Third Edition April 2010

QIAamp® DNA Blood BioRobot® 9604 Kit Handbook

For purification of genomic DNA from

- whole blood
- buffy coat
- bone marrow
- body fluids
- lymphocytes
- cultured cells

using the BioRobot 9604 workstation

QIAGEN

WWW.QIAGEN.COM

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

Contents

Kit Contents

QIAamp DNA Blood BioRobot 9604 Kit	**(12)**
Catalog no.	**965162**
Number of preps	**12 x 96**
QIAamp® 96 Plates	12
S-Blocks*	14
Tape Pad	1
AirPore tape	1 x 25 sheets
Caps for Elution Microtubes	3 x 50
Elution Microtubes CL	12 x 96
2 ml tubes for QIAGEN® Protease	50
Caps for 2 ml tubes	50
15 ml tubes for Buffer AL	50
QIAGEN Protease	6 vials†
Protease Solvent‡	6 x 10.2 ml
500 ml Bottle for Buffer AW2	1
Buffer AL¶	1 x 330 ml
Buffer AW1¶ (concentrate)	6 x 175 ml
Buffer AW2 (concentrate)	4 x 274 ml
Buffer AE‡	48 x 5 ml
Handbook	1

* Reusable; see page 13 for cleaning instructions.
† Resuspension volume 10.2 ml.
‡ Contains sodium azide as a preservative.
¶ Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 7 for safety information.

Storage

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

QIAGEN Protease is provided lyophilized. Reconstituted QIAGEN Protease is stable for 2 months when stored at 2–8°C. Keeping the QIAGEN Protease stock solution at room temperature for prolonged periods of time should be avoided. Storage at –20°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and freezing at –20°C is recommended.

Product Use Limitations

The QIAamp DNA Blood BioRobot 9604 Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAamp DNA Blood BioRobot 9604 Kit or QIAGEN products in general, please do not hesitate to contact us.

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

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

Quality Control

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

Safety Information

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

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

Buffers AL and AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the QIAamp DNA Blood BioRobot 9604 Kit.

Buffers AL and AW1

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

QIAGEN Protease

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

24-hour-emergency information

Emergency medical information can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R37/38: Irritating to respiratory system and skin; R41: Risk of serious damage to eyes; R42: May cause sensitization by inhalation; S13: Keep away from food, drink and animal 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 this container or label.

Introduction

The QIAamp DNA Blood BioRobot 9604 Kit enables rapid purification of genomic DNA for PCR and Southern blotting. DNA can be purified from whole blood, buffy coat, bone marrow, body fluids, lymphocytes, and cultured cells. A separate protocol is provided for isolation of DNA from buffy coat. For all other starting materials, follow the QIAamp 96 DNA Blood Purification Protocol. Note that for purification of viral nucleic acids from plasma or serum, use of the QIAamp Virus BioRobot 9604 Kit is recommended.

The QIAamp DNA Blood BioRobot 9604 Kit combines the selective binding properties of a silica-gel membrane with a high-throughput 96-well format. The kit is designed for automated processing of 200 µl samples on the BioRobot 9604 workstation. The workstation is capable of removing 200 µl samples from tubes of varying sizes, such as CryoTube®, BD VACUTAINER®, or 1.5 ml microcentrifuge tubes, and is equipped with a liquid-level detection system to facilitate aspiration of 200 µl aliquots directly from primary sample tubes. Special sample carrier racks are available upon request. Fresh or frozen samples that have been treated with citrate, heparin, CPD, CPDA, or EDTA can be used. Purification requires no phenol/chloroform extraction or alcohol precipitation, and involves few manual interactions.

