

# QIAamp<sup>®</sup> DNA Blood BioRobot<sup>®</sup> MDx Kit Handbook

For purification of genomic DNA from fresh or frozen whole blood or buffy coat using the BioRobot MDx workstation or BioRobot Universal System



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# Contents

<b>Kit Contents</b>	<b>4</b>
<b>Storage</b>	<b>5</b>
<b>Product Use Limitations</b>	<b>5</b>
<b>Product Warranty and Satisfaction Guarantee</b>	<b>5</b>
<b>Quality Control</b>	<b>6</b>
<b>Technical Assistance</b>	<b>6</b>
<b>Safety Information</b>	<b>7</b>
<b>Introduction</b>	<b>8</b>
Principle and procedure	8
<b>Equipment and Reagents to Be Supplied by User</b>	<b>10</b>
<b>Important Notes</b>	<b>11</b>
Storing blood samples	11
Sample volumes	11
Automated purification of high-molecular-weight DNA	11
Preparation of reagents	12
Worktable accessories	15
Setting up the BioRobot MDx	16
Setting up the BioRobot Universal System	20
<b>Protocols</b>	
■ <b>Purification of Genomic DNA from Whole Blood or Buffy Coat Using the BioRobot MDx</b>	<b>22</b>
■ <b>Purification of Genomic DNA from Whole Blood or Buffy Coat Using the BioRobot Universal System</b>	<b>28</b>
<b>Troubleshooting Guide</b>	<b>30</b>
<b>Appendix: Determination of Concentration, Yield, Purity, and Length of DNA</b>	<b>36</b>
<b>Ordering Information</b>	<b>37</b>

## Kit Contents

<b>QIAamp DNA Blood BioRobot MDx Kit</b>	<b>(12)</b>
<b>Catalog no.</b>	<b>965152</b>
<b>Number of preps</b>	<b>12 x 96</b>
QIAamp 96 Plates	12
S-Blocks	12
Caps for Elution Microtubes	3 x 50
Elution Microtubes CL	12 x 96
Disposable Troughs	4 x 10
Tape Pad	1 x 25 sheets
QIAGEN® Protease	12 vials*
Protease Solvent	12 x 6 ml
Buffer AL†	12 x 33 ml
Buffer AE	12 x 27 ml
Buffer AW1† (concentrate)	4 x 151 ml
Buffer AW2‡ (concentrate)	4 x 127 ml
Top Elute Fluid (orange caps)	48 x 1.48 ml
Q-Card§	1
Handbook	1

\* Resuspension volume 6 ml.

† 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.

## Storage

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

Lyophilized QIAGEN Protease can be stored at room temperature (15–25°C) until the expiration date printed 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. Keeping the QIAGEN Protease stock solution at room temperature for prolonged periods should be avoided.

## Product Use Limitations

The QIAamp DNA Blood 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 or visit [www.qiagen.com](http://www.qiagen.com) ).

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAamp DNA Blood 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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAamp DNA Blood BioRobot 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/goto/TechSupportCenter](http://www.qiagen.com/goto/TechSupportCenter) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## 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](http://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 these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the QIAamp DNA Blood BioRobot MDx Kit.

### Buffers AL and AW1

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

### QIAGEN Protease

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

### 24-hour emergency information

Emergency medical information 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.

## Introduction

The QIAamp DNA Blood BioRobot MDx Kit uses well-established technology for purification of genomic and mitochondrial DNA from whole blood or buffy coat. The procedure yields high-quality DNA that performs well in PCR and other enzymatic reactions.

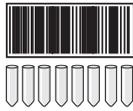
The QIAamp DNA Blood BioRobot MDx Kit combines the selective binding properties of a silica-based membrane with a high-throughput 96-well format, and is designed for fully automated, simultaneous processing of thirty-two to ninety-six 200  $\mu$ l samples (in increments of 8) on the BioRobot MDx or the BioRobot Universal System. Blood and buffy coat samples for processing can be fresh or frozen, provided they have not been frozen and thawed more than once (see "Storing blood samples", page 11). The procedure can be used for samples treated with citrate, heparin, or EDTA. Purification requires no organic extraction or alcohol precipitation. DNA is eluted in Buffer AE, and is free of proteins, nucleases, and other contaminants or inhibitors. It is ready for use in enzymatic reactions, such as PCR, or storage at  $-20^{\circ}\text{C}$ . DNA purified using the QIAamp DNA Blood BioRobot MDx Kit is up to 50 kb in size, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling and can be amplified very efficiently.

The fully automated procedure, 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 in the presence of QIAGEN Protease and Buffer AL under highly denaturing conditions at elevated temperatures. Ethanol is added to adjust lysate buffering conditions, allowing optimal binding of the DNA to the QIAamp 96 plate membrane. Lysates are transferred to a QIAamp 96 plate and nucleic acids are adsorbed onto the silica membrane as samples are drawn through the plate under vacuum. Salt and pH conditions in the lysate ensure that proteins and other impurities that can inhibit PCR and other downstream enzymatic reactions are not retained on the membrane. Nucleic acids bound to the membrane are efficiently washed in 3 steps under vacuum. Three different wash solutions are used, which considerably improves the purity of the eluted DNA. Pure DNA is eluted under vacuum in a single step in approximately 200  $\mu$ l of Buffer AE equilibrated to room temperature. DNA recovery is enhanced by overlaying the elution buffer with Top Elute Fluid.

