April 2021

QIAamp® UCP Pathogen Mini Handbook

For microbial DNA purification from whole blood, swabs, cultures, and body fluids

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp UCP Pathogen Mini Kit **Catalog no.** **Number of preps**	**(50)** **50214** **50**
QIAamp UCP Mini Columns	50
Collection Tubes (2 ml)	50
Tube Extenders (3 ml)	50
Elution Tubes (1.5 ml)	50
VacConnectors	50
Buffer ATL*	38 ml
Buffer APL2†	14 ml
Buffer APW1† (concentrate)	18 ml
Buffer APW2† (concentrate)	15 ml
Buffer AVE*	8 vials
Proteinase K	2.5 ml
Handbook	1

* Contains sodium azide as a preservative.

† Contains chaotropic salt. See page 6 for Safety Information.

Storage

The QIAamp UCP Mini Columns should be stored at 2–8°C upon arrival; however, short-term storage (up to 4 weeks) at room temperature (15–25°C) does not affect their performance. All buffers can be stored at room temperature (15–25°C).

The QIAamp UCP Pathogen Mini Kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially formulated storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature (15–25°C). To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

The QIAamp UCP Mini Columns and all buffers within the QIAamp UCP Pathogen Mini Kit undergo DNA decontamination processes and are certified to be free of contaminating microbial DNA at the time of delivery.

Intended Use

The QIAamp UCP Pathogen Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

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.

WARNING 	DO NOT add bleach or acidic solutions directly to waste containing Buffer APL2 or Buffer APW1.

Buffer APL2 and Buffer APW1 contain guanidine salts, 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 UCP Pathogen Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp UCP Pathogen Mini Kit provides fast and easy methods for purification of microbial DNA from a variety of sample materials such as whole blood, swabs, biological fluids, and cultures.

The QIAamp UCP Mini Columns and all buffers within the QIAamp UCP Pathogen Mini Kit undergo DNA decontamination processes and are certified to have no detectable contaminating microbial DNA at the time of delivery.

Tube extenders and vacuum processing on the QIAvac 24 Plus enable starting sample volumes of up to 400 µl. Flexible elution volumes between 50 and 200 µl allow concentration of nucleic acid.

The eluted nucleic acids are ready for use in common downstream reactions or storage at –15 to –30°C. Purified nucleic acids are free of contaminating DNA, proteins, nucleases, and other impurities.

Principle and procedure

The QIAamp UCP Pathogen Mini Protocols use tube extenders and vacuum processing on the QIAvac 24 Plus to enable starting sample volumes of up to 400 µl sample and flexible elution volumes from 50 µl to 200 µl.

The robust procedure helps to eliminate sample-to-sample cross-contamination and increases user safety when handling potentially infectious samples. The simple process is highly suited for simultaneous processing of multiple samples and provides pure nucleic acids in less than 2 hours per 24 samples.

Lysing samples

The QIAamp UCP Pathogen Mini Kit has been optimized for purification of microbial DNA from whole blood, swabs, or pelleted microbial cells from biological fluids or cultures.

While for some Gram-negative bacteria a chemical lysis is sufficient, the rigid cell wall from Gram-positive bacteria as well as from yeast and other fungi needs to be disrupted by additional methods. For maximal lysis efficiency of such cells, QIAGEN recommends the use of a mechanical disruption using Pathogen Lysis Tubes that are available as accessory products (see Table 1).

Pathogen Lysis Tubes are available with large (L) and small (S) beads. While large beads have a good lysing efficiency for fungi and some bacteria, the small beads are only recommended for lysing bacteria.

Samples are lysed under highly denaturing conditions at elevated temperatures in the presence of Proteinase K and Buffer APL2 to ensure inactivation of nucleases and complete release of nucleic acids from pre-lysed microbial cells.

Refer to Table 1 for appropriate starting materials and the recommended type of Pathogen Lysis Tubes.

