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QIAamp® Media MDx Handbook

For automated purification of nucleic acids from liquid media using the BioRobot® MDx workstation

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Contents

Kit Contents

QIAamp Media MDx Kit **Catalog no.** **Number of preps**	**(12 x 96)** **965752** **1152**
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
Proteinase K	24 x 1.4 ml
Buffer ATL	12 x 11.2 ml
Buffer AL*	12 x 33 ml
Buffer AW1* (concentrate)	2 x 151 ml
Buffer AW2† (concentrate)	4 x 127 ml
Buffer AVE† (tubes with 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

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 7 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 workstation.

Storage

QIAamp 96 Plates, lyophilized carrier RNA, proteinase K, 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.

Carrier RNA can only be dissolved in Buffer AVE. Mixed carrier RNA and Buffer AVE should be added immediately to Buffer AL as described in "Adding carrier RNA to Buffer AL", page 13. The Buffer AL–carrier RNA mixture should be prepared fresh, and is stable at 2–8°C for up to 48 hours.

Proteinase K solution can be stored at room temperature (15–25°C) until the expiration date stated on the Q-Card in the kit. For longer storage or if ambient temperatures exceed 25°C, proteinase K should be stored dry at 2–8°C.

Product Use Limitations

The QIAamp Media 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 or visit www.qiagen.com).

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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAamp Media MDx 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 see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

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

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/support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

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

Buffer AL and Buffer AW1 contain 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 Media MDx Kit.

Buffer AL and Buffer AW1

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

Proteinase K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

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; R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink and animal feedingstuffs; S23: Do not breathe spray; 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: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

The QIAamp Media MDx Kit uses well-established technology for purification of nucleic acids. 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 265 µl samples on the BioRobot MDx. The procedure is suitable for use with liquid media containing nucleic acids, such as cervical swab transport media (e.g., PreservCyt® or SurePath™ solution). 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 3 hours to process 96 samples, with no hands-on time. Turnaround time between consecutive runs is approximately 10 minutes.

Principle and procedure

Sample storage and sample volumes

Generally, samples should be stored at 2–8°C. Follow the sample storage conditions recommended by the downstream assay manufacturer. The automated procedure is optimized for use with 265 µl of sample. The BioRobot MDx is capable of removing this volume from tubes containing a minimum of 500 µl. The workstation's liquid detection system facilitates operation with volumes greater than 500 µl. Special tube holders for use with a range of tube sizes are available upon request.

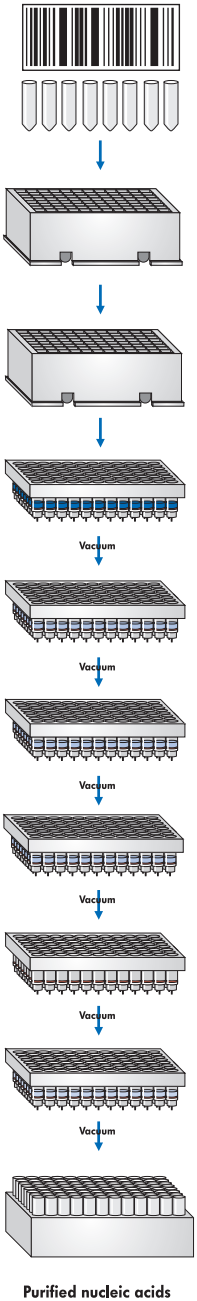
Lysis with QIAGEN Proteinase K

Samples are lysed under denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Proteinase K and Buffer ATL. Addition of Buffer AL enhances lysis efficiency.

Adsorption to the QIAamp 96 silica-gel membranes

Binding conditions are adjusted by adding ethanol to the lysates to ensure optimal binding of nucleic acids to the QIAamp 96 membrane. Lysates are applied to the QIAamp 96 plate, and nucleic acids are adsorbed onto the silica-gel membrane as the lysate is drawn through by vacuum pressure. Three different wash buffers are used to increase the purity of the eluted nucleic acids. Salt and pH conditions ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp 96 membrane. Highly pure nucleic acids are eluted under vacuum in a single step in approximately 120 µl Buffer AVE. Recovery is enhanced by overlaying with Top Elute Fluid.

