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

Σ 50

Version 3

IVD

For In Vitro Diagnostic Use

For use with QIAamp DSP DNA Blood Mini Kit

CE

REF 61104

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Sample to Insight

Contents

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

Intended User

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

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 or can be automated on the QIAcube® Connect MDx (Figure 1), and the vacuum procedure, which requires a centrifuge and a vacuum system (see the flowchart, page 9).

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.

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. Elution Buffer (AE) should be equilibrated to room temperature (15–25°C) before it is applied to the column.

Due to remaining elution buffer retained by the spin column membrane after centrifugation, the eluate volume recovered can be lower than the volume of Elution Buffer (AE) applied to the column. The volume of eluate recovered depends on the nature of the sample. Eluted DNA is collected in Elution Tubes (ET) and can be stored at 2–8°C for up to 4 weeks. For long-term storage, we recommend storage at –20°C.

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been evaluated for the QIAamp DSP DNA Blood Mini Kit in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

Yield and quality of genomic DNA

DNA yield depends on the sample and the quality of the starting material. Elution in smaller volumes increases the final DNA concentration in the eluate but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.

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

Automated purification on QIAcube Connect MDx

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

Sample preparation using 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 Blood Mini Kit for purification of high-quality DNA.

If automating the QIAamp DSP DNA Blood Mini Kit on the QIAcube Connect MDx, 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.

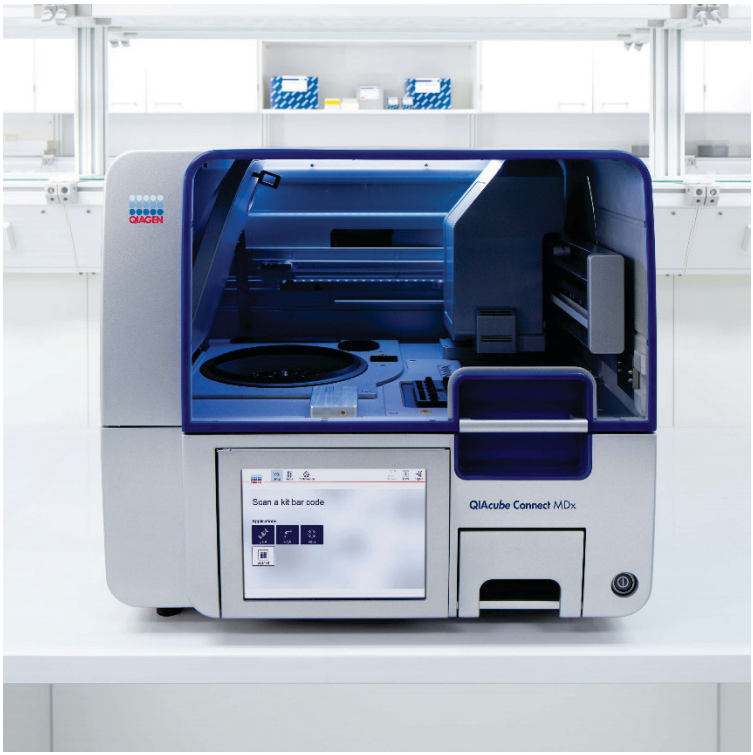
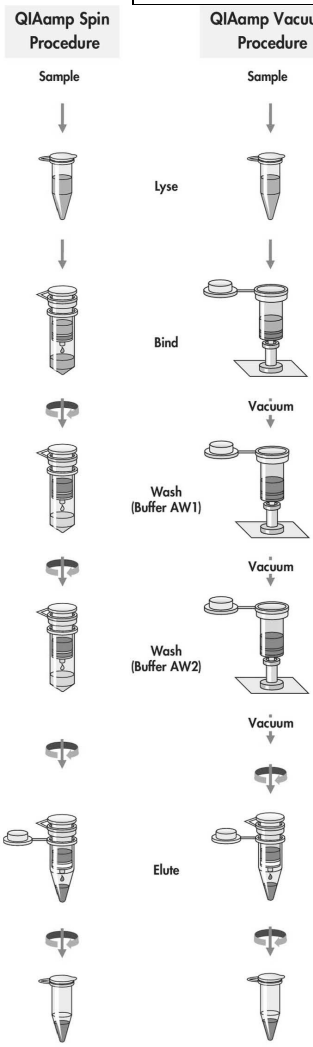


Figure 1. The QIAcube Connect MDx.

