the purification procedure with new samples.
- b) Degraded carrier RNA Carrier RNA reconstituted in Buffer AVE was stored at temperatures above –20°C, or 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, mix with Buffer AL immediately, and store appropriately. Repeat the purification procedure with new samples.
- c) Samples frozen and thawed more than once Repeated freezing and thawing should be avoided (see page 12). Always use fresh samples or samples that have been thawed once only.
- d) Degraded viral nucleic acids 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 temperatures for prolonged periods of time. Repeat the procedure using new samples and fresh QIAGEN Protease.
- f) Buffer AL–carrier RNA mixture prepared incorrectly Check Buffer AL for foaming after addition of carrier RNA. Remove any large bubbles present on the surface using a pipet.
- g) Buffer AL–carrier RNA mixture mixed insufficiently Mix Buffer AL with carrier RNA dissolved in Buffer AVE by gently inverting the tube of Buffer AL–carrier RNA at least 10 times.

Comments and suggestions

- h) Low-percentage ethanol used instead of 96–100% for reconstitution of buffers or the refilling of the ethanol bottle
- i) Isopropanol used instead of ethanol for reconstitution of buffers or the refilling of the ethanol bottle
- j) RNA is degraded
- k) RNase contamination in Buffer AVE
- l) Buffer AW2 prepared incorrectly
- m) Starting material contains phosphate
- 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.
- We recommend the use of ethanol, as use of isopropanol causes reduced yields. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
- 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.
- Each vial of Buffer AVE should be used once only. Repeat the purification procedure with a new vial of Buffer AVE.
- Check that Buffer AW2 concentrates were diluted with the correct volumes of ethanol. Repeat the purification procedure with new samples.
- Protease Resuspension Buffer is not compatible with phosphate-buffered solutions such as PBS.* Avoid phosphate by using Tris-buffered solutions* or RNase-free water instead. If phosphate cannot be avoided, resuspend QIAGEN Protease in Protease Solvent (see page 35 for ordering information).

* 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.

RNA or DNA does not perform well in downstream enzymatic reactions

- a) Little or no RNA or DNA in the eluate
See "Little or no nucleic acid in the eluate", page 24, 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", pages 13–14).
- 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 Buffer 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 readjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.
- f) Elution microtubes autoclaved before elution
Do not autoclave elution microtubes. Autoclaving may leach chemicals from the walls of elution microtubes that can inhibit some enzymatic reactions. Repeat the purification procedure with a new set of elution microtubes. Elution microtubes are delivered RNase- and DNase-free.
- g) Hemolytic plasma samples used
To completely remove hemoglobin, an additional wash step using Buffer AW1 is necessary. Contact QIAGEN Technical Services for an additional protocol version to use on the BioRobot MDx.

General handling

- a) Some bar codes not identified

Sample tubes were not positioned correctly in the tube holders of the sample tracking system. Turn the tubes so that the bar codes face the bar code reader on the left of the BioRobot MDx. 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 so that the bar code lines are horizontal. If some bar code labels were incorrectly oriented, remove the unidentified tubes from the sample tracking system tube holder and enter their identification codes into the table either manually or using the hand-held bar code reader. Put the sample tubes back in the sample tracking system tube holder and continue with the protocol.

Check that the type of bar code used can be read by the QIAsoft MDx Operating System (refer to the *BioRobot MDx User Manual* for a list of bar code systems that the software can interpret). Remove the unidentified tubes from the sample tracking system tube holder and manually enter their identification codes into the table. Replace the sample tubes in the sample tracking system tube holder and continue with the protocol.

- b) Blocked wells in the QIAamp 96 plate

Freezing of samples may lead to denaturation and precipitation of proteins. These cryoprecipitates can clog the QIAamp 96 plate 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. Do not use plasma that has been frozen and thawed more than once.

Comments and suggestions

- c) Variable elution volumes

The tubes containing Top Elute Fluid were not completely filled. Each vial of Top Elute Fluid should be used once only, even if only part of the plate was processed.
- d) Result of single samples marked as invalid in the report file

Different plasma samples can lead to different elution volumes. The quality of recovered nucleic acids is not affected.