QIAamp Media MDx Protocol



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 workstation
- 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 BD Falcon™ tube or equivalent

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

Important Notes

Yield and size of nucleic acids

Yields of nucleic acids purified from liquid media are normally low and are therefore difficult to determine using a spectrophotometer. Quantitative amplification methods are recommended to determine yields. When quantifying nucleic acids purified using the QIAamp Media MDx Kit, remember that there will be more carrier RNA than other nucleic acids in the sample.

The size distribution of nucleic acids 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 Laboratory Press).

Carrier RNA

Carrier RNA enhances binding of nucleic acids to the QIAamp 96 membrane, especially if there are very few target molecules in the specimen. Therefore, failure to add carrier RNA to Buffer AL may reduce the recovery of nucleic acids.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer AL supplied with the kit. If fewer than 96 samples are often processed in each run, additional Buffer AL (cat. no. 19075) and carrier RNA (cat. no. 1017647) must be purchased in order to process 1152 samples with the kit. The concentration of carrier RNA has been adjusted so that the QIAamp Media MDx procedure can be used as a generic purification system for a wide range of samples, 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 both target nucleic acids and carrier RNA. The amount of carrier RNA present will generally exceed the amount of other 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.

Addition of internal controls

Using the QIAamp Media 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 Buffer AL. For optimal purification, internal-control molecules should be more than 200 nucleotides long, since smaller molecules are not recovered efficiently.

Refer to the manufacturer's instructions in order to determine the optimal concentration. Using a concentration other than that recommended may reduce amplification efficiency.

Preparation of reagents

Important: Sufficient reagents are supplied to purify nucleic acids from 12 x 96 liquid-media samples. If fewer than 96 samples are processed in each run, additional reagents must be purchased in order to process 1152 samples with the kit (see below for details).

Mixing proteinase K with Buffer ATL

Two tubes of proteinase K and one bottle of Buffer ATL are required for each run of 96 samples. If fewer than 96 samples are processed in each run, additional proteinase K (cat. no. 19133) and Buffer ATL (cat. no. 19076) must be purchased in order to process 1152 samples with the kit. Equilibrate the proteinase K and Buffer ATL to room temperature before mixing. A white precipitate may form if the reagents are mixed while they are too cold.

For runs of 96 samples: Transfer the contents of two tubes of proteinase K into a full bottle of Buffer ATL, and mix gently by inverting the bottle 10 times, taking care to avoid foaming. Slowly pour the Buffer ATL–proteinase K mixture into a disposable trough in the reagent holder for 2 troughs (position ETOH). Remove any large bubbles with a pipet tip.

For runs of fewer than 96 samples: Transfer the appropriate volume of Buffer ATL (see Table 1, page 13) into a 50 ml BD Falcon tube (not supplied). Add the indicated volume of proteinase K (see Table 1, page 13), and mix gently by inverting the tube 10 times, taking care to avoid foaming. Slowly pour the Buffer ATL–proteinase K mixture into a disposable trough in the reagent holder for 2 troughs (position ETOH). Remove any large bubbles with a pipet tip.

Important: Always prepare a fresh Buffer ATL–proteinase K mixture before starting a purification run on the BioRobot MDx.

Table 1. Volumes of Buffer ATL and proteinase K to use per run with different numbers of samples

Number of samples	Vol. Buffer ATL (ml)	Vol. proteinase K (ml)
32	5.6	1.40
40	6.3	1.58
48	7.0	1.75
56	7.7	1.93
64	8.4	2.10
72	9.1	2.28
80	9.8	2.45
88	10.5	2.63
96	11.2	2.80

Adding 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 1350 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. If fewer than 96 samples are processed in each run, additional Buffer AL (cat. no. 19075) must be purchased in order to process 1152 samples with the kit.

For runs of 96 samples: Transfer 330 µl of carrier RNA reconstituted in Buffer AVE to one bottle containing 33 ml Buffer AL to give a final amount of 2.75 µg carrier RNA per preparation. Gently mix by inverting the bottle 10 times. To avoid foaming, do not vortex. Pour the Buffer AL–carrier RNA mix into a disposable trough in the reagent holder for 2 troughs (position AL) and remove any large air bubbles using a pipet tip.