## QIAamp DNA Blood BioRobot MDx Procedure



Check reagents  
Scan sample bar codes



Aliquot QIAGEN Protease,  
transfer samples,  
and add Buffer AL  
Lysis in cooling and heating system



Add ethanol



Transfer lysates

Vacuum



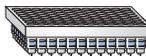
Wash with Buffer AW1

Vacuum



Wash with Buffer AW2

Vacuum



Wash with ethanol

Vacuum



Dry QIAamp 96 plate

Vacuum



Heating  
Add Buffer AE and  
Top Elute Fluid for elution

Vacuum



Purified genomic DNA

Fully automated genomic DNA purification

## 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.

### For DNA purification using the BioRobot MDx

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

### For DNA purification using the BioRobot Universal System

- BioRobot Universal System, cat. no. 9001094
- Application Pack, Genotyping, cat. no. 9016755
- QIAGEN conducting disposable filter-tips (1100 µl) for use with BioRobot systems, cat. no. 9012598
- BD Falcon™ Tubes, 14 ml, BD cat. no. 352051
- Ethanol (96–100%)\*
- Deionized water

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

## Important Notes

### Storing blood samples

Whole blood samples treated with EDTA,\* citrate,\* or heparin\* can be used, and may be fresh or frozen. Yield and quality of the purified DNA depend on storage conditions of the blood. Fresher blood samples yield better results. For long-term storage, we recommend collecting blood samples in tubes containing anticoagulant and storing at  $-70^{\circ}\text{C}$ .

Repeatedly frozen and thawed samples, samples stored at room temperature for extended periods, or samples containing visible precipitates should not be processed, as they may clog the QIAamp 96 plate membrane. Although clots and clogs are detected and managed by the BioRobot MDx and the BioRobot Universal System during runs, their presence will increase run time and consumption of tips (if using automatic clog detection). The number of tips loaded onto the worktable allows automatic detection and removal of up to 24 clogged samples in each run of 96 samples.

### Sample volumes

The QIAamp 96 plate membrane can bind nucleic acids greater than 200 bases in length. Actual yields will depend on sample storage and white blood cell content. The procedure is optimized for use with 200  $\mu\text{l}$  samples of human whole blood or buffy coat. Each blood or buffy coat sample must not exceed  $3 \times 10^7$  cells per ml.

The BioRobot MDx and the BioRobot Universal System are capable of removing 200  $\mu\text{l}$  samples from tubes of varying sizes, such as CryoTube® or BD Vacutainer™ tubes, containing a minimum sample volume of 500  $\mu\text{l}$ . Special tube holders are available upon request. Elution is performed in a single step using 200  $\mu\text{l}$  Buffer AE.

### Automated purification of high-molecular-weight DNA

To purify high-molecular-weight DNA larger than the 50 kb achieved with the QIAamp DNA Blood BioRobot MDx Kit, we recommend using the Autopure LS® workstation. The system provides automated purification from 8 or 16 blood or buffy coat samples per run. Purified DNA ranges from 100 to 200 kb and is highly stable. It is suitable for DNA archiving, PCR, SNP analysis, Southern blotting, and other demanding applications.

\* 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.

## Preparation of reagents

**Important:** Sufficient reagents are supplied to isolate DNA from 12 x 96 blood or buffy coat samples. If fewer than 96 samples are processed in each run, additional reagents must be purchased to process the same number of samples in total (see below for details).

### QIAGEN Protease

**For runs of 96 samples:** One vial of lyophilized QIAGEN Protease and protease solvent is supplied for each run of 96 samples. Add one bottle (6 ml) of protease solvent to a vial of lyophilized QIAGEN Protease and mix carefully to avoid foaming. Make sure that the protease solvent is transferred completely and the QIAGEN Protease is fully dissolved. For the BioRobot Universal System, transfer the reconstituted QIAGEN Protease to a 14 ml BD Falcon Tube before placing it into the reagent holder.

**For runs of fewer than 96 samples:** If runs contain 32–88 samples, each vial of lyophilized QIAGEN Protease and protease solvent has to be reused 2–3 times. Add one bottle (6 ml) of protease solvent to a vial of lyophilized QIAGEN Protease and mix carefully to avoid foaming. Make sure that the protease solvent is transferred completely and the QIAGEN Protease is fully dissolved. For the BioRobot Universal System, transfer the reconstituted QIAGEN Protease to a 14 ml BD Falcon Tube before placing it into the reagent holder.

After the run, seal the vial or tube 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.

**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

**Note:** If you also use the QIAamp Virus BioRobot MDx Kit, be aware that the QIAGEN Protease supplied with this kit is reconstituted in protease resuspension buffer and is not compatible with the QIAamp DNA Blood BioRobot MDx Kit. After reconstituting a vial of QIAGEN Protease, we recommend labeling the vial with the name of the compatible kit in order to avoid any mixup.

## Buffer AL

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

**For runs of 96 samples:** One bottle of Buffer AL is supplied for each run of 96 samples. Mix by shaking the bottle carefully before use, avoiding extensive foaming. Open the bottle and slowly pour the entire contents into a disposable trough in the reagent holder for 2 troughs (position AL). Remove any large bubbles with a pipet tip.