During aspiration of the samples from sample tubes, no sample was detected.

During aspiration of the samples from sample tubes, clots were detected.

Cryoprecipitates prevented transfer of correct sample volumes from sample tubes to the S-Block.

After sample transfer, not enough sample was detected in the S-Block.
- e) Some positions in the "Sample result" column in the report file are empty

A processing error occurred during sample preparation. Information about the status of the samples being processed when the protocol was interrupted may have been lost.
- f) Z-movement blocked during tip disposal

The tip disposal bag in the tip disposal container was not emptied, leading to a tip jam. After the protocol has stopped, carefully shake the container in the position beneath the tip-disposal station, and try to pull it out. Empty the tip disposal bag, remove the jammed tips, and continue the protocol.

The tip disposal bag was not inserted correctly into the tip disposal container, leading to a tip jam. The bag must fit tightly to the wall of the container so that ejected tips fall down freely. Carefully shake the container in the position beneath the tip-disposal station and try to pull it out. Empty the tip disposal bag, remove the jammed tips, and make sure that the bag fits tightly to the container. Continue the protocol.

Comments and suggestions

- The tip disposal container was not pushed back completely, leading to a tip jam. Remove the tip disposal container and the jammed tips, clean the container, and insert it again. Push it back until a metallic click is heard. Continue the protocol.
- Sufficient vacuum was not achieved. After the protocol has stopped, open the worktable hood, and check whether the QIAamp 96 plate fits flush to the elution microtubes. Correct the position of the QIAamp 96 plate, close the hood, and continue the protocol.
- There is no contact between the sensor and the system liquid container. Ensure that the container is positioned correctly in the container holder and that the outsides of container and sensor are dry.
- Drops adjacent to the sensor (inside or outside the waste container) cause sensing of a full container. Make sure that the container is positioned correctly in the container holder and that the outside of the container is dry.
- Drops adjacent to the sensor (inside or outside the vacuum trap) cause sensing of a full bottle. Make sure that the bottle is positioned correctly in the container holder and that the outside of the container is dry.

Appendix A: General Considerations

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. The BioRobot MDx procedure uses sterile, conductive disposable filter-tips (1100 µl); see page 35 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 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 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.

* 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.

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 CO₂. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer.

DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

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

Note: Buffers in the QIAamp Virus BioRobot MDx 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.

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

Appendix B: Calculating the Amount of Internal Control

To monitor the efficiency of sample preparation and downstream assay, an internal control, provided with the diagnostic downstream assay, is added to the lysis buffer (Buffer AL). To calculate the amount of internal control needed in QIAamp Virus BioRobot MDx protocols, the amount of Buffer AL and volume of the eluate must be taken into account.

Determining how much internal control will be in downstream reactions

To determine the volume of internal control that is present in the downstream assay, use the formula:

$$IC_{RXN} = \frac{IC_{LB} \times LB_{SAM} \times EL_{RXN}}{(LB_{TOT} + IC_{LB}) \times EL_{SAM}}$$

where:

- IC_{RXN} = Volume of internal control per downstream reaction
 IC_{LB} = Volume of internal control added to lysis buffer (Buffer AL, with added carrier RNA)
 LB_{SAM} = Volume of lysis buffer (Buffer AL, with added carrier RNA) per sample
 EL_{RXN} = Volume of eluate per downstream reaction
 LB_{TOT} = Total volume of lysis buffer (Buffer AL, with added carrier RNA) used in the protocol
 EL_{SAM} = Volume of elution buffer (Buffer AVE) per sample

As an example, User 1 is working with a diagnostic assay that is validated for use with 50 µl of internal control solution (IC_{LB}) added to 1400 µl lysis buffer (LB_{TOT}). The manual sample-preparation method provided with the diagnostic assay uses 100 µl of lysis buffer per sample (LB_{SAM}) with a 225 µl elution volume (EL_{SAM}). User 1 uses 25 µl of eluate per downstream reaction (EL_{RXN}). The volume of internal control solution in each downstream reaction (IC_{RXN}) for the standard assay with manual sample preparation is:

$$IC_{RXN} = \frac{50 \mu\text{l} \times 100 \mu\text{l} \times 25 \mu\text{l}}{(1400 \mu\text{l} + 50 \mu\text{l}) \times 225 \mu\text{l}} = 0.383 \mu\text{l}$$

The final downstream reactions contain 0.383 µl of internal control solution per reaction.