* Buffer AL contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.

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 appropriate volume of Buffer AL (see Table 2) into a 50 ml BD Falcon tube (not supplied). Add the indicated volume of carrier RNA (see Table 2), and mix gently by inverting the tube 10 times. To avoid foaming, do not vortex. Slowly pour the Buffer AL–carrier RNA mix into a disposable trough in the reagent holder for 2 troughs (position AL). Buffer AL is viscous, and a portion of the mix may therefore be lost during transfer, but this is accounted for in the procedure. Remove any large bubbles using a pipet tip.

Note: The purification procedure is optimized so that 2.75 µg carrier RNA is added per sample. If a different amount of carrier RNA has been shown to be better for a specific amplification system, transfer the required amount of reconstituted carrier RNA (in Buffer AVE) to the tubes containing Buffer AL. For each microgram of carrier RNA required per sample, add 120 µl of reconstituted carrier RNA to the bottle containing 33 ml Buffer AL (or 3.64 µl carrier RNA per milliliter of Buffer AL).

Table 2. Volumes of Buffer AL and carrier RNA to use per run with different numbers of samples

Number of samples	Vol. Buffer AL (ml)	Vol. carrier RNA (µl), dissolved in Buffer AVE
32	13.8	138
40	16.2	162
48	18.7	187
56	21.1	211
64	23.6	236
72	26.1	261
80	28.5	285
88	31.0	310
96	33.0	330

Buffer AW1*

Add 200 ml ethanol (96–100%) to a bottle containing 151 ml Buffer AW1 concentrate, as described on the bottle. Tick the check box on the label of the bottle to indicate that ethanol has been added. Reconstituted Buffer AW1 left over after a run should be stored capped at room temperature (15–25°C) for the next run.

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

For easy identification on the BioRobot MDx, the Buffer AW1 bottle has a bar code on its label. The open bottle should be placed in the reagent carousel with the bar code facing out.

Each bottle of Buffer AW1 contains sufficient buffer for 6 runs of 96 samples each. Approximately 30 ml of reconstituted Buffer AW1 is used to purge the system tubing during each run, regardless of the number of samples processed. If fewer than 96 samples are often processed in each run, additional Buffer AW1 (cat. no. 1021052) must be purchased in order to process 1152 samples with the kit.

Buffer AW2

Add 300 ml of ethanol (96–100%) to a bottle containing 127 ml Buffer AW2 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Reconstituted Buffer AW2 should be stored capped at room temperature (15–25°C) for the next run.

Note: Always mix reconstituted Buffer AW2 by shaking 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 out.

Sufficient Buffer AW2 is supplied for 12 runs of 96 samples each. Approximately 30 ml of reconstituted Buffer AW2 is used to purge the system tubing during each run, regardless of the number of samples processed. If fewer than 96 samples are often processed in each run, additional Buffer AW2 (cat. no. 1020955) must be purchased in order to process 1152 samples with the kit.

* Buffer AW1 contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.

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 the 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 out.

Buffer AVE (purple cap)

For each run of 96 samples, 8 tubes of Buffer AVE (2 ml each) are required. Buffer AVE is stable when stored at room temperature (15–25°C). Open the 8 Buffer AVE tubes and place them in the reagent holder for microtubes and trough (position AVE). Buffer AVE left over after a run should be discarded and should not be reused in subsequent runs.

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 processed at a time, it is still necessary to place all 8 tubes of Buffer AVE, containing 2 ml of Buffer AVE each, onto the worktable. If fewer than 96 samples are processed in each run, additional Buffer AVE (cat. no. 102953) must be purchased in order to process 1152 samples with the kit. If fewer than 48 samples are processed, only 4 tubes of Buffer AVE should be used. These should be inserted in the left row of the AVE-Slot on the reagent holder for microtubes and troughs.