**For runs of fewer than 96 samples:** Sufficient Buffer AL is supplied for 12 runs of 96 samples. For runs of 32–88 samples, one bottle of Buffer AL has to be reused 2–3 times. The QIAsoft Operating System indicates how much Buffer AL is required during setup of the BioRobot MDx or BioRobot Universal System before a purification run is started. Transfer the indicated volume of Buffer AL into a disposable trough. Remove any large bubbles with a pipet tip.

Note that processing 96 samples divided over more than one run will require more buffer than one 96-sample run. If runs of fewer than 96 samples are performed, additional Buffer AL must be purchased (see ordering information, page 37).

## Buffer AW1

Add 200 ml of ethanol (96–100%) to a bottle containing 151 ml of 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. Sufficient Buffer AW1 is supplied for 12 runs of 96 samples. One bottle of reconstituted Buffer AW1 contains enough wash buffer for 3 runs of 96 samples. Buffer AW1 left over after a run should be stored 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 and BioRobot Universal System, 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 the outside.

Approximately 30 ml of Buffer AW1 is used to flush the system tubing during each run, and this volume remains constant regardless of the number of samples to be purified. Note that processing 96 samples divided over more than one run (e.g., two 48-sample runs) will require more buffer than one 96-sample run. If runs of fewer than 96 samples are often performed, additional Buffer AW1 must be purchased (see ordering information, page 37).

## Buffer AW2

Add 300 ml of ethanol (96–100%) to a bottle containing 127 ml of Buffer AW2 concentrate, as described on the bottle. Tick the check box on the label of the bottle to indicate 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. 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 and BioRobot Universal System, 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. Note that processing 96 samples divided over more than one run (e.g., two 48-sample runs) will require more buffer than one 96-sample run. If runs of fewer than 96 samples are often performed, additional Buffer AW2 must be purchased (see ordering information, page 37).

## Ethanol

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

For the BioRobot MDx, 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. Ethanol left over after a run should be stored at room temperature (15–25°C) for the next run.

For the BioRobot Universal System, the QIAsoft 5 Operating System indicates how much ethanol is required during setup before a purification run is started. Reconstituted Buffer AW2 should be added to the bottle containing ethanol (1:1000 dilution), to allow the BioRobot Universal System to detect the fill level in the ethanol bottle by conductivity measurements. Ethanol left over after a run should be stored at room temperature for the next run.

For easy identification on the BioRobot MDx and BioRobot Universal System, 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 AE**

For each run of 96 samples, one bottle of Buffer AE (27 ml) is required. Sufficient Buffer AE is provided for 12 runs of 96 samples.

Mix by shaking the bottle of Buffer AE before use. During setup of the BioRobot MDx or BioRobot Universal System before a purification run is started, open the bottle and pour the entire contents into a disposable trough. Even if fewer than 96 samples are prepared at a time, it is still necessary to transfer a complete bottle of Buffer AE into the trough. Buffer AE left over after a run must be discarded and should not be reused for subsequent runs.

Note that processing 96 samples divided over more than one run (e.g., two 48-sample runs) will require more Buffer AE than one 96-sample run. If runs of fewer than 96 samples are performed, additional Buffer AE must be purchased (see ordering information, page 37).

## **Top Elute Fluid (orange cap)**

For one 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). During setup of the BioRobot MDx or BioRobot Universal System, the opened tubes of Top Elute Fluid should be placed on the worktable. Top Elute Fluid left over after a run should be discarded and should not be reused for subsequent runs.

Even if fewer than 96 samples are prepared at a time, it is still necessary to place all 4 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 ordering information, page 38).

## **Worktable accessories**

### **S-Blocks**

Twelve S-Blocks are supplied with the kit. When loading the BioRobot worktable before starting a protocol run, make sure that position A1 is located at the upper-left corner. Discard the S-Block after use.

### **Disposable troughs**

Forty disposable troughs are supplied with the kit. For each run, 2 disposable troughs are required. Discard the disposable troughs 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. During setup, make sure that the bar code of the elution microtube rack faces to the right. After sample preparation, the eluates should be directly used in downstream applications or stored for up to 24 hours at 2–8°C. For longer storage, DNA should be stored at –20 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 one QIAamp 96 plate is required. During setup, make sure that position A1 is located at the upper-left corner. Discard the QIAamp 96 plate after use.

## **Tape pad**

Tape sheets from the tape pad are used to seal 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 sheet from the tape pad and leave them sealed throughout the purification procedure. Ensure that complete columns of 8 samples are processed. After use, keep the unused wells sealed and store the QIAamp 96 plate at 4°C 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 previously used wells with adhesive tape before beginning the purification procedure.

## **Setting up the BioRobot MDx**

The QIAsoft MDx software guides you through worktable setup. For a summary of worktable setup, see Tables 2, 3, and 4 below and Figure 1 on page 19.