The QIAamp DSP DNA Blood Mini Spin and Vacuum Procedures



Read the protocols (pages 26 and 30) carefully before starting.

Into LT, add 20 µl QP, 200 µl sample, and 200 µl AL.
Vortex for 15 s.
Incubate for10 min at 56°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 for 1 min at full speed (approx. 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 (approx. 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 µl 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.

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 –20°C for long-term storage.

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 Connect MDx for increased standardization and ease of use (page 7).

For the vacuum procedure, a vacuum manifold (e.g., the QIAvac 24 Plus with the QIAvac Connecting System) and a vacuum pump capable of producing a vacuum of approximately 800–900 mbar (e.g., QIAGEN Vacuum Pump) are required for the protocol. Vacuum Regulator should be used (part of the QIAvac Connecting System) for easy monitoring of vacuum pressure and convenient vacuum release.

Materials Provided

Kit contents

QIAamp DSP DNA Blood Mini Kit			
Catalog no.			**61104**
Number of preps			**50**
	Identity	**Symbols**	**Quantity**
5	QIAamp Mini Spin Columns with Wash Tubes (WT) (2 ml)	COL	50
ET	Elution Tubes (1.5 ml)	ELU TUBE COL EXT	50
VC	VacConnectors	VAC CON	50
LT	Lysis Tubes (1.5 ml)	LYS TUBE	50
WT	Wash Tubes (2 ml)	WASH TUBE	3 x 50
AL	Lysis Buffer*	LYS BUF	12 ml
AW1	Wash Buffer 1† (concentrate)	WASH BUF 1 CONC	19 ml
AW2	Wash Buffer 2‡ (concentrate)	WASH BUF 2 CONC	13 ml
AE	Elution Buffer‡	ELU BUF	25 ml
PS	Protease Solvent‡	ELU BUF	2 ml
QP	QIAGEN Protease§	QPROT	1 vial
–	Instructions for Use (Handbook)		1

* If automating the QIAamp DSP DNA Blood Mini Kit on the 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 15.

‡ Contains sodium azide as a preservative.

§ Resuspension volume 1.2 ml. See "Preparing reagents and buffers" on page 22.

Components of the kit

The principal components of the kit containing active ingredients are explained below.

Reagent	Active Ingredients	Concentration (w/w) [%]
QIAGEN Protease	Subtilisin	≥0 to ≤100
AL	Guanidine hydrochloride Maleic acid	≥30 to <50 ≥0.1 to <1
AW1	Guanidine hydrochloride	≥50 to <70

Materials Required but Not Provided

Additional reagents

- Ethanol (96–100%)*

Consumables

- Pipettes† and pipette tips (to prevent cross-contamination, we strongly recommend the use of pipette tips with aerosol barriers)
- Disposable gloves

Equipment

- Heating block† for lysis of samples at 56°C (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†
- VacValves (cat. no. 19408)
- QIAvac Connecting System (cat. no. 19419)
- Vacuum Pump (cat. no. 84020)
- Vacuum Regulator (cat. no. 19530)

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

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

For the automated procedure only

- QIAcube Connect MDx instrument (cat. no. 9003070)*
- 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)

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

Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative and the regulatory authority in which the user and/or the patient is established.

For in vitro diagnostic use.

Read all instructions carefully before using the kit.

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.
- Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

Emergency information

CHEMTREC
USA & Canada 1-800-424-9300
Outside USA & Canada +1 703-527-3887

Precautions

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

Buffer AL

Contains: guanidine hydrochloride and maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. May cause an allergic skin reaction. Causes serious eye irritation. Wear protective gloves/protective clothing/eye protection/face protection. Call a POISON CENTER or doctor/physician if you feel unwell. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse. Dispose of contents/container to an approved waste disposal plant.

Buffer 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. Take off contaminated clothing and wash before reuse. Dispose of contents/container to an approved waste disposal plant.