The QIAamp 96 DNA Blood Protocol processes 96 samples in under 2 hours, with about 5 minutes hands-on time, including bar code reading and complete process documentation. Turnaround time between consecutive runs is about 10 minutes. DNA is eluted in 200 µl Buffer AE or water. The QIAamp 96 DNA Buffy Coat Protocol processes 96 samples in less than 2.5 hours, with about 10 minutes hands-on time. Turnaround time between consecutive runs is about 10 minutes. DNA is eluted in 400 µl Buffer AE or water. As higher volumes of Buffer AL and AE are used in this protocol, additional buffer must be ordered (see ordering information, page 24). DNA from both protocols can be used directly in amplification or other enzymatic reactions, or stored at –20°C, and is free of protein, nucleases, and other contaminants. It is sized up to 50 kb, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling, so is well suited for PCR.

QIAamp principle and procedure

Samples are lysed in the presence of QIAGEN Protease and Buffer AL under highly denaturing conditions. Lysate buffering conditions are adjusted to optimize conditions for binding of DNA to the QIAamp membrane and DNA is adsorbed onto the silica-gel membrane under vacuum. Salt and pH conditions in the lysate ensure that proteins and other impurities are not retained on the membrane. DNA bound to the membrane is efficiently washed in three steps using vacuum and centrifugation. Two different wash buffers are used, which considerably improves the purity of the DNA. Highly pure DNA is eluted in Buffer AE or water. Yields depend on sample storage and white blood cell content.

Sample storage

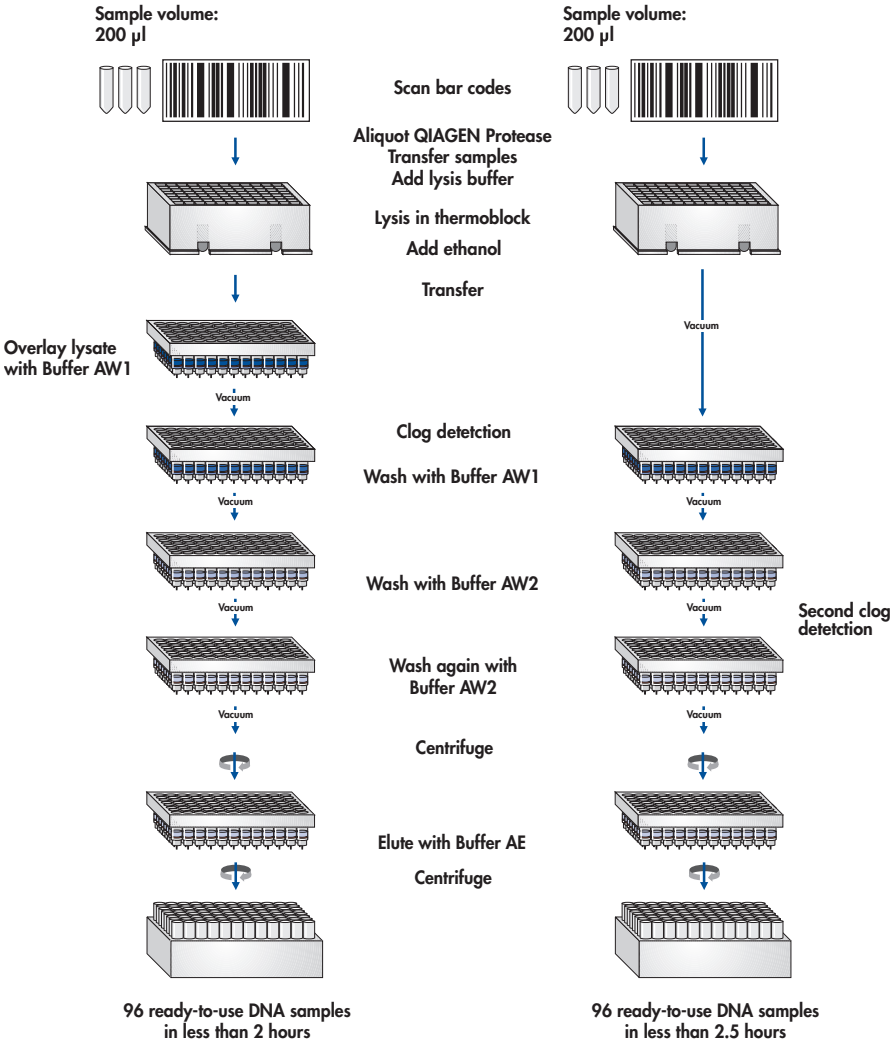
Repeatedly frozen and thawed samples, samples stored at room temperature for extended periods, or samples containing visible precipitates should not be used, as the QIAamp membranes are likely to become clogged. Although clots and clogs are detected by the BioRobot and do not disrupt runs, their presence will increase run time.

