Fourth Edition February 2015

QIAamp® DNA Blood Midi/Maxi Handbook

For large-scale genomic and viral DNA purification from whole blood, plasma, serum, body fluids, and lymphocytes

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

QIAamp DNA Blood Midi Kit	(20)	(100)
Catalog no.	**51183**	**51185**
Number of preps	**20**	**100**
QIAamp Midi Spin Columns	20	100
Collection Tubes (15 ml)	20	100
Buffer AL*	2 x 33 ml	330 ml
Buffer AW1* (concentrate)	19 ml	98 ml
Buffer AW2 (concentrate)	17 ml	81 ml
Buffer AE	15 ml	60 ml
QIAGEN® Protease	1 vial†	4 vials‡
Selection Guide	1	1

QIAamp DNA Blood Maxi Kit	(10)	(50)
Catalog no.	**51192**	**51194**
Number of preps	**10**	**50**
QIAamp Maxi Spin Columns	10	50
Collection Tubes (50 ml)	10	50
Buffer AL*	126 ml	2 x 330 ml
Buffer AW1* (concentrate)	27 ml	151 ml
Buffer AW2 (concentrate)	17 ml	81 ml
Buffer AE	2 x 15 ml	128 ml
QIAGEN Protease	1 vial‡	5 vials‡
Selection Guide	1	1

* Not compatible with disinfecting agents containing bleach. Contains a chaotropic salt. Take appropriate safety measures, and see page 5 for further safety information.

† Resuspension volume 4.4 ml

‡ Resuspension volume 5.5 ml

Storage

QIAamp Midi spin columns and buffers can be stored dry at room temperature (15–25°C). Upon arrival, store QIAamp Maxi spin columns at 2–8°C. All other QIAamp Maxi components can be stored at 2–8°C or at room temperature. Kits can be stored under these conditions for up to 1 year without showing any reduction in performance. For longer periods, storing the QIAamp DNA Blood Midi and Maxi Kits at 2–8°C is recommended.

Lyophilized QIAGEN Protease can be stored at room temperature for up to 1 year without any reduction in performance. For storage longer than 1 year or if ambient temperatures constantly exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

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 –30 to –15°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into usable aliquots and storage at –30 to –15°C is recommended.

Intended Use

QIAamp DNA Blood Kits are intended for molecular biology applications. These products are 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.

Safety Information

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

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The sample-preparation waste contains guanidine hydrochloride from Buffers AL and AW1, 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAamp DNA Blood Midi Kit and the QIAamp DNA Blood Maxi Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

QIAamp DNA Blood Midi and Maxi Kits provide the fastest and easiest way to purify total DNA for reliable PCR and Southern blotting. Total DNA (e.g., genomic, viral, mitochondrial) can be purified from up to 0.3–2 ml and 3–10 ml, respectively, of whole blood, plasma, serum, buffy coat, bone marrow, other body fluids, and lymphocytes.

The simple QIAamp DNA Blood Midi and Maxi procedures yield pure DNA ready for direct restriction digestion or amplification. With a suitable centrifuge rotor, eight samples can be prepared simultaneously in about 1.5 hours with approximately 30 minutes of hands-on time. Using the vacuum protocols minimizes manual handling and speeds up the entire process, enabling preparation of up to 192 samples in 8 hours. QIAamp DNA Blood Midi and Maxi Kits are suitable for use with whole blood that has been treated with EDTA, citrate, or heparin, and samples may be fresh or frozen. Separation of leukocytes is not necessary. The procedures require no phenol/chloroform extraction or alcohol precipitation and involve minimal handling. DNA is eluted in Buffer AE or water and is ready for direct addition to PCR or other enzymatic reactions, or it can be safely stored at –30 to –15°C for later use. The purified DNA is virtually free of protein, nucleases, and other contaminants or inhibitors of downstream applications. DNA purified using QIAamp DNA Blood Midi and Maxi Kits ranges in size up to 50 kb, with fragments of approximately 30 kb predominating. DNA of this length is suitable for use in restriction digestion and Southern blotting or related applications.

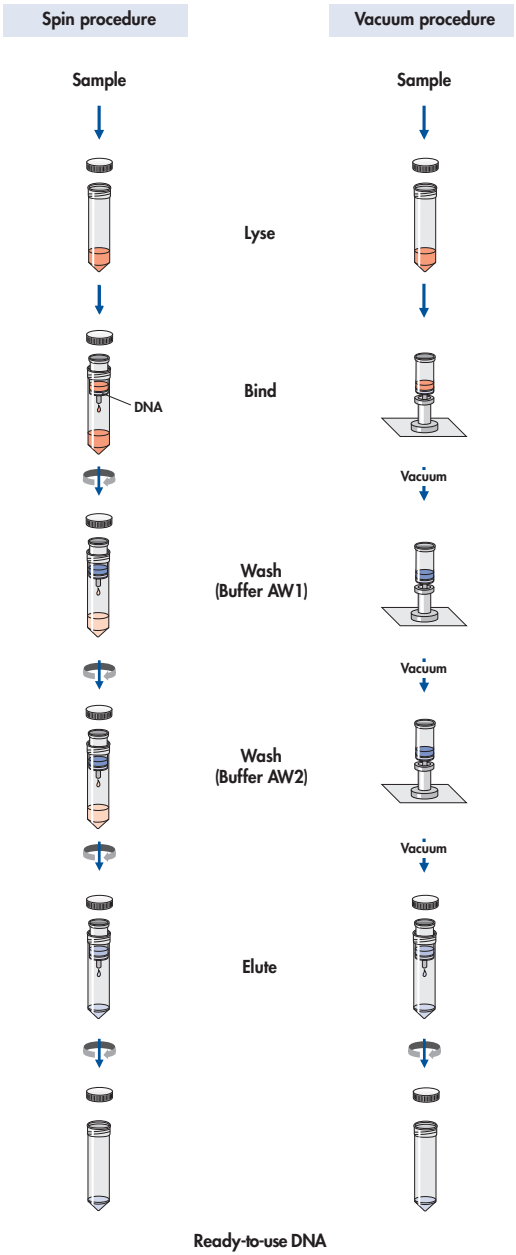
The QIAamp Midi/Maxi procedure

QIAamp DNA Blood Midi and Maxi Kits are intended for fast and efficient purification of total DNA (e.g., genomic, viral, mitochondrial) from medium to large volumes of human blood from a healthy donor (see flowcharts, page 8). After lysis, the lysate is loaded onto the QIAamp spin column. DNA binds to the QIAamp membrane while impurities are effectively washed away in two centrifugation or vacuum steps. Finally, ready-to-use DNA can be eluted using two different elution modes (see "Elution of pure nucleic acids", page 11).

Sample volumes

The QIAamp DNA Blood Midi and Maxi procedures have been adapted for large sample volumes of 0.3–2 ml and 3–10 ml, respectively. The Midi procedure is optimized for use with 2 ml sample volumes, but samples as small as 300 µl can be prepared. In this case sample volumes should be adjusted to 1 ml with PBS. Samples >1 ml should be adjusted to 2 ml with PBS, and the lysed sample should be loaded onto the QIAamp Midi column in two steps, loading half of the lysate in each step. Samples with volumes less than 300 µl should be processed using the QIAamp DNA Blood Mini Kit.

