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QIAamp® DSP DNA Blood Mini Kit Instructions for Use (Handbook)



Version 2



For In Vitro Diagnostic Use



61104



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Intended Use

The QIAamp DSP DNA Blood Mini Kit is a system that uses silica-membrane technology (QIAamp technology) for isolation and purification of genomic DNA from biological specimens.

The product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.

The QIAamp DSP DNA Blood Mini Kit is intended for in vitro diagnostic use.

Description and Principle

Each QIAamp DSP DNA Blood Mini procedure comprises 4 steps:

- Lysing the cells in the blood sample
- Binding the genomic DNA in the cell lysate to the membrane of a QIAamp Mini spin column
- Washing the membrane
- Eluting the genomic DNA from the membrane

This handbook contains protocols for 2 alternative QIAamp DSP DNA Blood Mini procedures: the spin procedure, which requires a centrifuge, and the vacuum procedure, which requires a centrifuge and a vacuum system (see flowchart, page 9). The spin procedure can be automated on the QIAcube and the QIAcube Connect MDx.

Lysing blood cells

Samples are lysed under denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Protease (QP) and Lysis Buffer (AL).

Binding genomic DNA to the QIAamp Mini spin column membrane

To optimize the binding of genomic DNA to the QIAamp Mini spin column membrane, ethanol is first added to the lysates. Each lysate is then applied to a QIAamp Mini spin column and genomic DNA is adsorbed onto the silica membrane as the lysate is drawn through by vacuum pressure or centrifugal force.

Eluting pure genomic DNA

Genomic DNA is eluted from the QIAamp Mini spin column membrane using 50–200 μ l Elution Buffer (AE). The eluted DNA is ready for use in different downstream assays.

Automated purification on QIAcube/QIAcube Connect MDx

The QIAcube and QIAcube Connect MDx perform automated isolation and purification of nucleic acids. It can process up to 12 samples per single run.

Sample preparation using the QIAcube and the QIAcube Connect MDx follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAamp DSP DNA Mini Kit for purification of high-quality DNA.

If automating the QIAamp DSP DNA Blood Mini Kit on the QIAcube or QIAcube Connect MDx instruments, they may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp DSP DNA Blood Mini Kit.



Figure 1. The QIAcube.

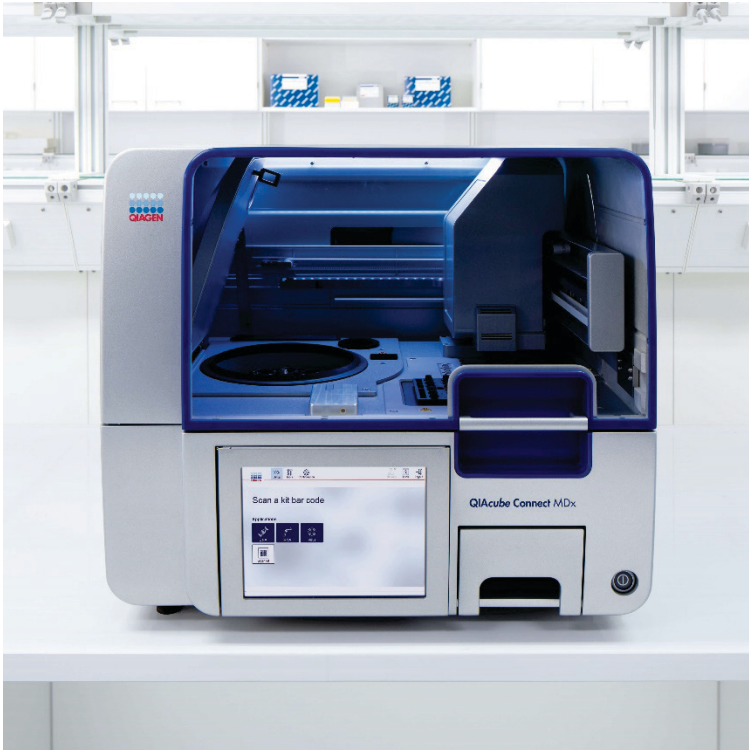
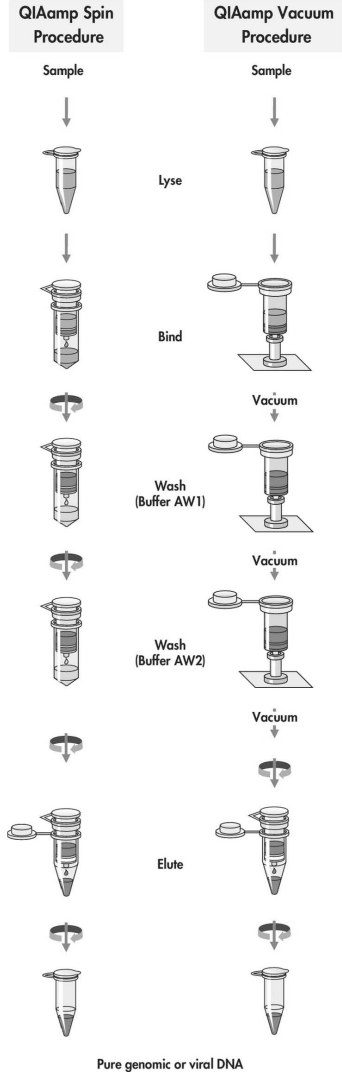