QIAGEN Protease

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 person to fresh air and keep comfortable for breathing.

Disposal

The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed properly. Refer to your local safety regulations for proper disposal procedures.

For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Reagent Storage and Handling

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

The 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.

Note: To ensure that kit components from different kits are not mixed, please label the QIAamp Mini spin columns with the respective kit lot number.

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.

In-use stability

Reconstituted QIAGEN Protease (QP) is stable for up to 1 year when stored at 2–8°C, but only until the kit expiration date. Keeping the QIAGEN Protease (QP) stock solution at room temperature for prolonged periods of time should be avoided.

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.

For preparation of buffers for the automated procedure, follow the instructions in the *QIAcube Connect MDx User Manual* (which can be found under the resource tab of the product page on **www.qiagen.com**).

Specimen Collection, Storage, and Handling

Note: Sample stability highly depends on various factors and relates to the specific downstream application. It has been assessed with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

For general collection, transport, and storage recommendations refer to the approved CLSI guideline MM13-A "Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods". Furthermore, the manufacturer's instructions for the selected sample collection device shall be followed during sample preparation, storage, transport, and general handling. Independent of the blood collection tube manufacturer's instructions, ISO 20186-2:2019 (E) should be considered for genomic DNA extraction from venous whole blood.

Note: According to ISO 20186-2:2019(E), heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates could cause inhibitions in some downstream applications. Therefore, we recommend usage of blood samples treated with EDTA or citrate as anticoagulant.

If using fresh blood samples in primary tubes, mix the blood samples thoroughly (e.g., by inverting the tubes several times) before sample transfer. Frozen samples (with a maximum of 3 freeze/thaw cycles) should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. Do not use blood samples that have been frozen and thawed more than 3 times. To ensure reliable sample transfer, avoid generating foam in sample tubes. Try to avoid blood clots in the samples and transfer the sample without clots. Cryoprecipitates formed during thawing of frozen samples will clog the QIAamp Mini spin column membrane or may impair the automated procedure on the QIAcube Connect MDx. If cryoprecipitates are visible, avoid aspirating them.

Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results. For short-term storage of up to 10 days, we recommend storage at 2–8°C. However, for applications requiring maximum fragment size, such as southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time. For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –20 or –80°C.

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 15). Do not use damaged kit components because 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.
- Always use disposable gloves throughout the entire procedure 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.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids. The user should ensure that traceability of the samples is kept during the entire procedure.
- All centrifugation steps are carried out at room temperature (15–25°C).
- 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. Pipette the sample into the QIAamp Mini spin column without wetting the rim of the column.
- Avoid touching the QIAamp Mini spin column membrane with the pipette tip.
- Open only one QIAamp Mini spin column at a time, and take care to avoid generating aerosols.

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 2).

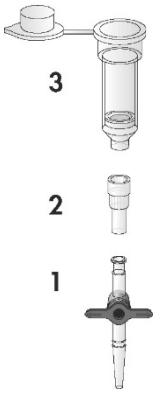


Figure 2. Assembly of components of the QIAamp DSP DNA Blood Mini Kit for vacuum processing of samples.
(**1**) VacValve, (**2**) VacConnector (VC), and (**3**) QIAamp Mini spin column.

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 3 (see next page) 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: _____

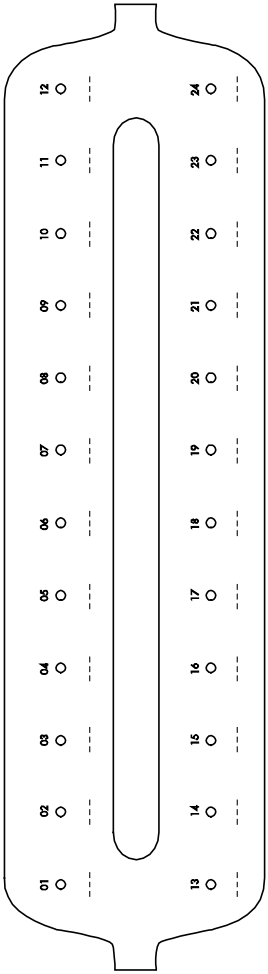


Figure 3. Labeling scheme for Lysis Tubes (LT), Elution Tubes (ET), and QIAamp Mini spin columns for use on the QIAvac 24 Plus vacuum system.