Purification of high molecular-weight DNA

To purify high-molecular-weight DNA, larger than the 50 kb achieved with QIAamp Kits, we recommend QIAGEN Genomic-tips (or ready-to-use Blood & Cell Culture DNA Kits, which contain QIAGEN Genomic-tips). QIAGEN Genomic-tips are available for purification of up to 500 µg genomic DNA from blood, cultured cells, tissue, yeast, and Gram-negative bacteria. The highly pure DNA prepared with QIAGEN Genomic-tips is up to 150 kb in size, and is suitable for use in Southern blotting, library construction, genomic mapping, and other demanding applications.

QIAamp 96 DNA Blood Procedure on the BioRobot 9604

QIAamp 96 DNA Buffy Coat Procedure on the BioRobot 9604



Equipment and Reagents to Be Supplied by User

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

- BioRobot 9604
- 96-well thermostat system for the BioRobot 9604
- Centrifuge 4-16 or 4-16K with Plate Rotor 2 x 96 (see page 24 for ordering information)
- Conductive 1.1 ml disposable filter tips from QIAGEN, for use with the BioRobot 9604
- Disposable gloves
- Ethanol (96–100%)*

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

Important Notes

QIAGEN Protease

QIAGEN Protease stock solution should be stored at 2–8°C or –20°C. Reconstitute by adding 10.2 ml protease solvent* to each vial containing lyophilized QIAGEN Protease. After the protease has been reconstituted, aliquot 8 x 1.25 ml into the 2 ml QIAGEN Protease tubes. Close the caps and transfer 4 tubes into positions A5–A8 of the 96-well thermostat on the BioRobot 9604. The remaining 4 tubes should be stored at –20°C until further use. Storage at –20°C will prolong the life of QIAGEN Protease, but repeated freezing and thawing should be avoided.

Note: After the run there may be some QIAGEN Protease left in the 2 ml tubes. All remainders should be collected and stored at 4°C for use during the next run. Once dissolved, QIAGEN Protease is stable for up to 2 months when stored at 4°C. Use only the tubes provided with the kit.

Buffer AL

Buffer AL is supplied as a single reagent in a stock bottle (store at 15–25°C). Mix by shaking the bottle carefully before use, avoiding extensive foaming. Distribute 6 ml (for the blood protocol) or 10 ml (for the buffy coat protocol) of Buffer AL into each of four 15 ml tubes (provided) and remove any large air bubbles with a pipet tip. Do not fill more than the required number of tubes. Only use the tubes provided with the kit.

The volume of Buffer AL supplied (330 ml) is sufficient to isolate DNA from 12 x 96 blood samples. To isolate DNA from 12 x 96 buffy coat samples, a total of 440 ml Buffer AL is required and additional buffer must be purchased (see ordering information, page 25).

Buffer AW1 (green label)†

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

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

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

Sufficient Buffer AW1 is supplied for 12 runs of 96 samples. One bottle of reconstituted Buffer AW1 contains enough wash buffer for two runs of 96 samples each. 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 25).

* Contains sodium azide as a preservative.

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

Buffer AW2 (red label)

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

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

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

Sufficient Buffer AW2 is supplied for 12 runs of 96 samples. 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 25).

Buffer AE*

For one run of 96 whole blood samples, 4 x 5 ml tubes of Buffer AE are required. For one run of 96 buffy coat samples, 4 x 10 ml tubes of Buffer AE are required. Even if fewer than 96 samples are prepared at a time, it is still necessary to attach all 4 Buffer AE tubes containing 5 or 10 ml of buffer each. If runs of fewer than 96 samples are performed, or if DNA is isolated from buffy coat samples, additional Buffer AE must be purchased (see ordering information, page 25).