Figure 2. The QIAcube Connect MDx.

The QIAamp DSP DNA Blood Mini Spin and Vacuum Procedures



Read the protocols (pages 23 and 27) carefully before starting.

Into LT, add 20 µl QP, 200 µl sample, and 200 µl AL
Vortex for 15 s.
Incubate for 10 min (± 1 min) at 56°C (±1°C)
Add 200 µl ethanol.
Vortex for 15 s.

Transfer lysate to QIAamp Mini spin column.
Spin Procedure: Centrifuge for 1 min at 6000 x g.

Vacuum Procedure: Apply vacuum.

Spin Procedure: Place QIAamp Mini spin column in new WT, add 500 µl AW1, and centrifuge for 1 min at 6000 x g.

Vacuum Procedure: Add 750 µl AW1, and apply vacuum.

Spin Procedure: place QIAamp Mini spin column in new WT, add 500 µl AW2, and centrifuge 1 min at full speed (approximately 20,000 x g or 14,000 rpm).

Vacuum Procedure: Add 750 µl AW2, and apply vacuum.

Place QIAamp Mini spin column in WT.

Centrifuge for 3 min at full speed (approximately 20,000 x g or 14,000 rpm).

Place QIAamp Mini spin column in ET.

Add 50–200 µl AE, and incubate for 1 min.

Centrifuge for 1 min at 6000 x g.

Summary and explanation

The QIAamp DSP DNA Blood Mini Kit uses well-established technology to provide a fast and easy way to isolate and purify genomic DNA from 200 μ l whole blood.








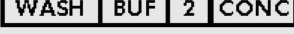




The QIAamp DSP DNA Blood Mini procedures, which are designed for simultaneous processing of multiple blood samples, yield purified DNA ready for use. The procedures are suitable for use with fresh or frozen whole blood and blood that has been treated with citrate or EDTA.

The simple QIAamp DSP spin and vacuum procedures are suitable for simultaneous processing of multiple samples. Some of the QIAamp spin procedures can be fully automated on the QIAcube or QIAcube Connect MDx for increased standardization and ease of use (page 7).

Prior separation of leukocytes is not necessary. The procedures require neither phenol/chloroform extraction nor alcohol precipitation and require minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedures are designed to minimize sample-to-sample cross-contamination. The purified DNA is ready for use in PCR or other applications, or alternatively, can be stored at -25°C to -15°C for later use.

Materials Provided

Kit contents

QIAamp DSP DNA Blood Mini Kit			
Catalog no.			61104
Number of preps			50*
5	QIAamp Mini Spin Columns with Wash Tubes (WT) (2 ml)		50
ET	Elution Tubes (1.5 ml)		50
VC	VacConnectors		50
LT	Lysis Tubes (1.5 ml)		50
WT	Wash Tubes (2 ml)		3 x 50
AL	Lysis Buffer [†]		12 ml
AW1	Wash Buffer 1 [†] (concentrate)		19 ml
AW2	Wash Buffer 2 [‡] (concentrate)		13 ml
AE	Elution Buffer [‡]		25 ml
PS	Protease Solvent [‡]		2 ml
QP	QIAGEN Protease [§]		1 vial
–	Instructions for Use (Handbook)		1

* If automating the QIAamp DSP DNA Blood Mini Kit on the QIAcube or QIAcube Connect MDx instrument, the instrument may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp DSP DNA Blood Mini Kit.