The QIAamp DNA Blood Midi and Maxi Spin and Vacuum Procedures



If the initial sample volume exceeds 2 ml we recommend using the QIAamp DNA Blood Maxi Kit. The Maxi procedure is optimized for use with 10 ml sample volumes, but samples as small as 3 ml can be prepared. In this case sample volumes should be adjusted to 5 ml with PBS. Samples >5 ml should be adjusted to 10 ml with PBS, and the lysed sample should be loaded onto the QIAamp Maxi column in two steps, loading half of the lysate in each step.

Note: All QIAamp buffers and QIAGEN Protease can be purchased separately (see ordering information on page 38). QIAGEN Protease (cat. nos. 19157 and 19155) is suitable for genomic DNA preparation from blood, cells, and body fluids in conjunction with Buffer AL.

Cell number

To achieve optimal binding conditions, it is important to avoid loading viscous lysates onto the QIAamp columns. Lysate viscosity will increase proportionally with the number of cells present in the sample. The cell number should be determined before starting. No more than 2 x 10^7 cells should be used with QIAamp DNA Blood Midi columns. If your sample contains more than 2 x 10^7 cells and up to 1 x 10^8 cells, use the QIAamp DNA Blood Maxi Kit to prepare genomic DNA. The QIAamp DNA Blood Maxi Kit can be used to extract genomic DNA from blood containing as little as 2.5 x 10^5 leukocytes per milliliter and up to 1 x 10^7 cells per milliliter (see Table 1, page 10).

If you wish to prepare genomic DNA from blood containing more than 1 x 10^8 cells, we suggest using doubled volumes of Buffer AL and ethanol, as this will reduce the viscosity of the lysate, and loading the lysate in repeated steps. Also, it is a good idea to increase the volume of Buffer AE used for elution when preparing DNA from more than 1 x 10^8 cells (see "Elution of pure nucleic acids", page 11). Yields in excess of 1 mg have been achieved from 2.5 x 10^8 cells although these yields are no longer quantitative since the amounts exceed the limits of linearity of the system.

In some cases it may be desired to process a large volume of blood to prepare DNA from a very small number of cells, for example, to monitor the success of therapy after treatment. Here, the QIAamp DNA Blood Maxi Kit should be used to avoid repeated loading steps. Typically, a sample containing 2.5 x 10^5 cells per milliliter will yield approximately 16 µg of genomic DNA using the QIAamp Maxi columns.

Table 1. DNA yields from whole blood with different cell densities

Leukocytes per ml	QIAamp DNA Blood Kits			
	Midi		Maxi	
	µg	%	µg	%
2.5×10^5	3.0	90.9	15.8	95.8
1.0×10^6	11.8	89.4	60.4	91.5
5.0×10^6	62.4	94.5	312.0	94.5
1.0×10^7	116.3	88.1	624.6	94.6

Genomic DNA was purified from 2 ml (Midi) and 10 ml (Maxi) of whole blood and eluted in 300 µl and 1 ml, respectively, of Buffer AE. Percentage figures are calculated on the basis of theoretical yields.

QIAGEN Protease

QIAamp DNA Blood Midi and Maxi Kits contain QIAGEN Protease. Intensive research has shown that QIAGEN Protease is the optimal enzyme for use with Buffer AL provided for sample lysis in the QIAamp DNA Blood Midi and Maxi Kits. QIAGEN Protease is completely free of DNase and RNase activities.

If you are using the QIAamp DNA Blood Midi or Maxi Kit for a sample that requires a modified protocol, please contact our Technical Service Group for information on whether your lysis conditions are compatible with QIAGEN Protease. When >8 mM EDTA is used in conjunction with >0.5% SDS, QIAGEN Protease activity will decrease. For samples which require an SDS-containing lysis buffer or contain high levels of EDTA, Proteinase K is recommended.

Adsorption to the QIAamp membrane

The lysate must be adjusted to binding conditions before loading onto the QIAamp Midi column or QIAamp Maxi column. Optimal binding conditions are achieved by thoroughly mixing the lysed sample with the appropriate quantity of ethanol. To prevent DNA loss due to inefficient binding, monitor the cell number present in a given volume of sample (see "Cell number", page 9). If the initial sample volume is larger than 1 ml or 5 ml, respectively, it will be necessary to load the lysate onto the QIAamp Midi column or QIAamp Maxi column in several steps. DNA is adsorbed onto the QIAamp silica membrane during a brief centrifugation or vacuum step. Salt and pH conditions in the lysate ensure that protein and other contaminants which may inhibit PCR and other enzymatic reactions are not retained on the membrane.

Removal of residual contaminants

DNA bound to the QIAamp membrane is washed in two centrifugation or vacuum steps. The use of two different wash buffers, AW1 and AW2, has significantly improved the purity of the eluted DNA. Wash conditions ensure complete removal of any residual contaminants without any effect on DNA binding.

Elution of pure nucleic acids

Purified DNA is eluted from the QIAamp Midi and Maxi columns in a concentrated form in either Buffer AE or water. Elution buffer should be equilibrated to room temperature (15–25°C) before applying to the column.

Two elution modes are possible: either elution with two separate volumes of elution buffer or elution with one volume of buffer and re-elution with the first eluate. Elution with two separate volumes increases DNA yield while re-elution increases DNA concentration (see Table 2A, page 12).

With QIAamp Maxi columns, elution volumes larger than 1 ml increase DNA yield but reduce the concentration. Conversely, elution with volumes of less than 1 ml increases the final DNA concentration in the eluate significantly, but reduces overall yield (see Table 2B, page 12). Similar results are achieved using QIAamp Midi columns.

If the total cell number exceeds 1×10^8, we recommend increasing the volume of Buffer AE up to 2 ml (QIAamp DNA Blood Maxi Kit). Conversely, if the cell number is less than 2.5×10^5, the volume of Buffer AE should be reduced. We therefore suggest adjusting elution volumes according to the cell number and the researcher's individual requirements.

Note: Do not reduce the elution volume below 100 µl when using QIAamp Midi columns or below 500 µl when using QIAamp Maxi columns.

Elution with buffer of pH less than 9.0 may reduce DNA yield. Since EDTA inhibits PCR reactions, TE buffer is not recommended for elution.

The eluted DNA ranges in size up to 50 kb (predominant fragment size 20–30 kb), and is suitable for direct use in PCR, restriction digestion, and Southern blotting applications.

Table 2A. Elution modes

Elution mode	DNA yield		DNA concentration
	µg	%	µg/µl
2 x 1000 µl	373.3	100	0.20
1000 µl re-eluted	331.4	88.8	0.41

Table 2B. Elution volumes

Elution volume	DNA yield		DNA concentration
	µg	%	µg/µl
800 µl re-eluted	303.6	81.3	0.47
1000 µl re-eluted	331.4	88.8	0.41
1200 µl re-eluted	347.4	93.1	0.35

Effect of elution modes (Table 2A) and volumes (Table 2B) on DNA yields and concentration using QIAamp Maxi columns with 10 ml of blood containing 6 x 10^6 white blood cells per milliliter. Values represent mean values from 16 DNA preparations. Percentage yields in Table 2B are calculated based on the values in Table 2A.

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 safety data sheets (SDSs), available from the product supplier.