Table 1. Pathogen Lysis Tubes

Sample type	Target nucleic acid	Recommended bead type
Whole blood	Bacteria and fungi	Pathogen Lysis Tubes (L)
Swabs	Bacteria (Gram-positive and Gram-negative)	Pathogen Lysis Tubes (L) or Pathogen Lysis Tubes (S)
Bacterial culture (up to 2 x 10^9 cells)	Bacteria (Gram-positive and Gram-negative)	Pathogen Lysis Tubes (L) or Pathogen Lysis Tubes (S)
Yeast culture (up to 5 x 10^7 cells)	Yeast	Pathogen Lysis Tubes (L)
Biological fluids (e.g., urine or bronchoalveolar lavage [BAL])	Bacteria (Gram-positive and Gram-negative)	Pathogen Lysis Tubes (L) or Pathogen Lysis Tubes (S)

Adsorption to the QIAamp UCP Mini membrane

Binding conditions are adjusted by adding ethanol to allow optimal binding of the pathogen nucleic acids to the membrane. Lysates are then transferred onto a QIAamp UCP Mini Column, and the pathogen nucleic acids are adsorbed onto the silica membrane as the lysate is drawn through by vacuum pressure or centrifugation. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit downstream enzymatic reactions, are not retained on the QIAamp UCP Mini membrane.

A vacuum manifold (e.g., the QIAvac 24 Plus with the QIAvac Connecting System) and a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN Vacuum Pump) are required for the vacuum protocol. A vacuum regulator should be used for easy monitoring of vacuum pressures and convenient vacuum releases.

Removal of residual contaminants

Nucleic acids remain bound to the membrane while contaminants are washed away efficiently during two wash steps. In a double elution step, highly pure pathogen nucleic acids are eluted in Buffer AVE, equilibrated to room temperature.

Elution of pure nucleic acids from pathogens

Elution is performed using Buffer AVE. The elution volume can be as low as 50 µl. If higher nucleic acid concentrations are required, the elution volume can be reduced to as low as 20 µl. Low elution volume leads to highly concentrated eluates.

For downstream applications that require small starting volumes, a more concentrated eluate may increase assay sensitivity.

For downstream applications that require a larger starting volume or for multiple analyses, the elution volume can be increased up to 200 µl. However, an increase in elution volume will decrease the concentration of nucleic acids in the eluate. The eluate volume recovered can be up to 5 µl less than the volume of elution buffer applied to this column; for example, an elution volume of 20 µl results in >15 µl final eluate. The volume of eluate recovered depends on the nature of the sample.

Eluted DNA is collected in 1.5 ml elution tubes (provided). If the purified nucleic acids are to be stored for up to 24 hours, storage at 2–8°C is recommended. For periods of storage longer than 24 hours, storage at –15 to –30°C is recommended.

Description of protocols

This handbook contains two types of protocols.

- Pretreatment protocols with or without mechanical disruption detail the preliminary steps needed before purification with vacuum or spin protocols.
- DNA preparation protocols describe purification of pretreated samples using a microcentrifuge (spin protocol) or a QIAvac (vacuum protocol, recommended).

Pretreatment protocols

Different pretreatment protocols are provided for different starting materials with or without the optional use of a mechanical disruption for the lysis of the microbial cells. After the individual pretreatment steps, generic sample prep protocols (see “Protocol: Sample Prep (Vacuum Protocol)” on page 30 and “Protocol: Sample Prep (Spin Protocol)” on page 32) are provided to purify the DNA from the pretreated samples.

The following pretreatment protocols provide mechanical pre-lysis with Pathogen Lysis Tubes and procedures without mechanical pre-lysis.

The "Protocol: Pretreatment of Pathogen DNA from 400 µl Whole Blood (Mechanical Pre-lysis Protocol)" on page 20 and the "Protocol: Pretreatment of Pathogen DNA from 400 µl Whole Blood (Protocol without Pre-lysis)" on page 22 provide protocols with mechanical disruption and without, designed for isolation of DNA from whole blood.

The "Protocol: Pretreatment of Microbial DNA from Biological Fluids or Cultures (Mechanical Pre-lysis Protocol)" on page 23 and "Protocol: Pretreatment of Microbial DNA from Biological Fluids or Cultures (Protocol without Pre-lysis)" on page 25 provide procedures for purification of microbial DNA from biological fluids or 2 x 10^9 bacterial or 5 x 10^7 yeast cells.

The "Protocol: Pretreatment of Microbial DNA from Eye, Nasal, Pharyngeal, or Other Swabs (Mechanical Pre-lysis Protocol)" on page 27 and "Protocol: Pretreatment of Microbial DNA from Eye, Nasal, Pharyngeal, or Other Swabs (Protocol without Pre-lysis)" on page 29 provide procedures to isolate microbial DNA from eye, nasal, pharyngeal or other swab samples.