**Table 2. Loading buffers and reagents**

<b>Item</b>	<b>Position</b>	<b>Volume</b>
Buffer AL*	Disposable trough in reagent holder for 2 troughs (position AL)	Varies with sample number (see Table 4, next page)
QIAGEN Protease	Reagent holder for 1 bottle (QP)	Varies with sample number (see Table 4, next page)
Buffer AE*	Disposable trough in reagent holder for microtubes and trough (position AE)	One bottle
Top Elute Fluid (TOPE)*†	Reagent holder for microtubes and trough (position TOPE)	Four 1.5 ml microcentrifuge tubes (orange caps)
Buffer AW1 *	Reagent carousel	Minimum of 164 ml (reconstituted)
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.

\* 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 and Buffers AL, AW1, AW2, and AE must be purchased.

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

**Table 3. Loading plasticware**

Item	Position	Holder/adaptor
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) Reagent holder for microtubes and trough (AE)
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 Table 4 below)	Red tip-tray holder

**Table 4. Variations with different numbers of samples**

	Number of samples								
	32	40	48	56	64	72	80	88	96
Tip position* (no clog check)	1, 2, 4-5	1, 2, 4-5	1, 2, 4-6	1, 2, 4-6	1, 2, 4-6	1, 2, 4-7	1, 2, 4-7	1, 2, 4-8	1, 2, 4-8
Tip position* (visual clog check)	1, 2, 4-5	1, 2, 4-6	1, 2, 4-6	1, 2, 4-7	1, 2, 4-7	1, 2, 4-8	1, 2, 4-8	1, 2, 4-8	1, 2, 4-8
Tip position* (automatic clog check)	1, 2, 4-6	1, 2, 4-6	1, 2, 4-7	1, 2, 4-7	1, 2, 4-8				
Volume of Buffer AL (ml)	14.0	16.2	18.7	21.1	23.6	26.1	28.5	31.0	33.0
Volume of reconstituted QIAGEN Protease (ml)	3.2	3.5	3.9	4.2	4.6	4.9	5.3	5.6	6.0

\* See Figure 1 for the tip positions.

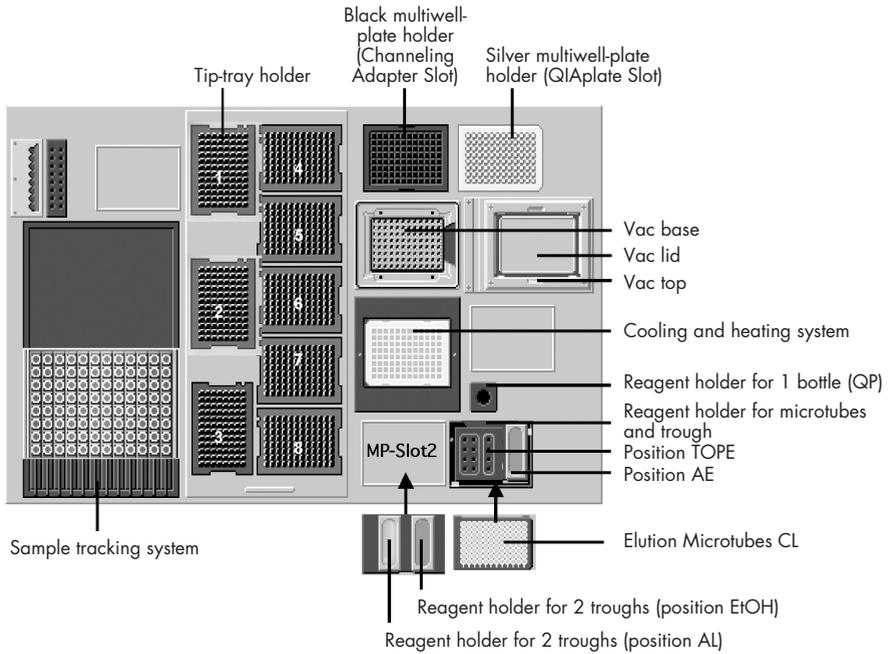


Figure 1. Overview of the BioRobot MDx worktable.

## Setting up the BioRobot Universal System

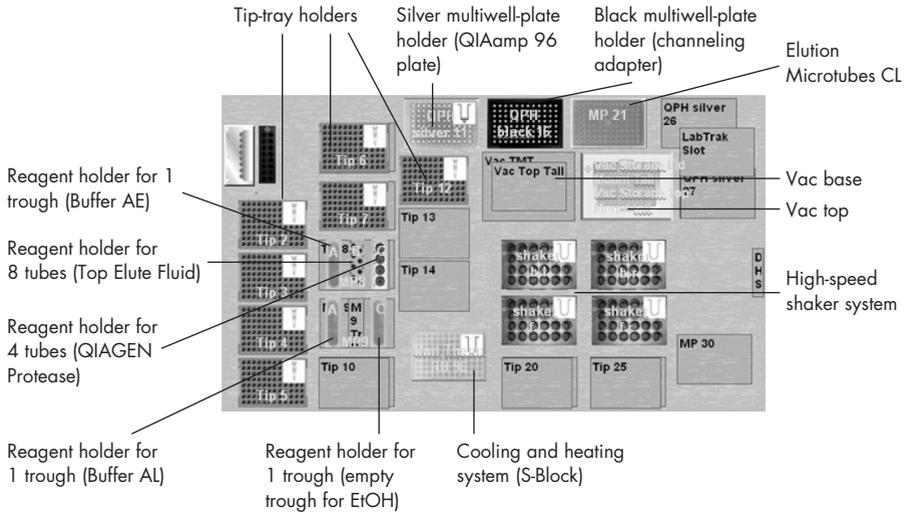
The QIAsoft 5 software guides you through worktable setup. For a summary of worktable setup, see Tables 5 and 6, below and Figure 2 on page 21.