- Centrifuge capable of attaining 4500 x *g* (5000 rpm), equipped with a swing-out rotor and buckets that can accommodate 15 ml (Midi) or 50 ml (Maxi) centrifuge tubes
- Water bath, heated to 70°C
- Phosphate-buffered saline (PBS) may be required for some samples
- Ethanol (96–100%)*

Spin protocols

- 15 ml centrifuge tubes (Midi) or 50 ml centrifuge tubes (Maxi)

Vacuum protocols

- QIAvac 24 Plus vacuum manifold (cat. no. 19413) or equivalent
- QIAvac Connecting System (cat. no. 19419) or equivalent
- Optional: VacConnectors (cat. no. 19407)
- Vacuum Pump (cat. no. 84000, 84010, or 84020) or equivalent pump capable of producing a vacuum of –800 to –900 mbar
- Additional Buffer AW1 (cat. no. 19081) and Buffer AW2 (cat. no. 19072)

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

Important Notes

Centrifugation

Centrifugation of QIAamp Midi or Maxi Columns is performed in 15 ml or 50 ml centrifuge tubes, at 1850 x *g* (3000 rpm) and 4500 x *g* (5000 rpm) respectively. Please ensure that your centrifuge is equipped with a swing-out rotor able to reach the required speed and centrifugal force. Do not use a fixed-angle rotor. All centrifugation steps are carried out at room temperature (15–25°C).

Note: When placing the centrifuge tubes into the rotor buckets make sure that lids of the tubes cannot touch each other during centrifugation. Failure to do so may cause tubes to break.

Note: Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.

Processing QIAamp Midi and QIAamp Maxi spin columns on the QIAvac 24 Plus

QIAamp Midi and Maxi spin columns can be processed using the QIAvac 24 Plus. Due to the large solution volumes used, the QIAvac Connecting System is required (Figure 1).

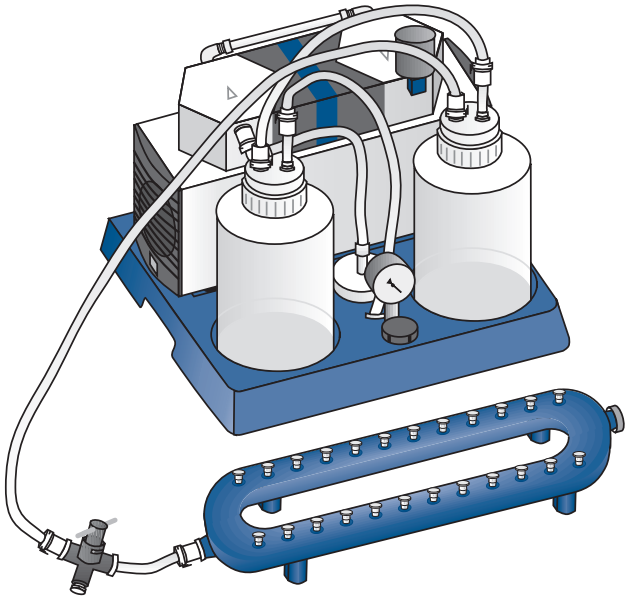


Figure 1. QIAvac 24 Plus, QIAvac Connecting System, and Vacuum Pump.

QIAamp Midi and QIAamp Maxi spin columns are processed on the QIAvac 24 Plus using VacConnectors (optional) and VacValves. VacValves are inserted directly into the luer slots of the QIAvac 24 Plus manifold (Figure 2) and ensure a steady flow rate, facilitating parallel processing of samples of different natures, volumes, or viscosities. VacConnectors are disposable connectors that fit between QIAamp spin columns and VacValves. They prevent direct contact between the spin column and VacValve during purification, thereby avoiding any cross-contamination between samples.

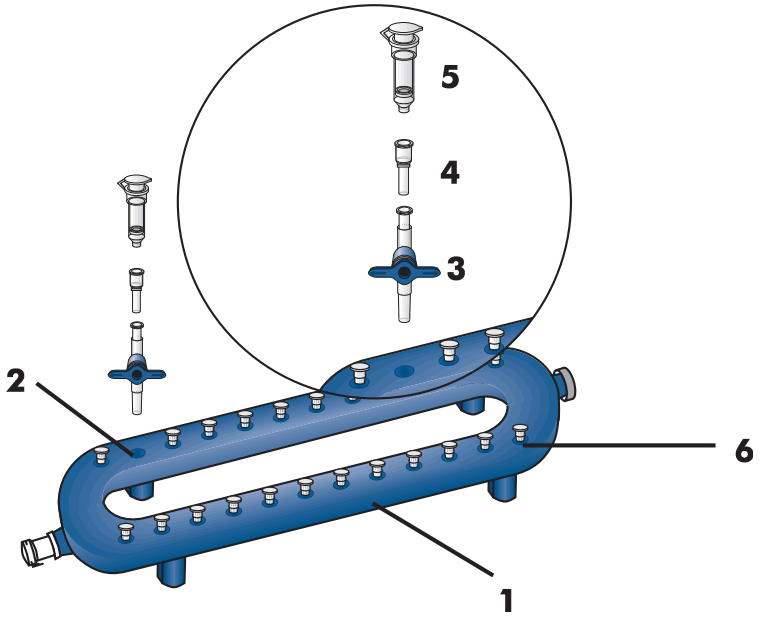


Figure 2. Setting up the QIAvac 24 Plus with QIAamp spin columns using VacValves and VacConnectors.

1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus
3. VacValve*
4. VacConnector (optional)†
5. QIAamp spin column (QIAamp Mini spin column shown)‡
6. Luer slot closed with luer plug

* Not included with the QIAvac 24 Plus. Supplied with QIAvac Connecting System or available separately (cat. no. 19408).

† Not included with the QIAvac 24 Plus. Cat. no. 19407

‡ Not included with the QIAvac 24 Plus. Included in QIAamp DNA Blood Kits.

Procedure

1. **Connect the QIAvac 24 Plus to the vacuum source using the QIAvac Connecting System (for more details, see the *QIAvac 24 Plus Handbook*).**
2. **Insert a VacValve into each luer slot of the QIAvac 24 Plus that is to be used. Close unused luer slots with luer plugs or close the inserted VacValve.**
3. **Optional: Insert a VacConnector into each VacValve.**

   Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.

   VacConnectors prevent direct contact between the spin column and VacValves during purification, avoiding cross-contamination between samples.
4. **Place a QIAamp spin column into each VacConnector (optional) or directly into the VacValve on the manifold.**
5. **For nucleic acid purification, follow instructions in the vacuum protocols.**

   Each VacValve can be closed individually when the sample is completely drawn through the spin column, allowing parallel processing of samples of different volumes or viscosities.

   To ensure that a consistent, even vacuum is applied during protocol steps, the vacuum pump should be switched on during the entire vacuum procedure, and the main vacuum valve should be closed between steps (see Figure 3).
6. **After processing of samples, clean and decontaminate the QIAvac 24 Plus (see "Cleaning and Decontaminating the QIAvac 24 Plus" in the *QIAvac 24 Plus Handbook*).**

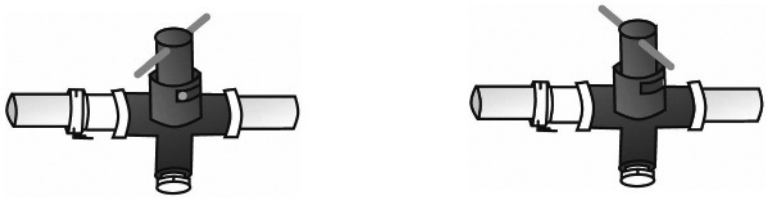


Figure 3. Main vacuum valve in the closed and open position.