[†] Contains guanidine hydrochloride. Not compatible with disinfectants containing bleach. For more information, see Safety information on page 14.

[‡] Contains sodium azide as a preservative.

[§] Resuspension volume 1.2 ml. See “Preparing reagents and buffers” on page 19.

Materials Required but Not Provided

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.

For the spin and vacuum procedures

- Ethanol (96–100%)
- Pipettes* and pipette tips (to prevent cross-contamination, we strongly recommend the use of pipette tips with aerosol barriers)
- Disposable gloves
- Heating block** for lysis of samples at 56°C (we recommend the Eppendorf® Thermomixer® comfort with thermoblock for 1.5 ml micro test tubes†)
- Microcentrifuge**
- Measuring cylinder (50 ml)
- Vortexer

For the vacuum procedure only

- QIAvac 24 Plus vacuum system (cat. no. 19413) or equivalent
- VacConnectors (cat. no. 19407)
- VacValves (cat. no. 19408)
- QIAvac Connecting System (cat. no. 19419)
- Vacuum Pump (cat. no. 84020)
- Vacuum Regulator (cat. no. 19530)

* To ensure that samples are properly processed in the QIAamp DSP DNA Blood Mini procedures, we strongly recommend that instruments (e.g., pipets and heating blocks) are calibrated according to the manufacturers' recommendations.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

For the automated procedure only

- Rotor Adapters, cat. no. 990394
- Rotor Adapter Holder, cat. no. 990392
- Sample Tubes CB, cat. no. 990382 (sample input tube)
- Shaker Rack Plugs, cat. no. 9017854
- Reagent Bottles, 30 ml, cat. no. 990393
- Filter Tips, 1000 µl, cat. no. 990352
- Filter Tips, 200 µl, cat. no. 990332
- SafeSeal Tube, 1.5 mL, Sarstedt® (Cat. no. 72.706)

Warnings and Precautions

Please be aware that you may be required to report serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

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 component.



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

Lysis Buffer (AL) and Wash Buffer 1 (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. If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid personal injury or injury to others.

QIAGEN has not tested the liquid waste generated by the QIAamp DSP DNA Blood Mini procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

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

Lysis Buffer (AL) and Wash Buffer 1 (AW1)



Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QIAGEN Protease (QP)



Contains: subtilisin. Danger! Harmful if swallowed. Causes skin irritation. Causes serious eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause respiratory irritation. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/ protective clothing/eye protection/face protection. Wear respiratory protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.



Reagent Storage and Handling

QIAamp Mini spin columns should be stored at 2–8°C upon arrival and can be used until the expiration date on the kit box.

All buffers can be stored at room temperature (15–25°C) until the expiration date on the kit box.

Lyophilized QIAGEN Protease (QP) can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. Reconstituted QIAGEN Protease is stable for up to 1 year when stored at 2–8°C, but only until the kit expiration date.

Reconstituted Wash Buffer 1 (AW1) and reconstituted Wash Buffer 2 (AW2) are stable for up to 1 year when stored at room temperature (15–25°C), but only until the kit expiration date.

Specimen Storage and Handling

Cryoprecipitates formed during thawing of frozen samples will clog the QIAamp Mini spin column membrane. If cryoprecipitates are visible, avoid aspirating them during aspiration of the sample. The effects of freezing and thawing blood samples on DNA purification using the QIAamp DSP DNA Blood Mini Kit has been determined (see Figure 3).

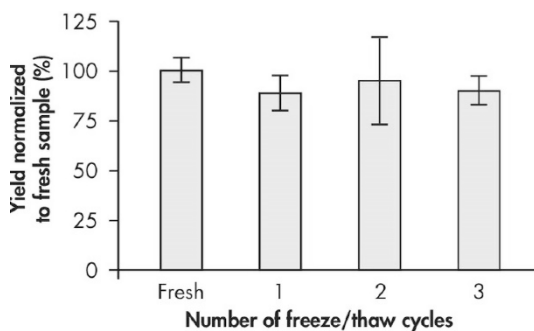


Figure 3. Effects of freezing and thawing blood samples. EDTA-treated blood was frozen and thawed up to 3 times, and then subjected to DNA purification using the QIAamp DSP DNA Blood Mini Kit. The calculated DNA yields are normalized to the yield from fresh sample (100%). Each bar on the graph represents the results from 32 replicates (mean \pm standard deviation).