**Table 5. Positions of reagents and buffers on the BioRobot Universal System**

Item	Position
Buffer AE	Disposable trough in the 1-trough reagent holder (Subslot A of MP Slot 8)
Top Elute Fluid	Positions A, C, E, and G of the 8-tube reagent holder (Subslot B of MP Slot 8)
QIAGEN Protease (reconstituted)	14 ml BD Falcon tube in position A of the 4-tube reagent holder (Subslot C of MP Slot 8)
Buffer AL	Disposable trough in the 1-trough reagent holder (Subslot A of MP Slot 9)
Ethanol (96–100%)	Reagent carousel
Buffer AW1	Reagent carousel
Buffer AW2	Reagent carousel
System liquid	Reagent carousel (Rotor Slot 8)
Samples	24-tube shaker adapter (high-speed shaker system)

**Table 6. Positions of accessories on the BioRobot Universal System worktable**

Item	Position	Holder/adapter
Elution Microtubes CL	MP Slot 21	Blue elution microtube adapter
Disposable trough	Subslot A of MP Slot 8, Subslot A of MP Slot 9, and Subslot C MP Slot 9	Trough holder in reagent-holder tray
QIAamp 96 plate	QIAplate Holder silver 11	Silver multiwell-plate holder
Channeling adapter	QIAplate Holder black 16	Black multiwell-plate holder
S-Block	Cooling and heating system (VariTherm Slot)	Silver heat transfer adapter
Disposable filter-tips	Varies with sample number	Red tip-tray holder
Samples	High-speed shaker system	24-tube shaker adapter



**Figure 2. Overview of the BioRobot Universal System worktable.**

# Protocol: Purification of Genomic DNA from Whole Blood or Buffy Coat using the BioRobot MDx

This protocol is for purification of total DNA from up to 96 samples of fresh or frozen whole blood or buffy coat using the BioRobot MDx. For purification using the BioRobot Universal System, see "Protocol: Purification of DNA from Whole Blood or Buffy Coat Using the BioRobot Universal System", page 28.

## Important points before starting

- Read "Important Notes" on pages 11–21.
- Each blood or buffy coat sample must not exceed  $3 \times 10^7$  cells per ml.
- Avoid processing blood and buffy coat samples in the same protocol run.
- Orient samples in the tube holders of the sample tracking system so that the bar codes face the bar code reader camera on the left side of the BioRobot MDx. 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  $\mu$ l of sample. Always use 12 tube holders.
- Ensure that you are familiar with operating the BioRobot MDx.

## Things to do before starting

- Check that the QIAGEN Protease and Buffers AL, AW1, and AW2 have been prepared according to the instructions on pages 12–14.

## Procedure

1. Follow step 1a if processing blood samples, or step 1b if processing buffy coat samples.
  - 1a. If processing blood samples, check whether they have separated into 2 phases: an upper plasma phase containing low amounts of DNA, and a lower cell-dense phase containing large amounts of DNA. If phase separation has occurred, mix the blood samples thoroughly (e.g., by inverting the tubes several times) before loading them onto the BioRobot MDx worktable.
  - 1b. Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at  $2500 \times g$  for 10 min at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Transfer at least 500  $\mu$ l of the intermediate buffy coat layer into a new tube, and use this sample for processing on the BioRobot MDx.

2. Equilibrate 32–96 whole blood or buffy coat samples to room temperature (15–25°C).

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

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

4. Switch on the computer and monitor.

5. Launch the QIAsoft MDx Operating System.

The QIAsoft MDx software can be started from the desktop.

6. Log in 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*.

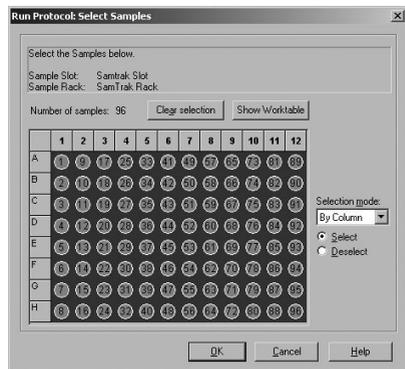
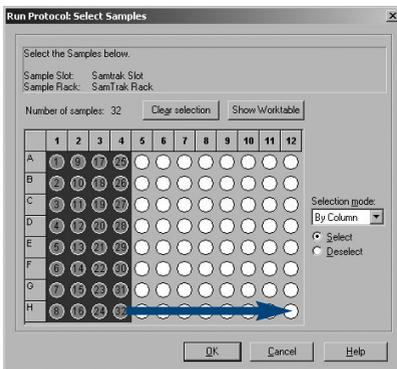
7. Select the QIAamp DNA Blood MDx Protocol (located in the Genomic DNA Purification package) using the protocol selection box in the “Execute” environment toolbar.

8. Click  to start the QIAamp DNA Blood MDx Protocol.

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

A minimum of 32 samples or a multiple of 8 between 32 and 96 samples should be selected. Mark the corresponding positions 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:** Please keep in mind that buffer consumption is calculated for processing runs of 96 samples. If runs of fewer than 96 samples are performed, it will be necessary to purchase additional Top Elute Fluid and Buffers AL, AW1, AW2, and AE.