DNA purification protocols

There are two DNA purification protocols that can be used in conjunction with the pretreatment protocols. The "Protocol: Sample Prep (Vacuum Protocol)" on page 30 and "Protocol: Sample Prep (Spin Protocol)" on page 32 provide procedures for DNA purification from 400 µl pretreated samples using a QIAvac (recommended) or microcentrifuge. Both protocols can be used with all sample types from the pretreatment protocols.

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.

- Pipets (adjustable)
- Sterile pipet tips (pipet tips with aerosol barriers are recommended to help prevent cross-contamination)
- Thermoshaker at 56°C (capable of holding 2 ml collection tubes)
- Heating block or similar at 70°C (capable of holding 2 ml collection tubes)
- Microcentrifuge
- Ethanol (96–100%)*
- 2 ml microcentrifuge tubes
- Vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413)†
- Vacuum Regulator (cat. no. 19530) for easy monitoring of vacuum pressures and easy releasing of vacuum†
- Optional: Vortexer with Microtube foam insert (cat. no. 504-0234-00) or TurboMix® Attachment (cat. no. SI-0563), both available from Scientific Industries; TissueLyser LT (cat. no. 85600); or Fastprep®-24 (cat. no. 6004500), which is available from MP Biomedicals‡
- Optional: VacValves (cat. no. 19408)†
- Optional: QIAvac Connecting System (cat. no. 19419)†
- Optional: 2 ml collection tubes (cat. no. 19201)§

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† For use with the Sample Prep — Vacuum Protocol. Not required for the Spin Protocol.
‡ Only required for use with the pretreatment protocols with mechanical disruption.
§ For use with the Sample Prep – Spin Protocol. Not required for the Vacuum Protocol.

Important Notes

For preparation of buffers and reagents

Buffer APW1 *

Before use, add 24 ml ethanol (96–100%) to 18 ml Buffer APW1 concentrate to obtain 42 ml Buffer APW1. Mix well after adding ethanol.

Buffer APW2†

Before use, add 35 ml ethanol (96–100%) to 15 ml Buffer APW2 concentrate to obtain 50 ml Buffer APW2. Mix well after adding ethanol.

Handling of QIAamp UCP Mini Columns

Owing to the sensitivity of downstream reactions for nucleic acids, the following precautions are necessary when handling QIAamp UCP Mini Columns to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp UCP Mini Column. Pipet the sample into the QIAamp UCP Mini Column without wetting the rim of the column.
- Change pipet tips between all liquid transfers. The use of aerosol-barrier pipet tips is recommended.
- Avoid touching the QIAamp membrane with the pipet tip.
- After all pulse-vortexing steps, briefly centrifuge the 1.5 ml microcentrifuge tubes to remove drops from the inside of the lid.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

* Contains chaotropic salt. See page 6 for Safety Information.
† Contains sodium azide as a preservative.

Centrifugation

Centrifugation of QIAamp UCP Mini Columns is performed at 6000 x *g* (8000 rpm) to reduce centrifuge noise. Centrifuging QIAamp UCP Mini Columns at full speed will not affect DNA yield. Centrifugation at lower speeds is also acceptable, provided that nearly all of each solution is transferred through the QIAamp UCP Mini membrane. All centrifugation steps should be carried out at room temperature (15–25°C).

The QIAvac 24 Plus

The QIAvac 24 Plus is designed for fast and efficient vacuum processing of up to 24 QIAGEN spin columns in parallel. Samples and wash solutions are drawn through the column membranes by vacuum instead of centrifugation, providing greater speed and reduced hands-on time in purification procedures.

In combination with the QIAvac Connecting System (optional), the QIAvac 24 Plus can be used as a flow-through system. The sample flow-through is collected in a separate waste bottle.

For maintenance of the QIAvac 24 Plus, refer to the handling guidelines in the *QIAvac 24 Plus Handbook*.

Processing QIAamp UCP Mini Columns on the QIAvac 24 Plus

The QIAamp UCP Mini Columns are processed on the QIAvac 24 Plus using disposable VacConnectors and reusable VacValves. VacValves (optional) are inserted directly into the luer slots of the QIAvac 24 Plus manifold and ensure a steady flow rate, facilitating parallel processing of samples of different natures (e.g., blood and body fluids), volumes, or viscosities. They should be used if sample flow rates differ significantly to ensure consistent vacuum. VacConnectors are disposable connectors that fit between QIAamp UCP Mini Columns and VacValves or between the QIAamp UCP Mini Columns and the luer slots of the QIAvac 24 Plus. They prevent direct contact between the spin column and VacValve during purification, thereby avoiding any cross contamination between samples. VacConnectors are discarded after a single use.