Top Elute Fluid (orange cap)

For each run of 96 samples, 4 tubes of Top Elute Fluid (1.48 ml each) are required. Top Elute Fluid is stable when stored at room temperature (15–25°C). Open the 4 Top Elute Fluid tubes and place them in the reagent holder for microtubes and trough (position TOPE). Top Elute Fluid left over after a run should be discarded and should not be reused in subsequent runs.

Even if fewer than 96 samples are processed at a time, it is still necessary to place all 4 opened tubes, containing 1.48 ml of Top Elute Fluid each, onto the worktable. If runs of fewer than 96 samples are performed, additional Top Elute Fluid (cat. no. 1020460) must be purchased in order to process 1152 samples with the kit.

Using plasticware on the BioRobot MDx

S-Blocks

Twelve S-Blocks are supplied with the kit. For each run, one S-Block must be placed onto the silver heat transfer adapter of the cooling and heating system. Make sure that position A1 is located at the upper-left corner. Discard the S-Block after use. If fewer than 96 samples are processed in each run, additional S-Blocks (cat. no. 19585) must be purchased in order to process 1152 samples with the kit.

Disposable troughs

Thirty disposable troughs are supplied with the kit. For each run, disposable troughs must be placed in the reagent holder for 2 troughs (positions AL and EtOH) on MP Slot 2. Discard the disposable troughs after use. If fewer than 96 samples are processed in each run, additional disposable troughs (cat. no. 9232764) must be purchased in order to process 1152 samples with the kit.

Elution Microtubes CL

Twelve racks of Elution Microtubes CL are supplied with the kit. For each run, one rack of elution microtubes must be placed into the blue elution microtube adapter. The assembly of elution microtubes and adapter frame must be placed on top of the reagent holder for microtubes and trough. Make sure that the bar code of the elution microtube rack faces to the right. After preparation the eluates should be directly used 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. 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. For each run, a QIAamp 96 plate must be placed in the silver multiwell-plate holder. The assembly of QIAamp 96 plate and holder is transferred to the QIAplate Slot. Make sure that position A1 is located at the upper-left corner. Discard the QIAamp 96 plate after use, unless the plate was only partially used (see “Partially using a QIAamp 96 plate”, page 18).

Tape pad

A tape sheet from the 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.

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 a tape sheet from the tape pad, 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.

Protocol: Purification of Nucleic Acids from Media Using the BioRobot MDx

Important points before starting

- Before beginning the procedure, read "Important Notes" on pages 11–18.

Things to do before starting

- Equilibrate the liquid-media samples, Buffer ATL, and proteinase K to room temperature (15–25ºC).
- Ensure that all reagents have been prepared according to instructions in "Preparation of reagents", page 12.
- Place 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 media.

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.**

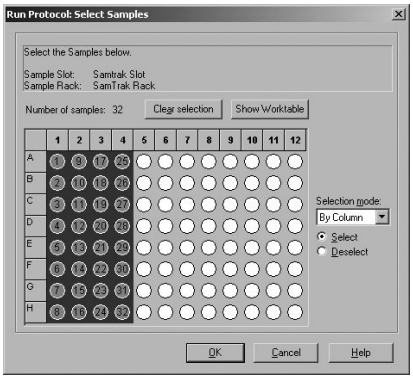
   The username and password for each user can be defined by the supervisor. For detailed information on how to assign a username and password to each user, refer to the *BioRobot MDx User Manual.*
5. **Select the QIAamp Media MDx Protocol (located in the Viral Nucleic Acid Purification package) using the protocol selection box in the "Execute" environment toolbar.**
6. **Click**  **to start the QIAamp Media MDx protocol.**

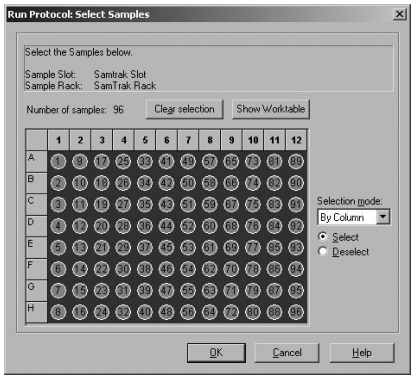
   Note: If the gray "RUN" button turns yellow, some maintenance activities are due. After these maintenance activities are completed and signed off in the maintenance environment, the "RUN" button turns gray. If a protocol is started while maintenance activities are due, this will be indicated in the report file generated with each protocol run.