Determining how much internal control solution to add before starting

After you know the amount of internal control that you want to have present in the downstream assay (IC_{RXN}), then you need to determine the amount to add to the lysis buffer before starting purification using the QIAamp Virus BioRobot MDx protocol (IC_{LB}). To calculate this value, use the formula:

$$IC_{LB} = \frac{IC_{RXN} \times LB_{TOT} \times EL_{SAM}}{(LB_{SAM} \times EL_{RXN}) - (IC_{RXN} \times EL_{SAM})}$$

Continuing the example above, User 1 needs 0.383 µl of internal control solution per reaction (IC_{RXN}) with 25 µl of eluate per reaction (EL_{RXN}). User 1 follows the QIAamp Virus BioRobot MDx protocol, which provides the volumes shown in Table 5.

Table 5. Volumes Used in the QIAamp Virus BioRobot MDx Protocol

Description	Variable	Volume
Volume of lysis buffer (Buffer AL, with added carrier RNA) per sample	LB_{SAM}	270 µl
Total volume of lysis buffer (Buffer AL, with added carrier RNA) used in the protocol (for 96 samples; see Table 2, page 14, for runs with fewer samples)	LB_{TOT}	33 ml (33,000 µl)
Sample input volume	SAM	263 µl
Volume of elution buffer (Buffer AVE) per sample	EL_{SAM}	91 µl
Final volume of eluate per sample	EV	60–90 µl (mean 83 µl)

The amount of internal control solution (IC_{LB}) that User 1 needs to add to 33 ml lysis buffer (LB_{TOT}) is:

$$IC_{LB} = \frac{0.383 \mu\text{l} \times 33,000 \mu\text{l} \times 91 \mu\text{l}}{(270 \mu\text{l} \times 25 \mu\text{l}) - (0.383 \mu\text{l} \times 91 \mu\text{l})} = 171 \mu\text{l}$$

User 1 needs to add 171 µl of internal control solution to 33 ml lysis buffer (Buffer AL, with added carrier RNA) before purification of 96 samples.

Adjusting for titer calculations

Titers are frequently calculated by comparison of target to internal control in amplification reactions. The factor used to determine titer for a commercial assay will be calibrated to the sample input volumes (SAM) and the final volume of the eluate (EV) in the sample-preparation method. If these volumes differ from the volumes in the QIAamp Virus BioRobot MDx protocol, then a conversion factor (CF) needs to be considered for accurate calculation of titers. To calculate this factor, use the formula:

$$CF = \frac{SAM_{MDx} \times EV_{CA}}{SAM_{CA} \times EV_{MDx}}$$

where:

SAM_{MDx} = Sample input volume for QIAamp Virus BioRobot MDx protocol

SAM_{CA} = Sample input volume for sample preparation according to the commercial assay

EV_{MDx} = Final volume of eluate with QIAamp Virus BioRobot MDx protocol

EV_{CA} = Final volume of eluate with sample preparation according to the commercial assay

Continuing the example above, if the manual sample-preparation method with the commercial assay has a sample input volume (SAM_{CA}) of 100 µl and a final eluate volume (EV_{CA}) of 225 µl, then User 1 calculates a conversion factor of

$$CF = \frac{263 \mu l \times 225 \mu l}{100 \mu l \times 83 \mu l} = 7.13$$

In this example, the purified nucleic acids are 7.13 times more concentrated using the QIAamp Virus BioRobot MDx protocol. User 1 needs to either divide the assay results by 7.13 or dilute the samples 7.13-fold prior to purification.