Handling guidelines for the QIAvac 24 Plus

Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the QIAvac 24 Plus manifold may crack.

Always store the QIAvac 24 Plus clean and dry. For cleaning procedures see the *QIAvac 24 Plus Handbook*.

The components of the QIAvac 24 Plus are not resistant to certain solvents (Table 2). If these solvents are spilled on the unit, rinse it thoroughly with water.

To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 Plus manifold.

Always use caution and wear safety glasses when working near a vacuum manifold under pressure. Contact QIAGEN Technical Services or your local distributor for information concerning spare or replacement parts.

The vacuum pressure is the pressure differential between the inside of the vacuum manifold and the atmosphere (standard atmospheric pressure: 1013 mbar or 760 mm Hg) and can be measured using the QIAvac Connecting System or a vacuum regulator (see Figure 1). The protocols require a vacuum pump capable of producing a vacuum or –800 to –900 mbar (e.g., QIAGEN Vacuum Pump). Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce nucleic acid yield and purity and increase the risk of clogged membranes.

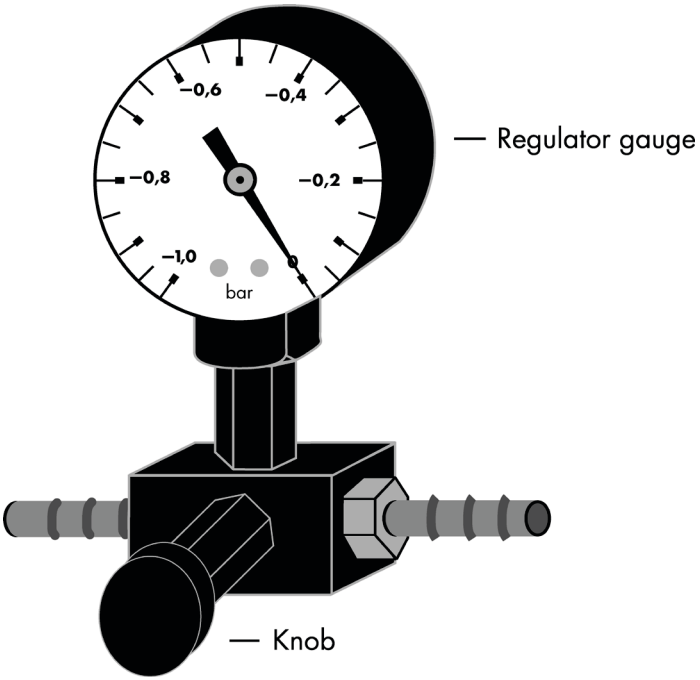

Figure 1. Schematic diagram of the Vacuum Regulator.

Table 2. Chemical resistance properties of QIAvac 24 Plus

Resistant to:		
Acetic acid	Chaotropic salt	Chlorine bleach
Chromic acid	Concentrated alcohols	Hydrochloric acid
SDS	Sodium chloride	Sodium hydroxide
Tween® 20	Urea	
Not resistant to:		
Benzene	Chloroform	Ethers
Phenol	Toluene	

Setup of the QIAvac Plus vacuum manifold

1. Connect the QIAvac 24 Plus to a vacuum source. If using the QIAvac Connecting System, connect the system to the manifold and vacuum source as described in Appendix A of the *QIAvac 24 Plus Handbook*.
2. Insert a VacValve (optional) into each luer slot of the QIAvac 24 Plus that is to be used (see Figure 2). Close unused luer slots with luer plugs or close the inserted VacValve.

   VacValves should be used if flow rates of samples differ significantly to ensure consistent vacuum.
3. Insert a VacConnector into each VacValve (see Figure 2).

   Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
4. Place the QIAamp UCP Mini Columns into the VacConnectors on the manifold (see Figure 2).

   Note: Save the collection tube from the blister pack for use in the purification protocol.
5. Insert a tube extender (3 ml) into each QIAamp UCP Mini Column (see Figure 2).