## 10. Enter the bar code of the kit.

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.

## 11. Choose whether to work without clog detection, with visual clog detection, or with automatic clog detection.

The QIAsoft MDx software can provide detection of clogged membranes. A choice box will appear allowing you to activate the clog detection function. Three options are available:

- **Sample processing without clog detection:** This is the fastest and most cost-effective option since no additional tips are used. However, there is a risk of clot formation and membrane clogging, particularly when using samples with high white blood cell counts ( $>3 \times 10^7$  cells per ml). A clogged membrane that is not detected may lead to overflow of wash buffer during the purification procedure, which may contaminate samples in adjacent positions.
- **Sample processing with visual detection of clogged membranes:** This option provides a safer and cost-effective solution, but requires user intervention after the lysate is loaded onto the QIAamp 96 plate. The process pauses after the lysate is drawn through the membrane under vacuum and the operator is asked to check visually whether any positions are clogged. If clogs are detected, the operator enters the clogged positions into a table. The QIAsoft MDx software then ensures that the marked well(s) are kept clear during the rest of the purification procedure. Adjacent samples can be safely processed without risk of contamination.
- **Sample processing with automatic clog detection:** This option is the safest but most time-consuming solution. It incurs higher costs because at least one extra tip is used for each sample. During the process, each position is automatically checked for clogging using disposable tips. The QIAsoft MDx software ensures that clogged wells are kept clear during the rest of the purification procedure. Adjacent samples can be safely processed without risk of contamination.

## 12. 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.

**13. 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.

**14. 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 DNA Blood BioRobot 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, where you decide if you will 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 has to 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 locked, the BioRobot MDx checks if every component needed for the protocol is in place and if sample bar codes can be read. If an error is found, the instrument pauses and a display message prompts you to recheck the cause of the error 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 interruptions.

- 15. A software message on the screen will indicate when the purification procedure is finished, and protocol messages will guide you through the steps for worktable cleanup.**
- 16. The purified DNA is ready to use in downstream applications or can be stored at 2–8°C for 24 h or at –20°C or –80°C for longer periods.**
- 17. Follow the maintenance instructions described in the “Maintenance” environment of the QIAsoft MDx software and in the *BioRobot MDx User Manual*.**

## Generation of a report file

After the run is completed, 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\QIAsoft MDx\UserData\ReportData**. The identification bar code of the processed elution microtube rack is used as a file name.

## Information in the header of report files

**Operator:**

**Date/Time:**

**Protocol name:**

**Run time:**

**Number of processed samples:**

**Bar code Elution Microtubes 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/1-n open maintenance action(s)

## Results table from example report file

General results:			
<i>Result table</i>	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 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 the sample (e.g., clots or clogs). For more detailed information, refer to the troubleshooting section on pages 30–35.

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

# Protocol: Purification of DNA from Whole Blood or Buffy Coat Using the BioRobot Universal System

This protocol is for purification of total DNA from up to 96 samples of fresh or frozen whole blood or buffy coat using the BioRobot Universal System. For purification using the BioRobot MDx, see "Protocol: Purification of DNA from Whole Blood of Buffy Coat Using the BioRobot MDx", page 22.

## Important points before starting

- Read "Important Notes" on pages 11–21.
- Each blood or buffy coat sample must not exceed  $3 \times 10^7$  cells/ml.
- Avoid processing blood and buffy coat samples in the same protocol run.
- Each tube should contain at least 500  $\mu$ l of sample.
- Ensure that you are familiar with operating the BioRobot Universal System.

## Things to do before starting

- Check that the QIAGEN Protease and Buffers AL, AW1, and AW2 have been prepared according to the instructions on pages 12–14.

## Procedure

1. Follow step 1a if processing blood samples or step 1b if processing buffy coat samples.
  - 1a. If processing blood samples, check whether they have separated into 2 phases: an upper plasma phase containing low amounts of DNA and a lower cell-dense phase containing large amounts of DNA. If phase separation has occurred, mix the blood samples thoroughly (e.g., by inverting the tubes several times) before loading them onto the BioRobot Universal System worktable.
  - 1b. Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at  $2500 \times g$  for 10 min at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Transfer at least 500  $\mu$ l of the intermediate buffy coat layer into a new tube, and use this sample for processing on the BioRobot Universal System.
2. Equilibrate 32–96 whole blood or buffy coat samples to room temperature (15–25°C).

**3. Make sure that the BioRobot Universal System is switched on.**

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

**4. Switch on the computer and monitor.**

**5. Launch the QIAsoft 5 Operating System.**

The QIAsoft 5 software can be started from the Microsoft® Windows® “Start” menu, where it is located under Programs/QIAsoft 5/QIAsoft 5.

**6. Enter your user name and password in the “Login” dialog box, and click “OK” to access the QIAsoft 5 software.**

**7. Select the QIAamp DNA Blood BioRobot UNIV Protocol from the protocol selection box in the “Execute” environment toolbar.**

**8. Click  to start the protocol.**

The QIAsoft 5 software will now take you through the remaining steps required to set up the BioRobot Universal System for the QIAamp DNA Blood BioRobot UNIV Protocol. Follow the steps detailed in each protocol message before continuing.