Procedure

Protocol: Isolation and purification of genomic DNA from blood samples using a microcentrifuge/automated purification on 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 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 Connect MDx instrument.
- For automation, follow the instructions on the user interface (QIAcube Connect MDx) and refer to the *QIAcube Connect MDx User Manual* (which can be found under the resource tab of the product page on **www.qiagen.com**).

Things to do before starting

- Equilibrate blood samples to room temperature, and ensure that they are well mixed.
- Make sure that all reagents and the QIAamp Mini spin columns (in closed blisters) are equilibrated to room temperature.
- Set a heating block to 56°C for use in step 4 (required for manual procedure and automated procedure with off-board manual lysis).
- 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 22.

- If a precipitate has formed in Lysis Buffer (AL), dissolve by incubating at 56°C.
- Quality control procedures at QIAGEN use 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 fully automated (automation starting from step 1)
    - Elution volume: 200 µl fully automated (automation starting from step 1)
    - Manual lysis: partly automated with off-board manual lysis and elution volumes of 100–200 µl in increments of 10 µl (automation starting after step 5)

1. Pipette 20 µl QIAGEN Protease (QP) into a Lysis Tube (LT).

    ⓘ 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.

    ⓘ Because Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully and by using a suitable pipette.

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

4. Incubate at 56°C for 10 min.
5. Centrifuge the Lysis Tube (LT) for ≥5 s at full speed to remove drops from the inside of the lid.

ⓘ If manual lysis (steps 1–5) was done off-board, the following steps (steps 6–15) can be automated on the 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.

   ⓘ 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 x *g* for 1 min. Place the QIAamp Mini spin column in a clean wash tube (WT), and discard the tube containing the filtrate.

   ⓘ If the lysate has not completely passed through the membrane after centrifugation at 6000 x *g* (8000 rpm), centrifuge again at full speed (up to 20,800 x *g*) for 1 min.

   ⓘ 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 27.
10. Carefully open the QIAamp Mini spin column, and add 500 µl 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 x *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 µl 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 (approx. 20,000 x *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.

    Centrifuge at full speed (approx. 20,000 x *g*, or 14,000 rpm) for 3 min to dry the membrane completely.

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

14. Place the QIAamp Mini spin column in a new 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 µl Elution Buffer (AE) to the center of the membrane.

    ⓘ It is important to use a new Elution Tube to avoid contamination with residual wash buffers that might lead to inhibition of the downstream assay.

    ⓘ Dispensing the Elution Buffer (AE) on the center of the membrane is especially important for smaller elution volumes to ensure optimal retrieval of nucleic acids and Elution Buffer (AE).

15. Close the lid and incubate at room temperature for 1 min. Centrifuge at approximately 6000 x *g* (8000 rpm) for 1 min to elute the DNA.

    ⓘ Orient the Elution Tube lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

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

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 point 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.
- Make sure that all reagents and the QIAamp Mini spin columns (in closed blisters) are equilibrated to room temperature.
- Set a heating block to 56°C for use in step 4.
- 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 22.
- If a precipitate has formed in Lysis Buffer (AL), dissolve by incubating at 56°C.
- To minimize cross-contamination, insert a VacConnector (VC) into each luer adapter of the vacuum system.
- 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.
- Quality control procedures at QIAGEN use 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

1. Pipette 20 µl QIAGEN Protease (QP) into a Lysis Tube (LT).

   ⓘ 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 homogeneous solution.

   ⓘ Because Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully and by using a suitable pipette.

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

4. Incubate at 56°C for 10 min.
5. Centrifuge the Lysis Tube (LT) for ≥5 s at full speed to remove drops from inside the lid.
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 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.

ⓘ 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.

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

    ⓘ 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.

    ⓘ 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 x *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 32.

    ⓘ 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 31.

11. Apply 750 µl 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 µl 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 (approx. 20,000 x *g*, or 14,000 rpm) for 3 min to dry the membrane completely.

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

14. Place the QIAamp Mini spin column in a new 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 µl Elution Buffer (AE) to the center of the membrane.