   Note: Make sure that the tube extender is firmly inserted into the QIAamp UCP Mini Column to avoid leakage of sample.
6. For nucleic acid purification, follow the instructions in the protocols. Discard the VacConnectors appropriately after use.

   Leave the lid of the QIAamp UCP Mini Column open while applying vacuum.

   Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during processing. For faster vacuum release, a vacuum regulator should be used (see Figure 1).

   Note: 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.
7. After processing samples, clean the QIAvac 24 Plus (see "Cleaning and Decontaminating the QIAvac 24 Plus" in the *QIAvac 24 Plus Handbook*).

Note: Buffers APL2 and APW1 are not compatible with disinfecting agents containing bleach. See page 6 for Safety Information.

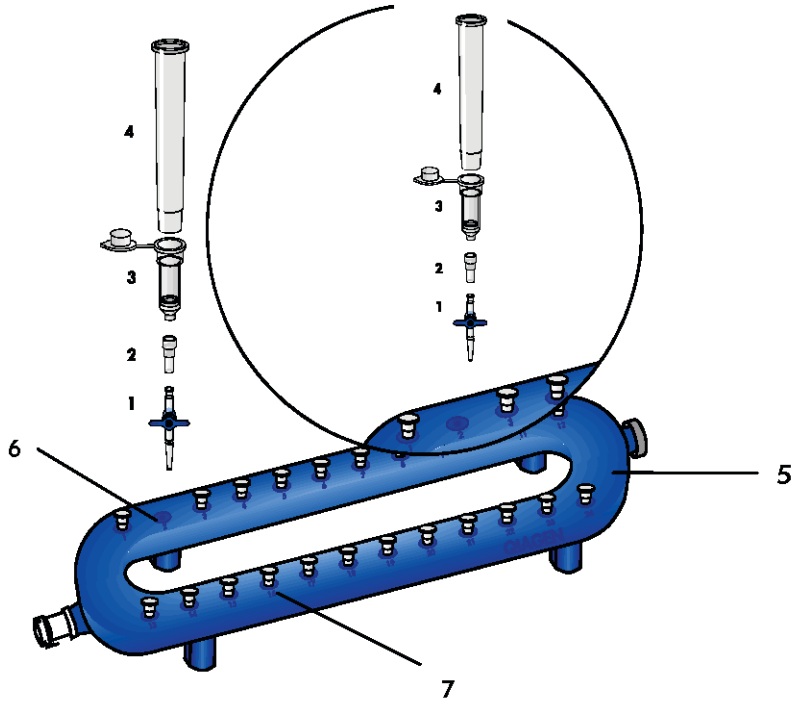

Figure 2. Setting up the QIAvac 24 Plus with QIAamp UCP Mini Columns using VacValves, VacConnectors, and 20 ml Tube Extenders. (1) VacValve*; (2) VacConnector; (3) QIAamp UCP Mini Column; (4) Tube Extender; (5) QIAvac 24 Plus vacuum manifold; (6) Luer slot of the QIAvac 24 Plus; (7) Luer slot closed with luer plug.

We recommend labeling the tubes and the QIAamp UCP Mini Columns for use on the QIAvac 24 Plus vacuum system according to the scheme in Figure 3 to avoid the mix-up of samples. This figure can be photocopied and labeled with the names of the samples.

* Must be purchased separately.

Date: _____
Operator: _____
Run ID: _____

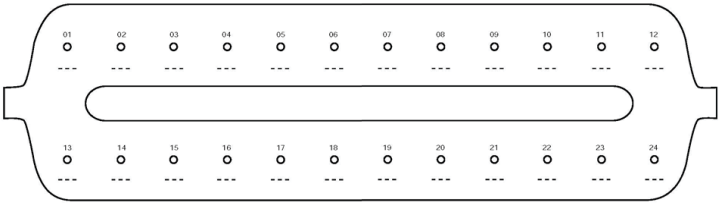

Figure 3. Labeling scheme for tubes and QIAamp UCP Mini Columns for use on the QIAvac 24 Plus vacuum system.

Processing QIAamp UCP Mini Columns using a microcentrifuge (spin protocols)

Close the QIAamp UCP Mini Column before placing it in the microcentrifuge. Centrifuge as described.