Preparation of reagents

QIAGEN Protease stock solution (store at 2–8°C or –30 to –15°C)

QIAamp DNA Blood Midi Kits: Pipet 4.4 ml distilled water into the vial of lyophilized QIAGEN Protease provided in the QIAamp DNA Blood Midi Kit (20), or 5.5 ml distilled water into a vial of lyophilized QIAGEN Protease provided in the QIAamp DNA Blood Midi Kit (100), as indicated on the labels.

QIAamp DNA Blood Maxi Kits: Pipet 5.5 ml distilled water into a vial of lyophilized QIAGEN Protease, as indicated on the label.

Once dissolved, QIAGEN Protease is stable for 2 months when stored at 2–8°C. Storage at –30 to –15°C will prolong the life of QIAGEN Protease, but repeated freezing and thawing should be avoided. For storage at –30 to –15°C, prepare and freeze QIAGEN Protease stock solution in aliquots.

Buffer AL* (store at room temperature, 15–25°C)

Mix Buffer AL thoroughly by shaking before use. Buffer AL is stable for at least 1 year when stored at room temperature.

Buffer AW1* (store at room temperature, 15–25°C)

Buffer AW1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle.

Buffer AW1 is stable for 1 year when stored closed at room temperature.

Buffer AW2 (store at room temperature, 15–25°C)

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle.

Buffer AW2 is stable for 1 year when stored closed at room temperature.

* Not compatible with disinfecting agents containing bleach. See page 5 for further safety information.

Optimized handling for higher throughputs

Several factors can be optimized for faster processing of large numbers of samples.

- To dispense reagents, use bottle-top dispensers (e.g., Brand Bottle Top Dispenser Seripettor® 2.5–25 ml, cat. no. 4720 150, www.brand.de) and/or hand dispensers (e.g., Eppendorf® Multipette plus, cat. no. 4981 000.019 with Eppendorf Combitips® plus 10 ml, cat. no. 0030 069.269, Eppendorf Combitips plus 25 ml, cat. no. 0030 069.293, 25 ml Adapter Standard, cat. no. 0030 069.528, www.eppendorf.com).
- Invert multiple tubes simultaneously by clamping tubes into a rack using another, empty rack, grasping both racks, and inverting them together.
- Use a multi-tube vortexer or a vortexer equipped with a platform head.
- Use a dedicated vacuum system suitable for QIAamp Midi and Maxi vacuum protocols (e.g., QIAvac 24 Plus with the QIAvac Connecting system) for handling the large solution volumes.
- Use a dedicated centrifuge equipped with a suitable rotor. We recommend the QIAGEN Centrifuge 4–16 or 4–16K (see www.qiagen.com for ordering information) or centrifuges with swing-out rotor (Sigma Laboratory Centrifuges, cat. no. 11150) and 4 buckets (Sigma Laboratory Centrifuges, cat. no. 13235). Each bucket holds 7 tubes (www.sigma-zentrifugen.de).

Protocol: Purification of DNA from Whole Blood using the QIAamp Blood Midi Kit (Spin Protocol)

This protocol is for purification of genomic DNA from up to 2 ml of whole blood.

Important points before starting

- Blue (marked with a ■) denotes values for 0.3–1 ml of whole blood; red (marked with a ▲) denotes values for 1–2 ml of whole blood.
- Do not use more than 2 x 10^7 white blood cells. For samples containing more than 2 x 10^7 white blood cells, use the QIAamp DNA Blood Maxi Kit.
- All centrifugation steps are carried out at room temperature (15–25°C). Do not use a fixed-angle rotor.
- For samples with low white blood cell counts (i.e., <20,000 genome equivalents), add carrier DNA at the start of the procedure. Since the carrier DNA will co-purify with the DNA from the blood, make sure that this will not interfere with any downstream analyses, such as PCR.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C) before starting.
- Prepare a 70°C water bath for use in step 4 of the protocol.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions (page 17).
- If a precipitate has formed in Buffer AL, redissolve by incubating at 56°C.

Procedure

1. **Pipet ■ 100 µl or ▲ 200 µl QIAGEN Protease into the bottom of a 15 ml centrifuge tube (not provided).**
2. **Add ■ 0.3–1 ml or ▲ 1–2 ml blood and mix briefly.**

   Bring the volume of the sample up to ■ 1 ml or ▲ 2 ml with PBS, if necessary, before adding to the centrifuge tube.

   Note: QIAGEN Protease (or proteinase K) can be added to samples that have already been dispensed into centrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.
3. **Add ■ 1.2 ml or ▲ 2.4 ml Buffer AL, and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min. Invert multiple tubes simultaneously by clamping them into a rack using another empty rack, grasping both racks, and inverting them together.**

   To ensure adequate lysis, the sample must be mixed thoroughly with Buffer AL to yield a homogenous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

4. **Incubate at 70°C for 10 min.**

   DNA yield reaches a maximum after lysis for 10 min at 70°C, but longer incubation times will not adversely affect yield.

5. **Add ■ 1 ml or ▲ 2 ml ethanol (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking.**

   To ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

   Note: Only use 96–100% ethanol. Other alcohols may result in reduced yield and purity. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

6. **Carefully transfer ■ all or ▲ one half of the solution from step 5 onto the QIAamp Midi column placed in a 15 ml centrifuge tube (provided), taking care not to moisten the rim. Close the cap and centrifuge at 1850 x *g* (3000 rpm) for 3 min. Proceed with ■ step 8 (skip step 7) or ▲ step 7.**

   ■ If the initial sample volume was ≤1 ml, apply all of the lysate to the QIAamp Midi column in the step above. Skip step 7 and go directly to step 8.

   If more convenient, lysate can be loaded onto the QIAamp Midi column up to the level of the top screw thread on the 15 ml centrifuge tube.

   If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

   Do not overtighten caps. If caps are tightened until they snap they may loosen during centrifugation and damage the centrifuge.

   Note: Always hold the closed QIAamp Midi columns in an upright position as liquid may pass through the ventilation slots on the rims of the columns even if caps are closed.

7. **▲ Remove the QIAamp Midi column, discard the filtrate, and place the QIAamp Midi column back into the 15 ml centrifuge tube. Load the remainder of the solution from step 5 onto the QIAamp Midi column. Close the cap and centrifuge again at 1850 x *g* (3000 rpm) for 3 min.**

   ■ This step is not necessary when starting with ≤1 ml blood. Proceed directly with step 8.

   Note: Wipe off any spillage from the thread of the 15 ml centrifuge tube before re-inserting the QIAamp Midi column.

   Do not wet the rim of the QIAamp Midi column. Close each column to avoid cross-contamination during centrifugation. If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

8. **Remove the QIAamp Midi column, discard the filtrate, and place the QIAamp Midi column back into the 15 ml centrifuge tube.**

   Note: Wipe off any spillage from the thread of the 15 ml centrifuge tube before re-inserting the QIAamp Midi column.