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 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 Buffers AL, ATL, AVE, AW1, AW2, Top Elute Fluid, carrier RNA, and proteinase K.





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 successfully completed. 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 Media MDx Protocol.

Refill the system liquid container, and empty the waste container, vacuum trap, and tip disposal bag. The tip disposal bag must be emptied after each run even if it is not completely full.

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 17 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. Tables 3 and 4 on pages 22 and 23 summarize the worktable setup.

After the worktable hood is closed the BioRobot MDx performs a load check to confirm 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.

At the end of a run, a report file is created 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\QIAsoft MDx\UserData\ReportData** . The identification bar code of the processed elution microtube rack is used as a file name. An example with the content of this file is given in the appendix, page 32.

Summary of worktable setup

Table 3. Plasticware setup

Item	Position	Holder/adapter
Elution Microtubes CL	Reagent holder for microtubes and troughs	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 (see below)	Red tip-tray holder

Table 4. Setup of buffers and reagents

Item	Position	Volume
Buffer AL*	Disposable trough in reagent holder for 2 troughs (position AL)	Varies with sample number (see Table 5, page 24)
Buffer ATL–proteinase K*	Disposable trough in reagent holder for 2 troughs (position EtOH)	Varies with sample number (see Table 5, page 24)
Buffer AVE*	Reagent holder for microtubes and trough (position AVE)	Varies with sample number (see Table 5, page 24)
Top Elute Fluid (TOPE)*†	Reagent holder for microtubes and trough (position TOPE)	Four 1.5 ml microcentrifuge tubes (orange cap)
Buffer AW1*	Reagent carousel	Minimum of 117 ml
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 per tube; always use 12 tube holders

* Remember that buffer consumption is calculated assuming that 96 samples are processed in each run. If fewer than 96 samples are processed in each run, additional Buffers AL, ATL, AVE, AW1, AW2, Top Elute Fluid, carrier RNA, and proteinase K must be purchased in order to process 1152 samples with the kit.

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

Table 5. Variations in worktable setup with different numbers of samples

Number of samples	Tip position	Buffer AL (ml)	Carrier RNA (µl)	Buffer ATL (ml)	Proteinase K (tubes)	Buffer AVE
32	1, 2, 4–6	13.8	138	5.6	1.40	4
40	1, 2, 4–7	16.2	162	6.3	1.58	4
48	1, 2, 4–7	18.7	187	7.0	1.75	4
56	1, 2, 4–7	21.1	211	7.7	1.93	8
64	1, 2, 4–8	23.6	236	8.4	2.10	8
72	1, 2, 4–8	26.1	261	9.1	2.28	8
80	1, 2, 4–8	28.5	285	9.8	2.45	8
88	1, 2, 4–8	31.0	310	10.5	2.63	8
96	1, 2, 4–8	33.0	330	11.2	2.80	8