You will be prompted to enter information about the following:

- Number and positions of samples to be processed.
- The kit bar code printed on the Q-Card supplied with the kit.
- Whether you want to work without clog detection, with visual clog detection, or with automatic clog detection.
- Whether sample bar codes should be read.
- Whether a load check should be performed.

**9. A software message on the screen will indicate when the purification procedure is finished, and protocol messages will guide you through the steps for worktable cleanup.**

**10. The purified DNA is ready to use in downstream applications or can be stored at 2–8°C for 24 h or at –20°C or –80°C for longer periods.**

**11. Follow the maintenance instructions described in the “Maintenance” environment of the QIAsoft 5 software and in the instrument 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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## Comments and suggestions

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### Little or no DNA in the eluate

- |    |                                                                  |                                                                                                                                                                                                                                                                                                                                                                                          |
|----|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) | Lysis buffer (Buffer AL) and elution buffer (Buffer AE) mixed up | Repeat the procedure with fresh samples.                                                                                                                                                                                                                                                                                                                                                 |
| b) | Samples left standing for too long at room temperature           | Genomic DNA in the samples has been degraded by nucleases. Repeat the purification procedure with new samples.<br><br>The sample has separated into 2 phases, an upper plasma phase (containing only low amounts of DNA) and a lower, cell-rich phase (containing high levels of DNA). Repeat the purification procedure, thoroughly mixing samples immediately before a run is started. |
| c) | Insufficient sample lysis in Buffer AL                           | QIAGEN Protease was subjected to elevated temperatures for prolonged periods. Repeat the procedure using new samples and fresh QIAGEN Protease.                                                                                                                                                                                                                                          |
| d) | If water was used for elution, the pH of the water was too low   | Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer AE for elution.                                                                                                                                                                                                                                                                               |
| e) | Low-percentage ethanol used instead of 96–100%                   | Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.                                                                                                                                                                                                             |
| f) | Isopropanol* used instead of ethanol                             | We recommend the use of ethanol, as use of isopropanol leads to reduced yields.                                                                                                                                                                                                                                                                                                          |

\* 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.

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## Comments and suggestions

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- g) DNA degraded      DNA may have been degraded by DNases in the starting material. Ensure that samples are processed quickly following collection or removal from storage. Ensure that no DNase is introduced into the reagents during the procedure. Use Buffer AE or DNase-free water for elution.
- h) Samples not equilibrated to room temperature      Using cold samples can lower the lysis temperature, leading to incomplete sample lysis.

### **$A_{260}/A_{280}$ ratio of purified nucleic acids is low**

- Buffer AW1 or AW2 prepared incorrectly      Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct amounts of ethanol. Repeat the purification procedure with new samples.

### **DNA does not perform well in downstream enzymatic reactions**

- a) Little or no DNA in the eluate      See "Little or no DNA in the eluate" for possible reasons. Increase the amount of eluate added to the reaction if possible. If necessary, concentrate the DNA under vacuum, or repeat the procedure using fresh samples.
- b) Reduced performance in PCR      Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.
- c) 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 AW1 or AW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
- d) Animal blood used      Hemoglobin can be difficult to remove from the blood of some animal species (e.g., monkey and mouse) and may interfere with downstream applications.
- e) Elution microtubes autoclaved before elution      Do not autoclave elution microtubes. Autoclaving may leach chemicals that can inhibit some downstream enzymatic reactions from the walls of elution microtubes. Repeat the procedure with a new set of elution microtubes. Elution microtubes are delivered RNase- and DNase-free.

### Colored residues remain on the QIAamp 96 plate after washing

- |    |                                                        |                                                                                                                                               |
|----|--------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| a) | Buffer AW1 or AW2 prepared incorrectly                 | Ensure that the correct amounts of ethanol were added to Buffer AW1 and AW2 concentrates. Repeat the purification procedure with new samples. |
| b) | Buffer AW1 or AW2 prepared with low percentage ethanol | Ensure that 96–100% ethanol was added to Buffer AW1 and AW2 concentrates. Repeat the purification procedure with new samples.                 |

### General handling

- |    |                                                    |                                                                                                                                                                                                                                                                                                     |
|----|----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) | BioRobot MDx: Some sample 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 into 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

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- b) BioRobot Universal System: Bar codes on sample tubes or elution microtubes not identified
- Sample tubes or elution microtubes were not positioned correctly. Turn the racks so that the bar codes are on the right side and enter the identification code manually.
- c) Blocked wells in the QIAamp 96 plate
- Blood has been stored at room temperature or frozen for extended periods. Repeat the procedure using fresh blood or use the feature for automatic clog detection.
- White blood cell count is too high. Dilute samples at least 1:1 with the anticoagulant solution used for blood collection, mix thoroughly (e.g., by inverting the tubes several times), and repeat the procedure. If the anticoagulant solution is not available, PBS can be used instead.
- Buffer AE used instead of Buffer AL. Repeat the procedure with fresh samples.
- Blood and buffy coat samples were processed together in the same protocol run. Blood samples have lower white blood cell counts compared with buffy coat samples, and pass more quickly through the membranes of the QIAamp 96 plate. This leads to a reduction in the vacuum applied to the QIAamp 96 plate, causing inefficient processing of buffy coat samples through the membranes of the QIAamp 96 plate. Repeat the procedure using only blood samples or only buffy coat samples in the same protocol run.
- Samples with white blood cell counts greater than  $3 \times 10^7$  cells/ml were processed. These samples can clog the membrane of the QIAamp 96 plate. Repeat the procedure using samples of lower cell counts.
- d) Overflowing wells in the QIAamp 96 plate
- Insufficient vacuum was applied. If fewer than 96 samples are purified simultaneously, ensure that unused wells in the QIAamp 96 plate are sealed with tape. Use the clog detection feature offered in the protocol.