   If the filtrate is not removed, the nozzle of the QIAamp Midi column will be submerged in the filtrate, and washing efficacy will be reduced.

9. **Carefully, without moistening the rim, add 2 ml Buffer AW1 to the QIAamp Midi column. Close the cap and centrifuge at 4500 x *g* (5000 rpm) for 1 min.**

   Note: Do not discard the flow-through at this stage. Continue directly with step 10.

10. **Carefully, without moistening the rim, add 2 ml Buffer AW2 to the QIAamp Midi column. Close the cap and centrifuge at 4500 x *g* (5000 rpm) for 15 min.**

    Note: The increased centrifugation time should remove all traces of Buffer AW2 from the QIAamp Midi column before elution. If the centrifugal force is below 4000 x *g*, incubating the QIAamp Midi column for 10 min at 70°C in an incubator to evaporate residual ethanol is recommended. Residual ethanol in the eluate may cause inhibition of PCR leading to false-negative results.

11. **Place the QIAamp Midi column in a clean 15 ml centrifuge tube (provided), and discard the collection tube containing the filtrate.**

    Note: Use a wet tissue paper to wipe any spillage off the QIAamp Midi column before insertion into the 15 ml centrifuge tube.

12. **Pipet ■ 200 µl or ▲ 300 µl Buffer AE or distilled water, equilibrated to room temperature (15–25°C), directly onto the membrane of the QIAamp Midi column and close the cap. Incubate at room temperature for 5 min, and centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    For long-term storage of DNA, eluting in Buffer AE and storing in aliquots at –30 to –15°C is recommended since DNA is subject to acid hydrolysis if dissolved in water.

13. **For highly concentrated DNA, continue with step 13a. For maximum DNA yield, continue with step 13b.**

    Note: Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable. (For detailed information regarding elution efficiency, see Tables 2A and 2B on page 12.)

13a. **For maximum concentration: Reload the eluate (■ 200 µl or ▲ 300 µl) containing the DNA onto the membrane of the QIAamp Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    Note: Less than ■ 200 µl or ▲ 300 µl will be eluted from the column, but this has no effect on DNA yield.

13b. For maximum yield: Pipet ■ 200 µl or ▲ 300 µl fresh Buffer AE or distilled water, equilibrated to room temperature, onto the membrane of the QIAamp Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 2 min.

Note: Less than ■ 200 µl or ▲ 300 µl will be eluted from the column, but this has no effect on DNA yield.

Protocol: Purification of DNA from Whole Blood using the QIAamp Blood Maxi Kit (Spin Protocol)

This protocol is for purification of genomic DNA from up to 10 ml of whole blood.

Important points before starting

- Blue (marked with a ■) denotes values for 3–5 ml of whole blood; red (marked with a ▲) denotes values for 5–10 ml of whole blood.
- Do not use more than 1 x 10^8 white blood cells.
- All centrifugation steps are carried out at room temperature (15–25°C). Do not use a fixed-angle rotor.
- For samples with low white blood cell counts (i.e., <100,000 genome equivalents), add carrier DNA at the start of the procedure. Since the carrier DNA will co-purify with the DNA from the blood, make sure that this will not interfere with any downstream analyses, such as PCR.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C) before starting.
- Prepare a 70°C water bath for use in step 4 of the protocol.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions (page 17).
- If a precipitate has formed in Buffer AL, redissolve by incubating at 56°C.

Procedure

1. **Pipet 500 µl QIAGEN Protease into the bottom of a 50 ml centrifuge tube (not provided).**
2. **Add ■ 3–5 ml or ▲ 5–10 ml blood and mix briefly.**

   Bring the volume of the sample up to ■ 5 ml or ▲ 10 ml with PBS, if necessary, before adding to the centrifuge tube.

   Note: QIAGEN Protease (or proteinase K) can be added to samples that have already been dispensed into centrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.
3. **Add ■ 6 ml or ▲ 12 ml Buffer AL, and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min. Invert multiple tubes simultaneously by clamping them into a rack using another empty rack, grasping both racks, and inverting them together.**

   To ensure adequate lysis, the sample must be mixed thoroughly with Buffer AL to yield a homogenous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

4. **Incubate at 70°C for 10 min.**

   DNA yield reaches a maximum after lysis for 10 min at 70°C, but longer incubation times will not adversely affect yield.

5. **Add ■ 5 ml or ▲ 10 ml ethanol (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking.**

   To ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

   Note: Only use 96–100% ethanol. Other alcohols may result in reduced yield and purity. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

6. **Carefully transfer ■ all or ▲ one half of the solution from step 5 onto the QIAamp Maxi column placed in a 50 ml centrifuge tube (provided), taking care not to moisten the rim. Close the cap and centrifuge at 1850 x *g* (3000 rpm) for 3 min. Proceed with ■ step 8 (skip step 7) or ▲ step 7.**

   ■ If the initial sample volume was ≤5 ml, apply all of the lysate to the QIAamp Maxi column in the step above. Skip step 7 and go directly to step 8.

   If more convenient, lysate can be loaded onto the QIAamp Maxi column up to the level of the top screw thread on the 50 ml centrifuge tube.

   If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

   Do not overtighten caps. If caps are tightened until they snap they may loosen during centrifugation and damage the centrifuge.

   Note: Always hold the closed QIAamp Maxi columns in an upright position as liquid may pass through the ventilation slots on the rims of the columns even if caps are closed.

7. **▲ Remove the QIAamp Maxi column, discard the filtrate, and place the QIAamp Maxi column back into the 50 ml centrifuge tube. Load the remainder of the solution from step 5 onto the QIAamp Maxi column. Close the cap and centrifuge again at 1850 x *g* (3000 rpm) for 3 min.**

   ■ This step is not necessary when starting with ≤5 ml blood. Proceed directly with step 8.

   Note: Wipe off any spillage from the thread of the 50 ml centrifuge tube before re-inserting the QIAamp Maxi column.

   Do not wet the rim of the QIAamp Maxi column. Close each column to avoid cross-contamination during centrifugation. If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

8. **Remove the QIAamp Maxi column, discard the filtrate, and place the QIAamp Maxi column back into the 50 ml centrifuge tube.**

   Note: Wipe off any spillage from the thread of the 50 ml centrifuge tube before re-inserting the QIAamp Maxi column.

   If the filtrate is not removed, the nozzle of the QIAamp Maxi column will be submerged in the filtrate, and washing efficacy will be reduced.

9. **Carefully, without moistening the rim, add 5 ml Buffer AW1 to the QIAamp Maxi column. Close the cap and centrifuge at 4500 x *g* (5000 rpm) for 1 min.**

   Note: Do not discard the flow-through at this stage. Continue directly with step 10.

10. **Carefully, without moistening the rim, add 5 ml Buffer AW2 to the QIAamp Maxi column. Close the cap and centrifuge at 4500 x *g* (5000 rpm) for 15 min.**

    Note: The increased centrifugation time should remove all traces of Buffer AW2 from the QIAamp Maxi column before elution. If the centrifugal force is below 4000 x *g*, incubating the QIAamp Maxi column for 10 min at 70°C in an incubator to evaporate residual ethanol is recommended. Residual ethanol in the eluate may cause inhibition of PCR leading to false-negative results.