The amount of DNA purified in the QIAamp DSP DNA Blood Mini procedures depends on the white blood cell content of each blood sample. Using the spin or vacuum procedure, genomic DNA is purified from 200 µl blood samples from healthy donors. Various different primary tubes and anticoagulants can be used to collect blood samples for the QIAamp DSP DNA Blood Mini procedures (Table 1).

Table 1. Average relative yields of DNA from blood samples collected using various primary tubes and anticoagulants

Primary tube	Manufacturer	Cat. no.	Nominal volume	Average yield*
BD® Vacutainer® 9NC	BD	366007	9 ml	6.4 µg
BD Vacutainer K3E	BD	36847	10 ml	6.6 µg
BD Vacutainer K2E	BD	367864	6 ml	6.4 µg
S-Monovette® EDTA	Sarstedt®	02.1066.001	9 ml	6.5 µg
S-Monovette CPDA1	Sarstedt	01.1610.001	8.5 ml	6.3 µg
Vacurette® K3E	Greiner Bio-One®	455036	9 ml	6.5 µg
Vacurette 9NC	Greiner Bio-One	454382	2 ml	6.3 µg

Genomic DNA was purified from 200 µl blood samples from healthy donors (4.0 to 9.0 × 10⁶ cells per ml).

* For each primary tube, the average yield is determined from 11 triplicate samples.

Removing residual contaminants

While the genomic DNA remains bound to the QIAamp Mini Spin Column membrane, contaminants are efficiently washed away using first Wash Buffer 1 (AW1) and then Wash Buffer 2 (AW2).

Eluting pure genomic DNA

Genomic DNA is eluted from the QIAamp Mini Spin Column membrane using 50–200 µl Elution Buffer (AE). The eluted DNA is ready for use in different downstream assays, including a variety of in vitro diagnostic downstream assays.

Important Notes

Important points before starting a protocol

- After receiving the kit, check the kit components for damage. If the blister packs or the buffer bottles are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Safety information” (page 14). Do not use damaged kit components, since their use may lead to poor kit performance.
- Always change pipette tips between liquid transfers. To minimize cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Always use disposable gloves and regularly check that they are not contaminated with sample material. Discard gloves if they become contaminated.
- To minimize cross-contamination, open only one tube at a time.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infection from potentially infectious material, we recommend working under laminar air-flow conditions until the samples are lysed.
- This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.

Preparing reagents and buffers

- Preparing QIAGEN Protease

Add 1.2 ml Protease Solvent (PS) to the vial of lyophilized QIAGEN Protease (QP) and mix carefully. To avoid foaming, mix by inverting the vial several times. Ensure that the QIAGEN Protease (QP) is completely dissolved.

Important: Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).

- Preparing Wash Buffer 1

Using a measuring cylinder, add 25 ml ethanol (96–100%) to the bottle containing 19 ml Wash Buffer 1 (AW1) concentrate. Store the reconstituted Wash Buffer 1 (AW1) at room temperature (15–25°C).

Important: Always mix the reconstituted Wash Buffer 1 (AW1) by inverting the bottle several times before starting the procedure.

- Preparing Wash Buffer 2

Using a measuring cylinder, add 30 ml ethanol (96–100%) to the bottle containing 13 ml Wash Buffer 2 (AW2) concentrate. Store the reconstituted Wash Buffer 2 (AW2) at room temperature (15–25°C).

Important: Always mix the reconstituted Wash Buffer 2 (AW2) by inverting the bottle several times before starting the procedure.

- Preparing Elution Buffer

One bottle of Elution Buffer (AE) is provided with the kit. To prevent contamination of Elution Buffer (AE), we strongly recommend using pipette tips with aerosol barriers when pipetting Elution Buffer (AE) from the bottle and replacing the cap of the bottle immediately afterwards.