- Remove the QIAamp UCP Mini Column and collection tube from the microcentrifuge. Place the QIAamp UCP Mini Column in a new collection tube (not provided). Discard the filtrate and the collection tube. Note that the filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp UCP Mini Column at a time, taking care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, fill a rack with collection tubes to which the QIAamp UCP Mini Columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the QIAamp UCP Mini Columns can be placed directly in the microcentrifuge.

Protocol: Pretreatment of Pathogen DNA from 400 µl Whole Blood (Mechanical Pre-lysis Protocol)

This protocol uses mechanical pre-lysis with Pathogen Lysis Tubes for purification of pathogen DNA from whole blood.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Heat a water bath or heating block to 56°C for use in step 2 of both sample prep protocols and for step 11 of the "Protocol: Sample Prep (Vacuum Protocol)" or step 12 of "Protocol: Sample Prep (Spin Protocol)".
- Heat a water bath or heating block to 70°C for use in step 4 of both sample prep protocols.
- Equilibrate Buffer AVE to room temperature for elution in step 12 of "Protocol: Sample Prep (Vacuum Protocol)" or step 13 of "Protocol: Sample Prep (Spin Protocol)".
- Ensure that Buffer APW1 and Buffer APW2 have been prepared according to the instructions on page 9.
- Before use, add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, before use transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after adding Reagent DX. After preparation the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. Add 100 µl Buffer ATL (containing Reagent DX) into a fresh Pathogen Lysis Tube.

   Pathogen Lysis Tubes (L) are recommended for the lysis of bacteria and fungi in blood.

2. Add 400 µl blood and mix by vortexing for 10 s.
3. Place the Pathogen Lysis Tube on a vortexer with the Microtube Foam Insert and vortex for 10 min at maximum speed.

   Alternatively, the Pathogen Lysis Tube can be vortexed for 10 min at 50 Hz on a TissueLyser LT or by using the FastPrep-24 instrument applying a velocity of 6.5 m/s for two times 45 s with a 5 min intermission.

4. Remove the Pathogen Lysis Tube from the vortexer and centrifuge the tube for 5 s at 8000 x *g* to remove drops from the inside of the lid.
5. Transfer 400 µl from the supernatant from step 4 into a fresh 2 ml microcentrifuge tube.
6. Continue with “Protocol: Sample Prep (Vacuum Protocol)” on page 30 or “Protocol: Sample Prep (Spin Protocol)” on page 32.

Protocol: Pretreatment of Pathogen DNA from 400 µl Whole Blood (Protocol without Pre-lysis)

This protocol is for purification of pathogen DNA from whole blood.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Heat a water bath or heating block to 56°C for use in step 2 of both sample prep protocols and for step 11 of the "Protocol: Sample Prep (Vacuum Protocol)" or step 12 of "Protocol: Sample Prep (Spin Protocol)".
- Heat a water bath or heating block to 70°C for use in step 4 of both sample prep protocols.
- Equilibrate Buffer AVE to room temperature for elution in step 12 of "Protocol: Sample Prep (Vacuum Protocol)" or step 13 of "Protocol: Sample Prep (Spin Protocol)".
- Ensure that Buffer APW1 and Buffer APW2 have been prepared according to the instructions on page 9.

Procedure

1. Add 400 µl of blood into a fresh 2 ml tube.
2. Continue with "Protocol: Sample Prep (Vacuum Protocol)" on page 30 or "Protocol: Sample Prep (Spin Protocol)" on page 32.

Protocol: Pretreatment of Microbial DNA from Biological Fluids or Cultures (up to 2 x 10^9 Bacterial or 5 x 10^7 Yeast Cells) (Mechanical Pre-lysis Protocol)

This protocol uses mechanical pre-lysis with Pathogen Lysis Tubes for purification of microbial DNA from biological fluids or cultures (up to 2 x 10^9 bacterial or 5 x 10^7 yeast cells).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Heat a water bath or heating block to 56°C for use in step 2 of both sample prep protocols and for step 11 of the "Protocol: Sample Prep (Vacuum Protocol)" or step 12 of "Protocol: Sample Prep (Spin Protocol)".
- Heat a water bath or heating block to 70°C for use in step 4 of both sample prep protocols.
- Equilibrate Buffer AVE to room temperature for elution in step 12 of "Protocol: Sample Prep (Vacuum Protocol)" or step 13 of "Protocol: Sample Prep (Spin Protocol)".
- Ensure that Buffer APW1 and Buffer APW2 have been prepared according to the instructions on page 9.
- Before use, add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, before use transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after adding Reagent DX. After preparation the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. Add up to 1.5 ml biological fluid or liquid culture to the Pathogen Lysis tube and centrifuge the tube for 5 min at maximum speed (>14,000 x *g*).
2. Remove and discard the supernatant. If necessary, repeat steps 1 and 2.