11. **Place the QIAamp Maxi column in a clean 50 ml centrifuge tube (provided), and discard the collection tube containing the filtrate.**

    Note: Use a wet tissue paper to wipe any spillage off the QIAamp Maxi column before insertion into the 50 ml centrifuge tube.

12. **Pipet ■ 600 µl or ▲ 1 ml Buffer AE or distilled water, equilibrated to room temperature (15–25°C), directly onto the membrane of the QIAamp Maxi column and close the cap. Incubate at room temperature for 5 min, and centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    For long-term storage of DNA, eluting in Buffer AE and storing in aliquots at –30 to –15°C is recommended since DNA is subject to acid hydrolysis if dissolved in water.

13. **For highly concentrated DNA, continue with step 13a. For maximum DNA yield, continue with step 13b.**

    Note: Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable. (For detailed information regarding elution efficiency, see Tables 2A and 2B on page 12.)

13a. **For maximum concentration: Reload the eluate (■ 600 µl or ▲ 1 ml) containing the DNA onto the membrane of the QIAamp Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 5 min.**

    Note: Less than ■ 600 µl or ▲ 1 ml will be eluted from the column, but this has no effect on DNA yield.

13b. For maximum yield: Pipet ■ 600 µl or ▲ 1 ml fresh Buffer AE or distilled water, equilibrated to room temperature, onto the membrane of the QIAamp Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 5 min.

Note: Less than ■ 600 µl or ▲ 1 ml will be eluted from the column, but this has no effect on DNA yield.

Protocol: Purification of DNA from Whole Blood using the QIAamp DNA Blood Midi Kit (Vacuum Protocol)

This protocol is for purification of genomic DNA from up to 2 ml of whole blood.

Important points before starting

- Blue (marked with a ■) denotes values for 0.3–1 ml of whole blood; red (marked with a ▲) denotes values for 1–2 ml of whole blood.
- Do not use more than 2 x 10^7 white blood cells. For samples containing more than 2 x 10^7 white blood cells, use the QIAamp DNA Blood Maxi Kit.
- To ensure that a consistent, even vacuum is applied during protocol steps, the vacuum pump should be switched on during the entire vacuum procedure, and the main vacuum valve should be closed between steps (see Figure 3 on page 16).
- All centrifugation steps are carried out at room temperature (15–25°C). Do not use a fixed-angle rotor.
- For samples with low white blood cell counts (i.e., <20,000 genome equivalents), add carrier DNA at the start of the procedure. Since the carrier DNA will co-purify with the DNA from the blood, make sure that this will not interfere with any downstream analyses, such as PCR.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C) before starting.
- Prepare a 70°C water bath for use in step 4 of the protocol.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions (page 17).
- If a precipitate has formed in Buffer AL, redissolve by incubating at 56°C.
- Set up the QIAvac 24 Plus as described in "Processing QIAamp Midi and QIAamp Maxi spin columns on the QIAvac 24 Plus" on pages 14–16.

Procedure

1. **Pipet ■ 100 µl or ▲ 200 µl QIAGEN Protease into the bottom of a 15 ml centrifuge tube (provided).**
2. **Add ■ 0.3–1 ml or ▲ 1–2 ml blood and mix briefly.**

   Bring the volume of the sample up to ■ 1 ml or ▲ 2 ml with PBS, if necessary, before adding to the centrifuge tube.

   Note: QIAGEN Protease (or proteinase K) can be added to samples that have already been dispensed into centrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

3. **Add ■ 1.2 ml or ▲ 2.4 ml Buffer AL, and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min. Invert multiple tubes simultaneously by clamping them into a rack using another empty rack, grasping both racks, and inverting them together.**

   To ensure adequate lysis, the sample must be mixed thoroughly with Buffer AL to yield a homogenous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

4. **Incubate at 70°C for 10 min.**

   DNA yield reaches a maximum after lysis for 10 min at 70°C, but longer incubation times will not adversely affect yield.

5. **Add ■ 1 ml or ▲ 2 ml ethanol (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking.**

   To ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

   Note: Only use 96–100% ethanol. Other alcohols may result in reduced yield and purity. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

6. **Insert the QIAamp Midi column into the VacConnector (optional) or directly into the VacValve on the vacuum manifold. Open the corresponding VacValve. Close the main vacuum valve and switch on the vacuum pump.**

   The 15 ml centrifuge tube can be saved for the centrifugation in step 12.

7. **Carefully transfer ■ all or ▲ one half of the solution from step 5 onto the QIAamp Midi column, taking care not to moisten the rim. Open the main vacuum valve. If sample flow rates differ, close the VacValves where the lysate has already passed through to ensure a consistent vacuum over the remaining columns. After all lysates have been drawn through the columns, close the main vacuum valve. Proceed with ■ step 9 (skip step 8) or ▲ step 8.**

   ■ If the initial sample volume was ≤1 ml, apply all of the lysate to the QIAamp Midi column in the step above. Skip step 8 and go directly to step 9.

8. **▲ Open the VacValve and load the remainder of the solution from step 5 onto the QIAamp Midi column, taking care not to moisten the rim. Open the main vacuum valve. If sample flow rates differ, close the VacValves where the lysate has already passed through to ensure a consistent vacuum over the remaining columns. After all lysates have been drawn through the columns, close the main vacuum valve.**

   ■ This step is not necessary when starting with ≤1 ml blood. Proceed directly with step 9.

9. **Open the VacValve carefully, and add 4 ml Buffer AW1 to the QIAamp Midi column, taking care not to moisten the rim. Open the main vacuum valve. After all of the Buffer AW1 has been drawn through the columns, close the main vacuum valve.**

   If sample flow rates differ, close the VacValves where Buffer AW1 has already passed through to ensure a consistent vacuum over the remaining columns.

10. **Open the VacValve, and add 4 ml Buffer AW2 to the QIAamp Midi column, taking care not to moisten the rim. Open the main vacuum valve. After all of the Buffer AW2 has been drawn through the columns close all VacValves.**

    If sample flow rates differ, close the VacValves where Buffer AW2 has already passed through to ensure a consistent vacuum over the remaining columns.

11. **To dry the membranes, open each individual VacValve for 10 s. Then open all VacValves and vacuum-dry the membranes for an additional 15 min.**

    Note: Residual ethanol in the eluate may cause inhibition of PCR, leading to false-negative results. Drying the membrane in this step helps to prevent this.

12. **Switch off the vacuum pump, and place the QIAamp Midi column in a 15 ml centrifuge tube (saved from step 6).**

    Note: Use a wet tissue paper to wipe any spillage off the QIAamp Midi column before insertion into the 15 ml centrifuge tube (saved from step 6).

13. **Pipet ■ 200 µl or ▲ 300 µl Buffer AE or distilled water, equilibrated to room temperature (15–25°C), directly onto the membrane of the QIAamp Midi column and close the cap. Incubate at room temperature for 5 min, and centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    For long-term storage of DNA, eluting in Buffer AE and storing in aliquots at –30 to –15°C is recommended since DNA is subject to acid hydrolysis if dissolved in water.

14. **For highly concentrated DNA, continue with step 14a. For maximum DNA yield, continue with step 14b.**

    Note: Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable. (For detailed information regarding elution efficiency, see Tables 2A and 2B on page 12.)

14a. For maximum concentration: Reload the eluate (■ 200 µl or ▲ 300 µl) containing the DNA onto the membrane of the QIAamp Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 2 min.