Important: 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 350 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, you should then prepare the blank by diluting 50 µl Elution Buffer (AE) with 100 µl water. Use fresh, distilled water for the dilutions.

Handling of QIAamp Mini spin columns

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp Mini spin columns to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp Mini spin column. Pipet the sample into the QIAamp Mini spin column without wetting the rim of the column.
- Always change pipette tips between liquid transfers. We recommend the use of aerosol-barrier pipette tips.
- Avoid touching the QIAamp Mini spin column membrane with the pipette tip.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids.
- Open only one QIAamp Mini spin column at a time, and take care to avoid generating aerosols.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Eluting genomic DNA

The volume of DNA eluted from a QIAamp Mini spin column can be up to 20 μ l less than the volume of Elution Buffer (AE) applied to the column. The volume of eluate recovered depends on the nature of the sample. Elution Buffer (AE) should be equilibrated to room temperature (15–25°C) before it is applied to the column. Eluted DNA is collected in elution tubes (ET). If storing the DNA for up to 4 weeks, we recommend storage at 2–8°C. For long-term storage, we recommend storage at –30 to –15°C.

Yield and quality of genomic DNA

The yield and quality of the isolated genomic DNA are suitable for many types of downstream detection procedures in molecular diagnostics. Diagnostic assays should be performed according to the manufacturers' instructions.

Setting up the QIAvac 24 Plus vacuum system

Ensure that you set up the QIAamp Mini spin column, the VacConnector (VC), and the VacValve correctly (see Figure 4).

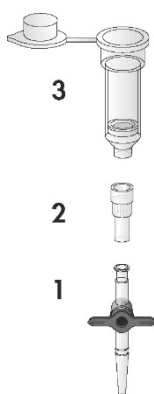


Figure 4. Assembly of components of the QIAamp DSP DNA Blood Mini Kit for vacuum processing of samples.
(1) VacValve (2) VacConnector (VC) (3) QIAamp Mini spin column

If using the vacuum procedure with the QIAvac 24 Plus vacuum system, we recommend labeling the lysis tubes (LT), elution tubes (ET), and the QIAamp Mini spin columns according to the scheme in Figure 5 (see next page) in order to avoid the mix-up of samples. This figure can be photocopied and labeled with the names of the samples. We recommend using a similar scheme if using other vacuum systems or if using the spin procedure.

Date: _____

Operator: _____

Run ID: _____

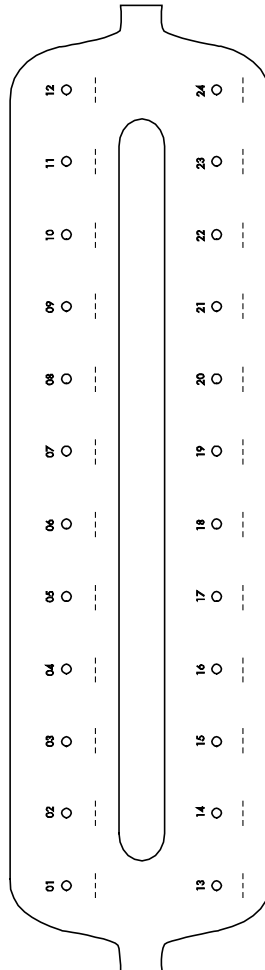


Figure 5. Labeling scheme for lysis tubes (LT), elution tubes (ET), and QIAamp Mini spin columns for use on the QIAvac 24 Plus vacuum system.

Procedure

Protocol: Isolation and purification of genomic DNA from blood samples using a vacuum system

For isolation and purification of genomic DNA from 200 µl whole blood samples treated with EDTA or citrate using a vacuum system such as the QIAvac 24 Plus vacuum system.

Important points before starting

The procedure below provides instructions for processing a single blood sample. However, up to 24 samples can be processed at the same time on the QIAvac 24 Plus vacuum system.