   Use a pipet to remove the supernatant, being careful to not remove any glass beads.
3. Add 500 µl Buffer ATL (containing Reagent DX) and resuspend the pellet.
4. Place the Pathogen Lysis Tube on a vortexer with the Microtube Foam Insert and vortex for 10 min at maximum speed.

   Alternatively, the Pathogen Lysis Tube may be vortexed on a Lab vortexer with an adapter (e.g., Microtube Foam Insert, cat. no. 504-0234-00, or TurboMix Attachment, cat. no. SI-0563, from Scientific Industries).
5. Remove the Pathogen Lysis Tube from the vortexer and centrifuge the tube for 5 s at 8000 x *g* to remove drops from the inside of the lid.
6. Carefully transfer 400 µl of the supernatant from the Pathogen Lysis Tube into a fresh 2 ml microcentrifuge tube without removing any glass beads.
7. Continue with “Protocol: Sample Prep (Vacuum Protocol)” on page 30 or “Protocol: Sample Prep (Spin Protocol)” on page 32.

Protocol: Pretreatment of Microbial DNA from Biological Fluids or Cultures (up to 2 x 10^9 Bacterial or 5 x 10^7 Yeast Cells) (Protocol without Pre-lysis)

This protocol is for purification of microbial DNA from biological fluids or cultures (up to 2 x 10^9 bacterial or 5 x 10^7 yeast cells).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Heat a water bath or heating block to 56°C for use in step 2 of both sample prep protocols and for step 11 of the "Protocol: Sample Prep (Vacuum Protocol)" or step 12 of "Protocol: Sample Prep (Spin Protocol)".
- Heat a water bath or heating block to 70°C for use in step 4 of both sample prep protocols.
- Equilibrate Buffer AVE to room temperature for elution in step 12 of "Protocol: Sample Prep (Vacuum Protocol)" or step 13 of "Protocol: Sample Prep (Spin Protocol)".
- Ensure that Buffer APW1 and Buffer APW2 have been prepared according to the instructions on page 9.

Procedure

1. Add up to 1.5 ml biological fluid into a 2 ml tube and centrifuge the tube for 5 min at maximum speed (>14,000 x *g*).
2. Remove and discard the supernatant. If necessary, repeat steps 1 and 2.
3. Add 400 µl Buffer ATL and resuspend the pellet.
4. Continue with "Protocol: Sample Prep (Vacuum Protocol)" on page 30 or "Protocol: Sample Prep (Spin Protocol)" on page 32.

Protocol: Pretreatment of Microbial DNA from Eye, Nasal, Pharyngeal, or Other Swabs (Mechanical Pre-lysis Protocol)

This protocol uses mechanical pre-lysis with Pathogen Lysis Tubes for purification of microbial DNA from nasal, pharyngeal, or other swabs.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Heat a thermoshaker to 56°C for use in step 3 of this pretreatment protocol and for step 2 of both sample prep protocols.
- Heat a water bath or heating block to 70°C for use in step 4 of both sample prep protocols.
- Equilibrate Buffer AVE to room temperature for elution in step 12 of "Protocol: Sample Prep (Vacuum Protocol)" or step 13 of "Protocol: Sample Prep (Spin Protocol)".
- Ensure that Buffer APW1 and Buffer APW2 have been prepared according to the instructions on page 9.
- Before use, add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, before use transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after adding Reagent DX. After preparation the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. Cut the tip off a swab and place in a 2 ml microcentrifuge tube.
2. Add 650 µl Buffer ATL (containing Reagent DX).
3. Place a tube on a thermoshaker and incubate for 10 min at 56°C with continuous shaking at 600 rpm.
4. Open the 2 ml microcentrifuge tube and carefully transfer all the liquid into a Pathogen Lysis Tube.
5. Place the Pathogen Lysis Tube on a vortexer with the Microtube Foam Insert and vortex for 10 min at maximum speed.