    ⓘ It is important to use a new Elution Tube (ET) to avoid contamination with residual wash buffers that might lead to inhibition of the downstream assay.

    ⓘ Dispensing the Elution Buffer (AE) on the center of the membrane is especially important for smaller elution volumes to ensure optimal retrieval of nucleic acids and Elution Buffer (AE).

15. 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.

    ⓘ Orient the Elution Tube (ET) lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

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

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.

Information on the use of the QIAamp DSP DNA Blood Mini Kit can be found within the "Description and Principle" section. The automated procedure is detailed under the section "Protocol: Isolation and purification of genomic DNA from blood samples using a microcentrifuge/automated purification on QIAcube Connect MDx".

It is the user's responsibility to validate system performance for any procedures used in their laboratory that 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.

Performance Characteristics

The applicable performance characteristics can be found under the resource tab of the product page on **www.qiagen.com**.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
General handling		
a)	Clogging of pipette tips during sample transfer	Mix the blood samples thoroughly (e.g., by inverting the tubes several times) before sample transfer. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. Try to avoid blood clots in the samples and transfer the sample without clots. Cryoprecipitates formed during thawing of frozen samples will clog the QIAamp Mini spin column membrane or may lead to problems during the automated procedure.
b)	Clogged QIAamp Mini spin column	**Spin workflow**: If the lysate has not completely passed through the membrane after centrifugation at 6000 x *g* (8000 rpm), centrifuge again at full speed (up to 20,800 x *g*) for 1 min. 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. **Vacuum workflow**: If the flow rate is reduced, vacuum time can be extended. Alternatively, close the VacValve, if used, and carefully remove the VacConnector–VacValve assembly from the QIAamp Mini spin column without losing any of the lysate. Remove the QIAamp Mini spin column from the vacuum manifold, place it in a 2 ml wash tube, and spin it at full speed until sample has completely passed through the membrane. Replace the VacConnector–VacValve assembly containing the remaining lysate. Switch on the vacuum pump, open the VacValve, and continue to load the remaining lysate. Repeat the above procedure if the QIAamp Mini spin column continues to clog. 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.

		Comments and suggestions
		General information Cryoprecipitates may have formed due to repeated freezing and thawing. These can block the QIAamp Mini spin column. Do not use blood samples that have been frozen and thawed more than 3 times. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure.
c)	Precipitate has formed in Lysis Buffer (AL)	Dissolve by incubation of Lysis Buffer (AL) at 56°C.
d)	Variable elution volumes	The volume of eluate recovered depends on the nature of the sample. Due to remaining Elution Buffer (AE) retained by the spin column membrane after centrifugation, the eluate volume recovered can be lower than the volume of elution buffer applied to the column. Apply the Elution Buffer (AE) to the center of the membrane. Dispensing the Elution Buffer (AE) on the center of the membrane is especially important for smaller elution volumes to ensure optimal retrieval of nucleic acids and Elution Buffer (AE).
e)	Vacuum pressure of approx. 800–900 mbar not reached	The vacuum manifold is not tightly closed. Press down on the lid of the vacuum manifold after the vacuum is switched on. Check if vacuum pressure is reached. Gasket of QIAvac lid has worn out. Check the seal of the manifold visually and replace it if necessary. VacValves have worn out. Remove all VacValves and insert VacConnectors (VC) directly into the luer extensions. Insert QIAamp Mini spin columns into VacConnectors (VC), close the lid of the columns, and switch on vacuum. Check if vacuum pressure is reached. Replace VacValves if necessary. Connection to vacuum pump is leaky. Close all luer extension with luer caps and switch on the vacuum pump. Check if vacuum pressure is stable after the pump is switched on (and the Vacuum Regulator valve is closed). Exchange the connections between pump and vacuum manifold if necessary. If the vacuum pressure is still not reached, replace the vacuum pump with a stronger one.
f)	For problems in the automated workflow	Refer to *QIAcube Connect MDx User Manual* (which can be found under the resource tab of the product page on **www.qiagen.com**).
Low DNA yield		
a)	Incomplete sample lysis	If QIAGEN Protease (QP) was subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh QIAGEN Protease (QP). Make sure to dissolve QIAGEN Protease (QP) with Protease Solvent (PS) according to the instructions above. To avoid foaming, mix by inverting the vial several times. Ensure that the QIAGEN Protease (QP) is completely dissolved. Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).