Reuse of S-Blocks

Fourteen S-Blocks are supplied per kit. One S-Block is required per run of 96 samples for lysis in the initial part of the protocol. S-Blocks used for this purpose should be discarded after use. The two remaining S-Blocks are used as supports for the QIAamp 96 plate during centrifugation. They collect flow-through and are intended for repeated use. To avoid cross-contamination, rinse thoroughly in tap water, incubate for 1 minute at room temperature in 0.4 M HCl,† empty the block, and wash thoroughly with distilled water before reusing. Used S-Blocks can also be autoclaved after washing. Additional S-Blocks can be ordered separately (see ordering information, page 24).

* Contains sodium azide as a preservative. This has no effect on downstream assays.

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

Adhesive tapes

Two kinds of adhesive tape are provided. AirPore Tape is used during the second centrifugation step to prevent aerosol cross-contamination. The other tape pad is used to seal the unused wells of the QIAamp 96 plate if fewer than 96 samples are to be processed in a run. It should not be used during centrifugation steps.

If you wish to reuse plates containing unused wells, label the used wells of the QIAamp 96 plate using a waterproof marker pen. Cover the unused wells with tape from the tape pad and store the QIAamp 96 plate in the blister pack it was supplied in. Before starting the next run, remove the tape and cover the previously used wells with fresh tape.

Centrifugation

Centrifugation of QIAamp 96 plates is performed at 6000 rpm (5788 x *g*). The speed limit of the centrifuge is programmed so that the required g-force will not be exceeded. All centrifugation steps are carried out at room temperature.

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

Abbreviated instructions for using the Centrifuge 4-16

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

   Entering the rotor number automatically sets time and speed limits for that rotor, thus eliminating the danger of the centrifuge running too fast.
3. **Select "Speed" by turning the knob. Press the knob and by turning it again, set the speed to "6000". Confirm entry by pressing the knob.**

   The corresponding relative centrifugal force (RCF) is calculated from the rotor number and speed and appears automatically in the RCF field. It is also possible to enter the RCF value "5788 x *g*" manually in the RCF field after selecting "RCF" in the same way.
4. **Select "Time" by turning the knob. Press once and by turning the knob again, set the time as recommended in the protocol step. Confirm entry by pressing the knob.**
5. **Open the lid, place the 96-well plates with the metal carriers in the buckets, and close the lid.**

   The start and lid keys light up.

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

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

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

   All preset parameters remain after a run has finished.

Protocol: Purification of DNA from Whole Blood

Important point before starting

- Before beginning the procedure, read "Important Notes" on pages 12–15.

Things to do before starting

- Equilibrate up to 96 whole blood samples to room temperature (15–25°C).
- Orient samples in the sample identification system (SIS) racks so that the bar codes face the bar code reader. Bar code labels should be stuck to the sample tubes such that the bar code lines are horizontal.
- Check that Buffers AL, AW1, and AW2 have been prepared according to the instructions on pages 12–13.

Procedure

1. **Ensure that the BioRobot 9604 is switched on.**

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

   Note: The BioRobot 9604 should be left on at all times.

2. **Ensure that the high-speed pipetting system is switched on.**
3. **Switch on the computer and monitor.**
4. **Launch QIAsoft 3.0 if necessary.**

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

   The computer controlling the BioRobot is normally set to launch QIAsoft 3.0 upon startup, but this setting may have been changed.

5. **Start the Execute environment by pressing "Start" in the QIAsoft Main Menu, if necessary.**

   By default, QIAsoft 3.0 is configured to start the Execute environment automatically, but this setting may have been changed.

6. **Select the QIAamp 96 DNA Blood Purification Protocol using the protocol button in the Execute environment toolbar.**
7. **Click "RUN" to start the QIAamp 96 DNA Blood Purification Protocol.**

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

Protocol: Purification of DNA from Buffy Coat

Important point before starting

- Before beginning the procedure, read "Important Notes" on pages 12–15.