Things to do before starting

- Equilibrate blood samples to room temperature, and ensure that they are well-mixed.
- If a precipitate has formed in Lysis Buffer (AL), dissolve by incubating at 56°C.
- Ensure that Wash Buffer 1 (AW1), Wash Buffer 2 (AW2), and QIAGEN Protease (QP) have been prepared according to the instructions in “Preparing reagents and buffers” on page 18.
- Equilibrate Elution Buffer (AE) to room temperature for use in step 14.
- Set a heating block to 56°C for use in step 4.
- To minimize cross-contamination, insert a VacConnector (VC) into each luer adapter of the vacuum system.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Therefore, do not mix reagents from different kit lots, and do not combine individual reagents from different reagent lots.
- Ensure that the waste bottle of the vacuum system is empty and all couplings are connected correctly.
- For details about operation of the vacuum system, especially maintenance, refer to the handbook supplied with it.

Procedure

1. Pipet 20 μ l QIAGEN Protease (QP) into a lysis tube (LT).

Note: Check the expiration date of the reconstituted protease before use.

2. Add 200 μ l blood sample to the lysis tube (LT).
3. Add 200 μ l Lysis Buffer (AL) to the lysis tube (LT), close the lid, and mix by pulse-vortexing for 15 s.

To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogeneous solution.

Note: Since Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully or by using a suitable pipette.

Note: Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).

4. Incubate at 56°C (\pm 1°C) for 10 min (\pm 1 min).
5. Centrifuge the lysis tube (LT) for \geq 5 s at full speed to remove drops from inside the lid.
6. Add 200 μ l ethanol (96–100%) to the lysis tube (LT), close the lid, and mix thoroughly by pulse-vortexing for \geq 15 s.
7. Centrifuge the lysis tube (LT) for \geq 5 s at full speed to remove drops from inside the lid.
8. Insert the QIAamp Mini spin column into the VacConnector (VC) on the vacuum system. Make sure that the main vacuum valve (between the vacuum system and the vacuum manifold) and the screw cap valve (on the vacuum manifold) are closed. Switch on the vacuum pump.

Discard the wash tube (WT) (2 ml) in which the QIAamp Mini spin column is placed in the blister.

The vacuum is applied only to the connecting system (if used) and not to the vacuum manifold.

9. Carefully apply the entire lysate from step 7 to the QIAamp Mini spin column without wetting the rim. Avoid touching the QIAamp Mini spin column membrane with the pipette tip.

Note: If processing several samples, open only one lysis tube (LT) at a time.

10. Open the main vacuum valve. After the lysate has been drawn through the QIAamp Mini spin column, close the main vacuum valve and open the screw cap valve on the vacuum manifold to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.

After closing the main vacuum valve, the vacuum is applied only to the connecting system (if used) and not the vacuum manifold.

Note: Use the screw cap valve of the vacuum manifold for rapid release of the vacuum.

Note: If processing several QIAamp Mini spin columns at the same time, we recommend closing the VacValve of each column after lysate has passed through in order to reduce the duration of this vacuum step.

Note: If the lysate has not completely passed through the membrane after 10 min, place the QIAamp Mini spin column into a clean wash tube (WT), close the lid, and centrifuge at $6000 \times g$ (8000 rpm) for 3 min or until the lysate has completely passed through. Place the QIAamp Mini spin column into another clean wash tube (WT) and continue with step 10 of the protocol on page 29.

Note: If the lysate still does not pass through the membrane during centrifugation, discard the sample and repeat the isolation and purification with new sample material beginning at step 1 on page 28.

11. Apply 750 μ l Wash Buffer 1 (AW1) to the QIAamp Mini spin column without wetting the rim. Avoid touching the QIAamp Mini spin column membrane with the pipette tip. Leave the lid of the column open, and open the main vacuum valve. After Wash Buffer 1 (AW1) has been drawn through the QIAamp Mini spin column, close the main vacuum valve and open the screw cap valve to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.
12. Apply 750 μ l Wash Buffer 2 (AW2) to the QIAamp Mini spin column without wetting the rim. Avoid touching the QIAamp Mini spin column membrane with the pipette tip. Leave the lid of the column open, and open the main vacuum valve. After Wash Buffer 2 (AW2) has been drawn through the QIAamp Mini spin column, close the main vacuum valve and open the screw cap valve to vent the manifold. Close the screw cap valve after the vacuum is released from the manifold.

13. Close the lid of the QIAamp Mini spin column, remove it from the vacuum system, and discard the VacConnector (VC). Place the QIAamp Mini spin column in a clean wash tube (WT), and centrifuge at full speed (approximately 20,000 x *g*, or 14,000 rpm) for 3 min to dry the membrane completely.