Note: Less than ■ 200 µl or ▲ 300 µl will be eluted from the column, but this has no effect on DNA yield.

14b. For maximum yield: Pipet ■ 200 µl or ▲ 300 µl fresh Buffer AE or distilled water, equilibrated to room temperature, onto the membrane of the QIAamp Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 2 min.

Note: Less than ■ 200 µl or ▲ 300 µl will be eluted from the column, but this has no effect on DNA yield.

Protocol: Purification of DNA from Whole Blood using the QIAamp DNA Blood Maxi Kit (Vacuum Protocol)

This protocol is for purification of genomic DNA from up to 10 ml of whole blood.

Important points before starting

- Blue (marked with a ■) denotes values for 3–5 ml of whole blood; red (marked with a ▲) denotes values for 5–10 ml of whole blood.
- Do not use more than 1 x 10^8 white blood cells.
- To ensure that a consistent, even vacuum is applied during protocol steps, the vacuum pump should be switched on during the entire vacuum procedure, and the main vacuum valve should be closed between steps (see Figure 3 on page 16).
- All centrifugation steps are carried out at room temperature (15–25°C). Do not use a fixed-angle rotor.
- For samples with low white blood cell counts (i.e., <100,000 genome equivalents), add carrier DNA at the start of the procedure. Since carrier DNA will co-purify with the DNA from the blood, make sure that this will not interfere with any downstream analyses, such as PCR.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C) before starting.
- Prepare a 70°C water bath for use in step 4 of the protocol.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions (page 17).
- If a precipitate has formed in Buffer AL, redissolve by incubating at 56°C.
- Set up the QIAvac 24 Plus as described in "Processing QIAamp Midi and QIAamp Maxi spin columns on the QIAvac 24 Plus" on pages 14–16.

Procedure

1. **Pipet 500 µl QIAGEN Protease into the bottom of a 50 ml centrifuge tube (provided).**
2. **Add ■ 3–5 ml or ▲ 5–10 ml blood and mix briefly.**

   Bring the volume of the sample up to ■ 5 ml or ▲ 10 ml with PBS, if necessary, before adding to the centrifuge tube.

   Note: QIAGEN Protease (or proteinase K) can be added to samples that have already been dispensed into centrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

3. **Add ■ 6 ml or ▲ 12 ml Buffer AL, and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min. Invert multiple tubes simultaneously by clamping them into a rack using another empty rack, grasping both racks, and inverting them together.**

   To ensure adequate lysis, the sample must be mixed thoroughly with Buffer AL to yield a homogenous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

4. **Incubate at 70°C for 10 min.**

   DNA yield reaches a maximum after lysis for 10 min at 70°C, but longer incubation times will not adversely affect yield.

5. **Add ■ 5 ml or ▲ 10 ml ethanol (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking.**

   To ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

   Note: Only use 96–100% ethanol. Other alcohols may result in reduced yield and purity. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

6. **Insert the QIAamp Maxi column into the VacConnector (optional) or directly into the VacValve on the vacuum manifold. Open the corresponding VacValve. Close the main vacuum valve and switch on the vacuum pump.**

   The 50 ml centrifuge tube can be saved for the centrifugation in step 12.

7. **Carefully transfer ■ all or ▲ one half of the solution from step 5 onto the QIAamp Maxi column, taking care not to moisten the rim. Open the main vacuum valve. If sample flow rates differ, close the VacValves where the lysate has already passed through to ensure a consistent vacuum over the remaining columns. After all lysates have been drawn through the columns, close the main vacuum valve. Proceed with ■ step 9 (skip step 8) or ▲ step 8.**

   ■ If the initial sample volume was ≤5 ml, apply all of the lysate to the QIAamp Maxi column in the step above. Skip step 8 and go directly to step 9.

8. **▲ Open the VacValve and load the remainder of the solution from step 5 onto the QIAamp Maxi column, taking care not to moisten the rim. Open the main vacuum valve. If sample flow rates differ, close the VacValves where the lysate has already passed through to ensure a consistent vacuum over the remaining columns. After all lysates have been drawn through the columns, close the main vacuum valve.**

   ■ This step is not necessary when starting with ≤5 ml blood. Proceed directly with step 9.

9. **Open the VacValve, and add 15 ml Buffer AW1 to the QIAamp Maxi column, taking care not to moisten the rim. Open the main vacuum valve. After all of the Buffer AW1 has been drawn through the columns, close the main vacuum valve.**

   If sample flow rates differ, close the VacValves where Buffer AW1 has already passed through to ensure a consistent vacuum over the remaining columns.

10. **Open the VacValve, and add 15 ml Buffer AW2 to the QIAamp Maxi column, taking care not to moisten the rim. Open the main vacuum valve. After all of the Buffer AW2 has been drawn through the columns close all VacValves.**

    If sample flow rates differ, close the VacValves where Buffer AW2 has already passed through to ensure a consistent vacuum over the remaining columns.

11. **To dry the membranes, open each individual VacValve for 10 s. Then open all VacValves and vacuum-dry the membranes for an additional 15 min.**

    Note: Residual ethanol in the eluate may cause inhibition of PCR, leading to false-negative results. Drying the membrane in this step helps to prevent this.

12. **Switch off the vacuum pump, and place the QIAamp Maxi column in a 50 ml centrifuge tube (saved from step 6).**

    Note: Use a wet tissue paper to wipe any spillage off the QIAamp Maxi column before insertion into the 50 ml centrifuge tube (saved from step 6).

13. **Pipet ■ 600 µl or ▲ 1 ml Buffer AE or distilled water, equilibrated to room temperature (15–25°C), directly onto the membrane of the QIAamp Maxi column and close the cap. Incubate at room temperature for 5 min, and centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    For long-term storage of DNA, eluting in Buffer AE and storing in aliquots at –30 to –15°Cis recommended since DNA is subject to acid hydrolysis if dissolved in water.

14. **For highly concentrated DNA, continue with step 14a. For maximum DNA yield, continue with step 14b.**

    Note: Highly concentrated DNA may be desirable for applications such as restriction digestion and Southern blotting. For other applications, increasing the total DNA yield may be preferable. (For detailed information regarding elution efficiency, see Tables 2A and 2B on page 12.)

14a. **For maximum concentration: Reload the eluate (■ 600 µl or ▲ 1 ml) containing the DNA onto the membrane of the QIAamp Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    Note: Less than ■ 600 µl or ▲ 1 ml will be eluted from the column, but this has no effect on DNA yield.

14b. **For maximum yield: Pipet ■ 600 µl or ▲ 1 ml fresh Buffer AE or distilled water, equilibrated to room temperature, onto the membrane of the QIAamp Maxi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x *g* (5000 rpm) for 2 min.**

    Note: Less than ■ 600 µl or ▲ 1 ml will be eluted from the column, but this has no effect on DNA yield.