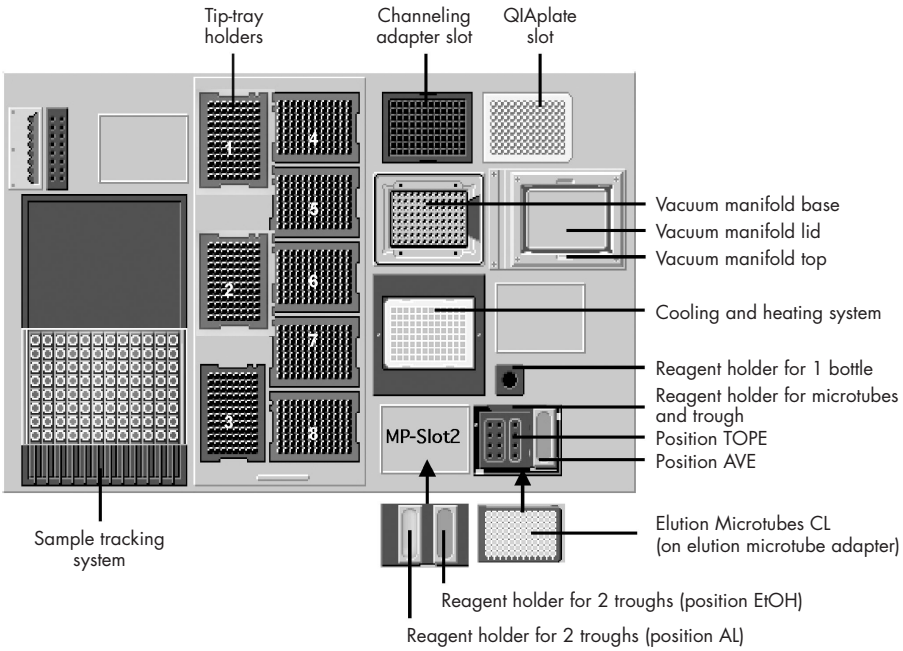


Figure 1. Overview of the BioRobot MDx worktable and its main components. Refer to Tables 3, 4, and 5 for more details.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Little or no nucleic acids 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 13. Repeat the purification procedure with new samples.
b)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –20°C or underwent multiple freeze–thaw cycles. Alternatively, Buffer AL–carrier RNA mixture was stored for more than 48 hours at 2–8°C. Prepare a new tube of carrier RNA dissolved in Buffer AVE and mix with Buffer AL. Repeat the purification procedure with new samples.
c)	Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided (see page 8). Always use fresh samples or samples that have been thawed once only.
d)	Degraded nucleic acids	Samples were left at room temperature (15–25°C) for too long. Repeat the purification procedure with new samples. Check the integrity of nucleic acids in the original samples. Nucleic acids are often degraded by nucleases in the starting material. Ensure that samples are processed quickly following collection or removal from storage. Ensure that no nucleases are introduced during the procedure, and use Buffer AVE for elution.

		Comments and suggestions
e)	Insufficient sample lysis	Proteinase K was subjected to elevated temperatures for a prolonged time. Repeat the purification procedure using new samples and fresh proteinase K. Buffer ATL and proteinase K should be mixed immediately before staring the BioRobot MDx protocol. Do not reuse Buffer ATL–proteinase K mixtures from previous runs.
f)	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. Check Buffer AL for foam after mixing. Remove any large bubbles using a pipet.
g)	Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and use 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
h)	Isopropanol used instead of ethanol for reconstitution of buffers or refilling of the ethanol bottle	We recommend the use of ethanol, as use the of isopropanol results in reduced yields.
i)	Buffer ATL or proteinase too cold when mixed	Buffer ATL may form a white precipitate when stored below room temperature. If white precipitates are visible, dissolve them by incubating at 56 C. Before mixing Buffer ATL with proteinase K, always make sure that there are no precipitates. If Buffer ATL is mixed with proteinase K immediately after removing the proteinase K solution from cold storage, a white precipitate may form. Always make sure that proteinase K is equilibrated to room temperature before mixing with Buffer ATL.
j)	Nuclease contamination in Buffer AVE	Each vial of Buffer AVE should be used only once. Repeat the purification procedure using a new vial of Buffer AVE.

		Comments and suggestions
k)	Buffers AW1 or AW2 prepared incorrectly	Check that Buffers AW1 and AW2 concentrates were diluted with the correct volumes of ethanol. Repeat the purification procedure with new samples.
l)	Buffers AW1 or AW2 prepared with 70% ethanol	Check that Buffers AW1 and AW2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with new samples.
Nucleic acids do not perform well in downstream enzymatic reactions		
a)	Little or no nucleic acids in the eluate	See "Little or no nucleic acids in the eluate" (above) 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 "Adding carrier RNA to Buffer AL", page 13).
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. The elution volume can be adjusted proportionally.
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 or AW2 may have separated out after being unused for a long period between runs. Always mix buffers thoroughly before each run.
e)	A new combination of reverse transcriptase and Taq DNA polymerase used	If enzymes are changed, it may be necessary to readjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.

		Comments and suggestions
f)	Elution Microtubes CL autoclaved before elution	Do not autoclave Elution Microtubes CL. Autoclaving may cause chemicals to leach from the walls of elution microtubes that can inhibit some enzymatic reactions. Repeat the purification procedure with a new set of Elution Microtubes CL. Elution Microtubes CL are delivered RNase- and DNase-free.
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 such 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.