Things to do before starting

- Equilibrate up to 96 buffy coat samples to room temperature (15–25°C).
- Orient samples in the sample identification system (SIS) racks so that the bar codes face the bar code reader. Bar code labels should be stuck to the sample tubes such that the bar code lines are horizontal.
- Check that Buffers AL, AW1, and AW2 have been prepared according to the instructions on pages 12–13.

Procedure

1. **Ensure that the BioRobot 9604 is switched on.**

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

   Note: The BioRobot 9604 should be left on at all times.
2. **Ensure that the high-speed pipetting system is switched on.**
3. **Switch on the computer and monitor.**
4. **Launch QIAsoft 3.0 if necessary.**

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

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

   By default, QIAsoft 3.0 is configured to start the Execute environment automatically, but this setting may have been changed.
6. **Select the QIAamp 96 DNA Buffy Coat Purification Protocol using the protocol button in the Execute environment toolbar.**
7. **Click "Run" to start the QIAamp 96 DNA Buffy Coat Purification Protocol.**

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 molecular biology applications (see back cover for contact information).

	Comments and suggestions
General handling	
a) Some bar codes not identified	1. Sample tubes were not positioned correctly in the sample identification system racks. Turn the tubes so that the bar codes face the bar code reader. Scan the sample tubes again and continue with the run once all samples have been correctly identified. 2. Bar code labels should be stuck to the sample tubes so that the bar code lines are horizontal. If some bar code labels were incorrectly oriented, remove the unidentified tubes from the sample identification system rack and enter their identification codes into the report file either manually or using the hand-held bar code scanner. Put the sample tubes back in the sample identification system rack and continue with the protocol. 3. Check that the type of bar code used can be read by the QIAsoft Operating System (refer to the BioRobot manual for a list of bar code systems that QIAsoft 3.0 can interpret). Remove the unidentified tubes from the sample identification system rack and manually enter their identification codes into the report file. Replace the sample tubes in the sample identification system rack and continue with the protocol.

	Comments and suggestions
b) Clogged wells in the QIAamp 96 plate	Problems with starting material: 1. Blood has been stored at room temperature or frozen for extended periods, and precipitates have formed. Repeat the procedure using fresh blood. 2. White blood cell count too high. Dilute samples at least 1:1 with PBS* and repeat the procedure. Insufficient lysis: 1. Buffer AE used instead of Buffer AL. Repeat the procedure with fresh samples. 2. 96-well thermostat system was not switched on before distribution of Buffer AL. Repeat the procedure with fresh samples. Insufficient vacuum: 1. If fewer than 96 samples are purified in a single QIAamp 96 plate, ensure that unused wells in the plate are sealed with tape. 2. Ensure that a normal tape sheet was used to seal unused wells in the QIAamp 96 plate. Do not use AirPore tape for this purpose as it will allow air to pass through, reducing vacuum pressure. If AirPore tape was used, replace it with a normal tape sheet. Continue with the purification if possible or repeat the purification with new samples. 3. Check that the vacuum trap has not overflowed. If this is the case, the filter between the vacuum pump and the vacuum trap will be wet. Change the filter, empty the vacuum trap, and repeat the purification.

* 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.

	Comments and suggestions
Colored residues remain on the QIAamp 96 plate after washing	
a) Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct amount of ethanol. Repeat the purification procedure with new samples.
b) 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. Optimize the procedure by reducing the starting volume of blood used. Performing extra washes with Buffer AW2 may also be helpful (see ordering information, page 25).
Little or no DNA in eluates	
a) Cassettes not fitted to the peristaltic pump	Fit the cassettes and flush the tubing with 50 ml system liquid to remove air bubbles. Repeat the purification procedure with new samples.
b) Buffer bottles not connected	Connect the buffer bottles to the color-coded adapters and repeat the purification with new samples.
c) pH of water 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.
d) Some buffers have run out, so that no DNA was purified from the last samples in a run	At the end of a 96-sample run, if any of the buffers, QIAGEN Protease, or ethanol have run out, inadequate amounts were supplied at the start of the run. Repeat the purification, increasing the volume of the relevant solution.
A_{260}/A_{280} ratio for purified nucleic acids is low	
a) Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct amount of ethanol. Repeat the purification procedure with new samples.