Troubleshooting Guide

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

		Comments and suggestions
Colored residues remain on the QIAamp Midi/Maxi column after washing		
a)	Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL	Repeat the DNA purification procedure with new samples.
b)	No alcohol added to the lysate before loading onto the QIAamp Midi/Maxi column	Repeat the purification procedure with new samples.
c)	Buffers AW1 or AW2 prepared incorrectly	Ensure that both Buffer AW1 and AW2 concentrates were diluted with the correct amount of ethanol. Repeat the purification procedure with new samples.
d)	Animal blood prepared	Hemoglobin can be difficult to remove from the blood of certain animal species (e.g., monkey and mouse), and may interfere with downstream applications. Perform extra wash steps with Buffer AW1. Use less blood in future preparations. Use proteinase K instead of QIAGEN Protease.
Little or no DNA in the eluate		
a)	Low concentration of cells in the sample	Increase the sample volumes and load the QIAamp Midi/Maxi column several times. Reduce volume of Buffer AE used for elution (see page 11). Repeat the DNA purification procedure with a new sample.
b)	Inefficient cell lysis due to insufficient mixing with Buffer AL	Repeat the DNA purification procedure with new samples.

		Comments and suggestions
c)	Inefficient binding due to high viscosity of lysate	Repeat the DNA purification procedure with new samples. Use double the volume of Buffer AL. Mix the sample and Buffer AL immediately and thoroughly. After incubation, add double the amount of ethanol and mix thoroughly. Load sample several times onto the same QIAamp Midi/Maxi column.
d)	Inefficient cell lysis or protein degradation in Buffer AL due to insufficient incubation time	Repeat the procedure with new samples.
e)	No alcohol added to the lysate before loading onto the QIAamp Midi/Maxi column	Repeat the purification procedure with new samples.
f)	QIAamp Midi/Maxi column not incubated at room temperature for 5 min	After addition of Buffer AE or distilled water, the QIAamp Midi/Maxi column should be incubated at room temperature (15–25°C) for 5 min.
g)	DNA not eluted efficiently	To increase elution efficiency, pipet the Buffer AE or water containing the eluted DNA once more onto the QIAamp Midi/Maxi column and incubate the column for 5 min at room temperature before centrifugation.
h)	pH of water incorrect (acidic)	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0.
Low concentration of DNA in the eluate		
a)	DNA eluted with 300 µl (Midi) or 1 ml (Maxi) Buffer AE or distilled water	Elution with volumes of less than 300 µl (Midi), or 1 ml (Maxi) increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield. For samples containing less than 4 µg (Midi) or 8 µg (Maxi) of DNA, re-elution in 100 µl (Midi) or 400 µl (Maxi) of Buffer AE or distilled water is recommended.

A_{260}/A_{280} ratio for purified nucleic acids is low

a)	Inefficient cell lysis due to insufficient mixing with Buffer AL	Repeat the procedure with new samples.
b)	Inefficient cell lysis or protein degradation in Buffer AL due to insufficient incubation time	Repeat the procedure with new samples. Extend the incubation time.
c)	No alcohol added to the lysate before loading onto the QIAamp Midi or QIAamp Maxi column	Repeat the purification procedure with new samples.
d)	Buffers AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 or AW2 concentrates were diluted with the correct amounts of ethanol. Repeat the purification procedure with new samples.
e)	Animal blood prepared	Repeat the purification procedure with new samples. Use less blood. Use proteinase K instead of QIAGEN Protease.

DNA does not perform well in subsequent enzymatic reaction

a)	Not enough DNA in sample	See "Little or no DNA in the eluate", page 33, for possible reasons. Increase the amount of eluate added to the reaction, if possible. If necessary, vacuum-concentrate the DNA, or increase the amount of sample used and repeat the purification procedure. If the amount of purified DNA is still expected to be low, reduce the elution volume. Lowering the elution volume will slightly reduce overall yield, but will result in a higher concentration of nucleic acids in the eluate. DNA remaining on the QIAamp Midi/Maxi column can be recovered in a subsequent elution step by applying the same eluate, heated to 70°C, to the column.
b)	Animal blood prepared	Repeat the purification procedure with new samples. Use less blood. Use proteinase K instead of QIAGEN Protease.

		Comments and suggestions
c)	Residual Buffer AW2 in the eluate	Repeat the purification procedure with new samples. Ensure that centrifugation is carried out exactly as recommended in step 9 (spin protocols). Ensure that the spin column does not come into contact with the filtrate prior to elution in step 10 (spin protocols).
d)	Drops of liquid on column nozzle after wash steps	Discard the flow-through. Replace QIAamp Midi/Maxi column in tube and centrifuge at 4500 x *g* for 1 min.
White precipitate in Buffer AL		
	A white precipitate may form after storage at low temperature or prolonged storage	Dissolve the precipitate by incubation at 56°C.
General handling		
	Lysate did not completely pass through the column when using the vacuum protocol	Insufficient vacuum was applied or the VacValve was closed during the vacuum step. Increase the vacuum pressure, and open the VacValve while applying the vacuum. If the vacuum pressure cannot be increased, place the QIAamp Maxi column in a clean 50 ml collection tube, close the cap, and centrifuge at 1850 x *g* (3000 rpm) for 3 min or until the lysate has completely passed through the membrane. Continue with step 8 of the QIAamp DNA Blood Maxi Spin protocol on page 25.

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.

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 (15–25°C) 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 safety data sheets (SDSs), available from the product supplier.

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Blood Midi Kit (20)	20 QIAamp Midi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (15 ml)	51183
QIAamp DNA Blood Midi Kit (100)	100 QIAamp Midi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (15 ml)	51185
QIAamp DNA Blood Maxi Kit (10)	10 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 ml)	51192
QIAamp DNA Blood Maxi Kit (50)	50 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 ml)	51194
Related products		
QIAamp DNA Blood Mini Kits — for small-scale DNA purification from blood and body fluids		
QIAamp DNA Blood Mini Kit (50)*	50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers and Collection Tubes (2 ml)	51104
QIAamp DNA Mini Kits — for small-scale DNA purification from tissue, blood, and body fluids		
QIAamp DNA Mini Kit (50)*	50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304

* Other kit sizes are available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
QIAamp 96 DNA Blood Kits* — for high-throughput DNA purification from blood and body fluids		
QIAamp 96 DNA Blood Kit (4)†	4 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, Collection Vessels	51161
QIAamp DNA Blood BioRobot® Kits*		
QIAamp DNA Blood BioRobot MDx Kit (12)	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
QIAamp DNA Blood BioRobot 9604 Kit (12)	12 QIAamp 96 Plates, Buffers, Reagents, Lysis Blocks, Collection Vessels	965162
QIAamp RNA Blood Mini Kit — for total RNA purification from blood and body fluids		
QIAamp RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304
FlexiGene® DNA Kits — for isolation of DNA from whole blood, buffy coat, and cultured cells		
FlexiGene DNA Kit (50)†	For purification of DNA from 50 ml whole blood: Buffers, QIAGEN Protease	51204

* Requires use of the QIAGEN 96-Well-Plate Centrifugation System.

† Other kit sizes are available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
Accessories		
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Buffer AL (216 ml)	216 ml Lysis Buffer	19075
Buffer AE (240 ml)	240 ml Elution Buffer	19077
QIAGEN Protease (7.5 AU)	7.5 Anson units per vial (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson units per vial (lyophilized)	19157
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubings, Couplings, Valve, Gauge, 24 VacValves	19419
VacConnectors (500)	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407
VacValves (24)	24 valves for use with the QIAvac 24 and QIAvac 24 Plus	19408
Vacuum Pump	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84010* 84000 † 84020 ‡

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.

* US and Canada. † Japan. ‡ Rest of world.

Notes

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