		Comments and suggestions
b)	Samples stored too long before processing	Depending on the type of liquid-cytology medium, cells can chemically cross-link, making them difficult to lyse. Make sure that samples are not stored too long before processing.
c)	Variable elution volumes	The tubes containing Top Elute Fluid were not completely filled. Each vial of Top Elute Fluid should only be used once, even if just part of the plate was processed. Different samples can lead to slightly different elution volumes. The quality of the recovered nucleic acids is not affected.
d)	Result of single samples marked as invalid in the report file	During aspiration of the samples from sample tubes, no sample was detected. During aspiration of the samples from sample tubes, clots were detected. After sample transfer, not enough sample was detected in the S-Block.
e)	Some positions in the Sample Result column in the file report 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.

		Comments and suggestions
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. 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.
g)	Vacuum error during elution	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.
h)	QIAsoft MDx software prompts operator to refill the system liquid container although the container is filled	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.

		Comments and suggestions
i)	QIAsoft MDx software prompts operator to empty the waste container although the container is empty	Drops adjacent to the sensor (inside or outside of 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.
j)	QIAsoft MDx software prompts operator to empty the vacuum trap although the bottle is empty	Drops adjacent to the sensor (inside or outside of 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: Report File (Example)

At the end of a run, a report file is created 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\QIAsoft MDx\UserData\ReportData .

The fields in this example report file are empty. The QIAsoft MDx Operating System fills in these fields at the end of the protocol run.

Operator:

Date/Time:

Protocol full name:

Run Time:

Number of processed samples:

Bar code Elution Microtube CL:

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/number* open maintenance action(s)

General Results:

Result table	Position	Sample bar codes	Sample result
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 successfully copied from "Sample bar codes" to "Sample results" column have been processed successfully.

Errors

* 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, page 25.

† 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.*

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
QIAamp Media MDx Kit (12)	For 12 x 96 preps: 12 QIAamp 96 Plates, Buffers, Proteinase K, S-Blocks, Disposable Troughs, Racks with Elution Microtubes CL (0.4 ml), Carrier RNA, Top Elute Fluid, Caps, Tape Pad	965752
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
Warranty PLUS 2	3-year warranty, 2 preventive maintenance visits per year, 48-hour priority response, all labor, travel, and parts	9238868
Disposable Filter Tips, 1100 µl (960)	Conducting disposable filter-tips, pack of 960	9012598
Accessories		
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer	19076
Buffer AL (216 ml)	216 ml Lysis Buffer AL	19075
Buffer AW1 (concentrate, 151 ml)	151 ml Wash Buffer (1) Concentrate in bar-coded bottle	1021052
Buffer AW2 (concentrate, 127 ml)	127 ml Wash Buffer (2) Concentrate in bar-coded bottle	1020955
Carrier RNA	12 x 1350 µg Carrier RNA	1017647
Buffer AVE	108 x 2 ml Buffer AVE	102953
Top Elute Fluid (48 x 1.48 ml)	48 x 1.48 ml Top Elute Fluid	1020460

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
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes caps	19588
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Disposable Troughs (10)	Disposable troughs for Buffers AL, AD, and ethanol	9232764
Related products		
QIAamp MinElute® Media Kit*	For 50 manual minipreps: 50 QIAamp MinElute Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	57414
QIAamp Virus BioRobot MDx Kit (12)	For 12 x 96 preps on the BioRobot MDx: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Carrier RNA	965652
QIAamp DNA Blood BioRobot MDx Kit (12)	For 12 x 96 DNA preps on the BioRobot MDx: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Tape Pad	965152

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* Requires use of a vacuum manifold, such as the QIAvac 24 Plus (cat. no. 19413).

Notes

Notes

Trademarks: QIAGEN®, QIAamp®, BioRobot®, MinElute® (QIAGEN Group); BD Falcon™ (Becton, Dickinson and Company); PreservCyt® (Cytyc Corporation); SurePath™ (TriPath Imaging, Inc.).

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