		Comments and suggestions
		To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogeneous solution. Because Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully and by using a suitable pipette.
b)	Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
c)	Buffer AW1 or Buffer AW2 prepared incorrectly	Make sure that the Buffer AW1 and Buffer AW2 concentrates were diluted with the correct volume of 96–100% ethanol and mixed by inverting the bottle several times before starting the procedure.
d)	Blood samples were not stored correctly	Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results. For short-term storage of up to 10 days, we recommend storage at 2–8°C. However, for applications requiring maximum fragment size, such as southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time. For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –20 or –80°C.
e)	Frozen blood samples were not mixed properly after thawing	Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure.
DNA does not perform well in downstream reactions		
a)	Little or no DNA in the eluate	See "Low DNA yield" above for possible reasons. Increase the amount of eluate added to the reaction if possible.
b)	Inappropriate elution volume used	Determine the maximum volume of eluate suitable for your downstream application. Reduce or increase the volume of eluate added to the downstream application accordingly. The elution volume can be adapted proportionally. Elution with smaller volumes of Buffer AE leads to higher nucleic acid concentrations but may result in a lower total yield.
c)	Insufficient DNA used	Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm.
d)	Excess DNA used	Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm.
e)	Potential inhibitor carryover	Be sure to perform dry centrifugation step prior to elution to prevent potential inhibition of the downstream assay. It is important to use a new Elution Tube (ET) to avoid contamination with residual wash buffers that might lead to inhibition of the downstream assay.

Symbols

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

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	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

Symbol	Symbol definition
NUM	Number
GTIN	Global Trade Item Number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Manufacturer
	Consult instructions for use
VOL	Volume
	Write down the current date after adding ethanol to the bottle
ADD	Adding
LYOPH	Lyophilized
RCNS	Reconstitute in
EtOH	Ethanol

Symbol	Symbol definition
GuHCl	Guanidine hydrochloride
SUBT	Subtilisin
	Leads to
	Consult instructions for use
	Important note
UDI	Unique device identifier

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP DNA Blood Mini Kit (50)	For 50 preps: QIAamp Mini Spin Columns, Buffers, Reagents, Tubes, VacConnectors	61104
Related products		
QIAcube Connect MDx*	Instrument and 1 year warranty on parts and labor	9003070
Accessories		
QIAvac 24 Plus†	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
Vacuum Pump (230 V, 50 Hz)†	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84020
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 Regulator	For use with QIAvac manifolds	19530
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubing, Couplings, Valve, Gauge, 24 VacValves	19419
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters and 240 Elution Tubes (1.5 ml); for use with the QIAcube Connect MDx	990394

Product	Contents	Cat. no.
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube Connect MDx	990392
Sample Tubes CB (2 ml)	1000 conical screw-cap tubes without skirted base (2 ml) for use with the QIAcube Connect MDx	990382
Shaker Rack Plugs	Shaker Rack Plugs (12)	9017854
Reagent Bottles, 30 ml (6)	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube Connect MDx	990393
Filter-Tips, 1000 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube Connect MDx	990352
Filter-Tips, 1000 µl, wide-bore (1024)	Disposable Filter-Tips, wide-bore, racked; (8 x 128); not required for all protocols. For use with the QIAcube Connect MDx	990452
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube Connect MDx 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 Instructions for Use. QIAGEN kit Instructions for Use are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Revision	Description
R1, June 2022	Version 3, Revision 1 ● Update to Kit Version 3 for compliance to IVDR ● Update of Description and Principle ● Update of Materials Provided (addition of active ingredients) and Material Required but Not Provided ● Update of Warnings and Precautions (Addition of emergency information and Disposal section) ● Update of Reagent Storage and Handling ● Update of Specimen Collection, Storage, and Handling ● Update of Important Notes and Procedure ● Update of Limitations ● Update of Performance Characteristics ● Update of Symbols section ● Update of Ordering Information

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