Note: Omission of the dry centrifugation might lead to inhibition of the downstream assay.

14. Place the QIAamp Mini spin column in a clean elution tube (ET), and discard the wash tube (WT) containing the filtrate. Carefully open the lid of the QIAamp Mini spin column, and apply 50 to 200 μ l Elution Buffer (AE) to the center of the membrane. Close the lid and incubate at room temperature for 1 min. Centrifuge at 6000 x *g* (8000 rpm) for 1 min to elute the DNA.

Note: Follow the maintenance procedure for the vacuum system after performing this protocol (see the handbook supplied with the vacuum system for more details).

Protocol: Isolation and purification of genomic DNA from blood samples using a microcentrifuge or QIAcube/QIAcube Connect MDx

For isolation and purification of genomic DNA from 200 µl whole blood samples treated with EDTA or citrate using a microcentrifuge or automated on the QIAcube or QIAcube Connect MDx.

Important points before starting

- The procedure below provides instructions for processing a single blood sample. However, several samples can be processed at the same time; the number depends on the capacity of the microcentrifuge used.
- Automated processing of 2 – 10 or 12 samples can be performed on QIAcube or *QIAcube or QIAcube connect MDx User Manual*.

Things to do before starting

- Equilibrate blood samples to room temperature, and ensure that they are well mixed.
- If a precipitate has formed in Lysis Buffer (AL), dissolve by incubating at 56°C.
- Ensure that Wash Buffer 1 (AW1), Wash Buffer 2 (AW2), and QIAGEN Protease (QP) have been prepared according to the instructions in “Preparing reagents and buffers” on page 18.
- Equilibrate Elution Buffer (AE) to room temperature for use in step 15.
- Set a heating block to 56°C for use in step 4.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Therefore, do not mix reagents from different kit lots, and do not combine individual reagents from different reagent lots.

Procedure

- For the manual procedure with a microcentrifuge follow steps 1 – 15.

- This procedure can be automated in 3 different versions:
 - Elution volume: 100 μL full automation with 100 μL elution volume (starting from step 1)
 - Elution volume: 200 μL full automation with 200 μL elution volume (starting from step 1)
 - Manual lysis: partly automated with off-board manual lysis (starting after step 5)
1. Pipet 20 μL QIAGEN Protease (QP) into a lysis tube (LT).

Note: Check the expiration date of the reconstituted protease before use.
 2. Add 200 μL blood sample to the lysis tube (LT).
 3. Add 200 μL Lysis Buffer (AL) to the lysis tube (LT), close the lid, and mix by pulse-vortexing for 15 s.

To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogenous solution.

Note: Since Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully or by using a suitable pipet.

Note: Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).
 4. Incubate at 56°C ($\pm 1^\circ\text{C}$) for 10 min (± 1 min).
 5. Centrifuge the lysis tube (LT) for ≥ 5 s at full speed to remove drops from the inside of the lid.

Note: If manual lysis (steps 1 – 5) was done off-board, the following steps (steps 6 – 15) can be automated on the QIAcube or QIAcube Connect MDx using the protocol for manual lysis.
 6. Add 200 μL ethanol (96–100%) to the lysis tube (LT), close the lid, and mix thoroughly by pulse-vortexing for ≥ 15 s.
 7. Centrifuge the lysis tube (LT) for ≥ 5 s at full speed to remove drops from the inside of the lid.
 8. Carefully apply the entire lysate from step 7 to the QIAamp Mini spin column without wetting the rim. Avoid touching the QIAamp Mini spin column membrane with the pipette tip.

Note: If processing several samples, open only one lysis tube (LT) at a time.
 9. Close the lid of the QIAamp Mini spin column, and centrifuge at approximately 6000 $\times g$ for 1 min. Place the QIAamp Mini spin column in a clean wash tube (WT), and discard the tube containing the filtrate.

Note: If the lysate has not completely passed through the membrane after centrifugation at $6000 \times g$ (8000 rpm), centrifuge again at full speed (up to $20,800 \times g$) for 1 min.