Ordering Information

Product	Contents	Cat. no.
QIAamp Virus BioRobot MDx Kit (12)	For 12 x 96 preps on the BioRobot MDx: 12 QIAamp 96 Plates, RNase-free Buffers,* QIAGEN Protease, S-Blocks, Disposable Troughs, Racks with Elution Microtubes CL (0.4 ml), Carrier RNA, Top Elute Fluid, Caps, Tape Pad	965652
BioRobot MDx	BioRobot MDx System includes: robotic workstation with 8 dilutor units, variable spacing system, sample tracking system, computer-controlled vacuum pump, automated vacuum manifold, cooling and heating system, QIAsoft MDx Operating System, computer, laboratory cabinet, accessory cabinet, 1-year warranty on parts and labor [†]	900600
Accessories		
Disposable Filter-Tips, 1100 µl (960)	Conducting disposable filter-tips, pack of 960	9012598
QIAGEN Protease (lyophilized)	7.5 Anson units per vial (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson units (lyophilized)	19157
Protease Solvent (6 ml)	6 ml of Protease Solvent	1021055
Buffer AW1 (concentrate, 151 ml)	151 ml Wash Buffer (1) Concentrate	1021052
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Buffer AL (216 ml)	216 ml Lysis Buffer AL	19075

* Wash buffers are labeled with bar codes, and expiration date is stated on the Q-Card in the kit.

[†] Warranty PLUS 2 (cat. no. 9238868) recommended: 3-year warranty, 2 preventive maintenance visits per year, 48-hour priority response, all labor, travel, and parts.

Ordering Information

Product	Contents	Cat. no.
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
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes; 2304 in racks of 96; includes cap strips	19588
Top Elute Fluid (48 x 1.48 ml)	48 x 1.48 ml Top Elute Fluid	1020460
Disposable Troughs (10)	Disposable troughs for Buffer AL, Buffer AE, and ethanol	9232764
Carrier RNA (12 x 1350 µg)	12 x 1350 µg Carrier RNA	1017647
Buffer AVE (108 x 2 ml)	108 x 2 ml Buffer AVE	1020953
Related products		
QIAamp Blood BioRobot MDx Kit (12)	For 12 x 96 preps on the BioRobot MDx: 12 QIAamp 96 Plates, Buffers,* QIAGEN Protease, S-Blocks, Racks with Elution Microtubes CL (0.4 ml), Top Elute Fluid, Caps, Tape Pad	965152
QIAamp Media MDx Kit (12)	For 12 x 96 preps on the BioRobot MDx: 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

* Wash buffers are labeled with bar codes, and expiration date is stated on the Q-Card in the kit.

Ordering Information

Product	Contents	Cat. no.
PAXgene™ Blood RNA MDx Kit (4)	For 4 x 96 RNA preps on the BioRobot MDx: 4 PAXgene 96 RNA Plates, 4 PAXgene 96 Filter Plates, Buffers,* Proteinase K, RNase-Free DNase Set, Plasticware, Collection Vessels. To be used with PAXgene Blood RNA Tubes	762431
QIAamp One-For-All Nucleic Acid Kit (12)	For 12 x 96 preps on the BioRobot MDx or the BioRobot Universal System: 12 QIAamp 96 Plates, RNase-Free Buffers (wash buffers are labeled with bar codes, and expiration date is stated on the Q-Card in the kit), QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Carrier RNA	965672
QIAamp 96 DNA Swab BioRobot Kit (12)	For 12 x 96 DNA preps on the BioRobot Universal System: 12 QIAamp 96 Plates, Buffers, QIAGEN Proteinase K, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Collection Microtubes (1.2 ml), Caps	965842
BioRobot Universal System	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft 5 Operating System, installation, 1-year warranty on parts and labor [†]	9001094

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.

* Wash buffers are labeled with bar codes, and expiration date is stated on the Q-Card in the kit.

[†] Warranty PLUS 2 (cat. no. 9239573) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

Notes

Notes

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)