## Comments and suggestions

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- e) 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 only part of the QIAamp 96 plate is processed.
- Processing of different blood samples may lead to variations in elution volume.
- Blood and buffy coat samples were processed together in the same protocol run. Repeat the procedure using only blood samples or only buffy coat samples in the same protocol run.
- f) Result of single samples marked as invalid in the report file    During sample aspiration from sample tubes, no sample was detected.
- During sample aspiration from sample tubes, clots were detected.
- Small clots 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.
- A clog was detected by the automatic clog detection system.
- g) 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.
- h) 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, replace the tip disposal container, and continue the protocol.

## Comments and suggestions

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The tip disposal bag was not inserted thoroughly into the tip disposal container, leading to a tip jam. The bag must fit tightly to the walls 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. Replace the tip disposal container and 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, empty the container, and insert it again. Push it back until a metallic click is heard. Continue the protocol.

- i) Vacuum error during elution  
Sufficient vacuum was not reached. After the protocol has paused, open the worktable hood, and check if the QIAamp 96 plate fits well to the elution microtubes. If necessary, correct the position of the QIAamp 96 plate, close the hood, and continue the protocol.
- j) QIAsoft 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 the container and sensor are dry.
- k) QIAsoft software prompts operator to empty the waste container although the container is empty  
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.
- l) QIAsoft software prompts operator to empty the vacuum trap although the bottle is empty  
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: Determination of Concentration, Yield, Purity, and Length of DNA

## Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an  $A_{260}/A_{280}$  ratio of 1.7–1.9.

Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. In order to measure only DNA, a fluorometer must be used.

## Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol precipitation and reconstituted by gentle agitation in approximately 30  $\mu$ l TE buffer,\* pH 8.0, for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature since over-dried genomic DNA is very difficult to redissolve. Load 3–5  $\mu$ g DNA per well. Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer
- Switch intervals: 5–40 s
- Run time: 17 h
- Voltage: 170 V

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

# Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Blood BioRobot MDx Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, S-Blocks, Disposable Troughs, Racks with Elution Microtubes CL (0.4 ml), Top Elute Fluid, Caps, Tape Pad	965152
BioRobot MDx	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft MDx Operating System, laboratory cabinet, accessory cabinet, installation and training, 1-year warranty on parts and labor*	900600
BioRobot Universal System	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft 5 Operating System, installation, 1-year warranty on parts and labor†	9001094
Application Pack, Genotyping	Protocols and application-specific accessories for genomic DNA purification and PCR setup on the BioRobot Universal System	9016755
Disposable Filter Tips 1100 µl (960)	Conducting Disposable Filter-Tips, pack of 960	9012598
<b>Accessories</b>		
QIAGEN Protease (lyophilized)	7.5 Anson units per vial (lyophilized)	19155
Protease Solvent (6 ml)	Solvent for reconstituting QIAGEN Protease	1021055
Buffer AW1 (conc., bar code, 151 ml)	151 ml Wash Buffer (1) Concentrate in bar code labeled bottle	1021052
Buffer AW2 (conc., bar code, 127 ml)	127 ml Wash Buffer (2) Concentrate in bar code labeled bottle	1020955
Buffer AL (216 ml)	216 ml Lysis Buffer AL	19075
Buffer AE (240 ml)	240 ml Elution Buffer AE	19077

\* 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.

† Warranty PLUS 2 (cat. no. 9237714) recommended: 3-year warranty, 1 preventive maintenance visit 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, QIAamp 96 BioRobot, and DNeasy® 96 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
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
Elution Microtubes CL (24 x 96)	Racked elution microtubes for use with QIAamp 96 Plates	19588

### Related products

#### **QIAamp Virus BioRobot MDx Kit — for purification of viral DNA and RNA from cell-free body fluids using the BioRobot MDx**

QIAamp Virus BioRobot MDx Kit (12)	For 12 x 96 preps: 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
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#### **QIAamp 96 DNA Blood Kit — for high-throughput purification of DNA from blood and cell-free body fluids**

QIAamp 96 DNA Blood Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, Collection Vessels	51162
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#### **QIAamp DNA Blood Mini Kit — for purification of DNA from blood and cell-free body fluids**

QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
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\* Fully automatable on the QIAcube. See [www.qiagen.com/MyQIAcube](http://www.qiagen.com/MyQIAcube) for protocols.

## Ordering Information

Product	Contents	Cat. no.
<b>QIAcube® — for fully automated sample preparation using QIAGEN spin-column kits</b>		
QIAcube (110 V)*	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor†	9001292*
QIAcube (230 V)†		9001293†

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