Note: If the lysate still does not pass through the membrane during centrifugation, discard the sample and repeat the isolation and purification with new sample material beginning at step 1 on page 28.

10. Carefully open the QIAamp Mini spin column, and add 500 μ l Wash Buffer 1 (AW1) without wetting the rim. Avoid touching the QIAamp Mini spin column membrane with the pipette tip.
11. Close the lid of the QIAamp Mini spin column, and centrifuge at approximately $6000 \times g$ for 1 min. Place the QIAamp Mini spin column in a clean wash tube (WT), and discard the tube containing the filtrate.
12. Carefully open the QIAamp Mini spin column, and add 500 μ l Wash Buffer 2 (AW2) without wetting the rim. Avoid touching the QIAamp Mini spin column membrane with the pipette tip.
13. Close the lid of the QIAamp Mini spin column, and centrifuge at full speed (approximately $20,000 \times g$, or 14,000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean wash tube (WT), and discard the tube containing the filtrate.
14. Centrifuge at full speed (approximately $20,000 \times g$, or 14,000 rpm) for 3 min to dry the membrane completely.

Note: Omission of the dry centrifugation might lead to inhibition of the downstream assay.

15. Place the QIAamp Mini spin column in a clean elution tube (ET), and discard the wash tube (WT) containing the filtrate. Carefully open the lid of the QIAamp Mini spin column, and apply 50 to 200 μ l Elution Buffer (AE) to the center of the membrane. Close the lid and incubate at room temperature for 1 min. Centrifuge at approximately $6000 \times g$ (8000 rpm) for 1 min to elute the DNA.

Important Note: In case of all automated procedures, remove the eluates from the instrument directly after the finished run and store them properly.

Quality Control

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

Limitations

The system performance has been established using whole blood for isolation of genomic DNA.














It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies.














To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonization of Technical Requirements (ICH) in ICH Q2(R1) Validation Of Analytical Procedures: Text And Methodology are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
 Σ <N>	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Upon arrival
	Open on delivery; store QIAamp Mini spin columns at 2–8°C
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components
	Contains
	Number
	Global Trade Item Number
R_n	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation

Symbol	Symbol definition
	Manufacturer
	Consult instructions for use
	Volume
	Write down the current date after adding ethanol to the bottle
	Adding
	Lyophilized
	Reconstitute in
	Ethanol
	Guanidine hydrochloride
	Subtilisin
	Leads to
	Consult instructions for use
	Important note

Type equation here.

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP DNA Blood Mini Kit (50)	For 50 DNA preps: QIAamp Mini spin columns, VacConnectors, QIAGEN Protease, Reagents, Buffers, and collection tubes	61104
Related products		
QIAcube Connect MDx*	Instrument and 1-year warranty on parts and labor	9003070
Accessories		
QIAvac 24 Plus vacuum manifold†	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
Vacuum Pump†	Universal vacuum pump	84020
VacConnectors†	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407
Rotor Adapters	For 240 preps: 240 Disposable Rotor Adapters and 240 Elution Tubes (1.5 ml); for use with the QIAcube	990394
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube	990392
Sample Tubes CB	1000 conical screw-cap tubes without skirted base (2 ml) for use with the QIAcube and QIAcube Connect MDx	990382
Shaker Rack Plugs	For loading the QIAcube shaker rack	9017854

Product	Contents	Cat. no.
Reagent Bottles, 30 ml	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube	990393
Filter-Tips, 1000 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube	990352
Filter-Tips, 1000 µl, wide-bore	Disposable Filter-Tips, wide-bore, racked; (8 x 128); not required for all protocols. For use with the QIAcube	990452
Filter-Tips, 200 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments	990332

* The QIAcube connect MDx is not available in all countries. For further details, please contact QIAGEN technical services.

† For use with vacuum protocols.

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.

Document Revision History

Revision	Description
R2, 01/2021	<p>Updates to the Automated purification on QIAcube/QIAcube Connect MDx, Warnings and Precautions, and the Protocol: Isolation and purification of genomic DNA from blood samples using a microcentrifuge or QIAcube/QIAcube Sections.</p> <p>Added references to the QIAcube Connect MDx and its accessories.</p> <p>Removed reference to the CD in the Kit contents section.</p> <p>Editorial and layout changes.</p>

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