	Comments and suggestions
b) 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. Optimize the procedure by reducing the starting volume of blood used. Performing extra washes with Buffer AW2 may also be helpful (see ordering information, page 25).
DNA does not perform well in downstream applications	
a) Not enough DNA in sample	Increase the amount of eluate added to the reaction, if possible. If necessary, concentrate the DNA under vacuum, or increase the amount of sample used, and repeat the purification procedure.
b) Too much DNA used in downstream application	Reduce the amount of eluate added to the downstream application (excess DNA can inhibit some enzymatic reactions).
c) Purified DNA contaminated with inhibitory substances	Check "A_{260}/A_{280} for purified nucleic acids is low" for possible reasons. Bring the eluate volume to 200 µl if necessary, and repeat the purification procedure.
d) DNA contaminated with RNA	Add 20 µl RNase A (20 mg/ml) to the eluate and incubate for 10 minutes at room temperature. Repeat the purification procedure as follows: Add 200 µl Buffer AL to the eluate and mix by vortexing. Transfer the sample to an S-block and then follow the protocol from the step "Distribute 250 µl ethanol from reagent slot E1 to samples on the thermostat slot."

	Comments and suggestions
e) Centrifuge was set to 4°C instead of 40°C	To develop the heat required to evaporate the ethanol, centrifuge at room temperature. Residual ethanol may inhibit downstream enzymatic reactions and must be removed by evaporation prior to elution. When using the Centrifuge 4-16K, set the centrifugation temperature to 40°C. Transfer the eluates into new 14 ml round-bottomed sample tubes and place in the sample identification system racks. Repeat the purification procedure to remove re sidual ethanol.
f) 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. Optimize the procedure by reducing the starting volume of blood used. Performing extra washes with Buffer AW2 may also be helpful (see ordering information, page 25).
White precipitate in Buffer AL	
A white precipitate may form after storage at low temperature or prolonged storage	Dissolve any precipitate by incubating Buffer AL at 70°C.

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. To measure only DNA, a fluorometer must be used.

Elution Buffer AE contains the preservative sodium azide, which shows absorbance at 260 nm. Therefore, when quantifying DNA in the eluate by absorbance measurement at 260 nm, when determining DNA purity in the eluate by absorbance measurements at 260 nm and 280 nm, or when scanning absorbance in the range between 220 nm and 250 nm, ensure that the blank contains the same concentration of sodium azide as the eluate. For example, if preparing eluate for absorbance measurements by diluting 50 µl eluate with 100 µl water, then prepare the blank by diluting 50 µl Buffer AE with 100 µl water. Use fresh, distilled water for the dilutions.

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 µl 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 µg 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 9604 Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Elution Microtubes CL (maximum elution volume 0.4 ml), Caps	965162
BioRobot 9604*		Inquire
Disposable Filter Tips, 1100 µl (960)	Conducting disposable filter-tips, pack of 960	9012598
QIAGEN 96-Well Centrifugation System		
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81300†
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (120 V, 60 Hz)	81310‡
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (220 V, 50 Hz)	81320¶
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81400†
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (120 V, 60 Hz)	81410‡
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81420¶
Plate Rotor 2 x 96**	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031

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

Ordering Information

Product	Contents	Cat. No.
Accessories		
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 cap strips	19588
Buffer AL	216 ml Lysis Buffer	19075
Buffer AE	240 ml Elution Buffer	19077
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Related products		
QIAamp 96 DNA Swab BioRobot Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Proteinase K, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Elution Microtubes (maximum volume 0.4 ml), Caps	965842
QIAamp DNA Blood BioRobot MDx Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers; QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Tape Pad	965152

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

Notes

www.qiagen.com

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

QIAGEN