   Alternatively, the Pathogen Lysis Tube may be vortexed on a Lab vortexer with an adapter (e.g., Microtube Foam Insert, cat. no. 504-0234-00, or TurboMix Attachment, cat. no. SI-0563, from Scientific Industries).
6. Remove the Pathogen Lysis Tube from the vortexer and centrifuge the tube for 5 s at 8000 x *g* to remove drops from the inside of the lid.
7. Carefully transfer all of the supernatant (approx. 400 µl) from the Pathogen Lysis Tube into a fresh 2 ml tube without removing any glass beads.
8. Continue with “Protocol: Sample Prep (Vacuum Protocol)” on page 30 or “Protocol: Sample Prep (Spin Protocol)” on page 32.

Protocol: Pretreatment of Microbial DNA from Eye, Nasal, Pharyngeal, or other Swabs (Protocol without Pre-lysis)

This protocol is for purification of microbial DNA from nasal, pharyngeal, or other swabs.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Heat a thermoshaker to 56°C for use in step 3 of this pretreatment protocol and for step 2 of both sample prep protocols.
- Heat a water bath or heating block to 70°C for use in step 4 of both sample prep protocols.
- Equilibrate Buffer AVE to room temperature for elution in step 12 of "Protocol: Sample Prep (Vacuum Protocol)" or step 13 of "Protocol: Sample Prep (Spin Protocol)".
- Ensure that Buffer APW1 and Buffer APW2 have been prepared according to the instructions on page 9.

Procedure

1. Cut the tip off a swab and place it in a 2 ml microcentrifuge tube.
2. Add 500 µl Buffer ATL.
3. Place the tube on a thermoshaker and incubate for 10 min at 56°C with continuous shaking at 600 rpm.
4. Open the 2 ml tube and carefully transfer all the liquid into a fresh 2 ml tube.
5. Continue with "Protocol: Sample Prep (Vacuum Protocol)" on page 30 or "Protocol: Sample Prep (Spin Protocol)" on page 32.

Protocol: Sample Prep (Vacuum Protocol)

This protocol is for purification of microbial DNA from 400 µl pretreated samples using a vacuum (recommended). Purification of DNA from 400 µl pretreated samples can be performed using a spin protocol see page 32, "Protocol: Sample Prep (Spin Protocol)".

Procedure

1. Add 40 µl Proteinase K and mix the sample by vortexing for 10 s.
2. Incubate the sample at 56°C for 10 min.
3. Add 200 µl of Buffer APL2 to the sample. Close the cap and mix by pulse-vortexing for 30 s.

   Note: To ensure efficient pathogen lysis, it is essential that the sample and Buffer APL2 are mixed thoroughly to yield a homogeneous solution.
4. Incubate at 70°C for 10 min.
5. Briefly spin the tube to remove drops from the inside of the lid.
6. Add 300 µl ethanol to the lysate. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7. Carefully apply the lysate from step 6 into the tube extender of the QIAamp UCP Mini Column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

   To avoid cross-contamination be careful to not cross-neighboring QIAamp UCP Mini Columns while tube extenders are removed.
8. Apply 600 µl of Buffer APW1 to the QIAamp UCP Mini Column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer APW1 has been drawn through the column, switch off the vacuum pump and release the pressure to 0 mbar.

9. Apply 750 µl of Buffer APW2 to the QIAamp UCP Mini Column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer APW2 has been drawn through the column, switch off the vacuum pump and release the pressure to 0 mbar.
10. Close the lid of the QIAamp UCP Mini Column. Remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp UCP Mini Column in a clean 2 ml collection tube and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
11. Place the QIAamp UCP Mini Column into a new 2 ml collection tube. Open the lid and incubate the assembly at 56°C for 3 min to dry the membrane completely.
12. Place the QIAamp UCP Mini Column in a clean 1.5 ml elution tube and discard the collection tube. Carefully apply 20–100 µl of Buffer AVE to the center of the QIAamp UCP Mini membrane. Close the lid and incubate at room temperature for 1 min.

    Important: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl), the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will up to 5 µl less than the elution volume applied onto the column.
13. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the DNA.
14. Repeat steps 12 and 13.

Protocol: Sample Prep (Spin Protocol)

This protocol is for purification of microbial DNA from 400 µl pretreated samples using a microcentrifuge. Purification of DNA from 400 µl pretreated samples can be performed using a vacuum protocol see page 30, "Protocol: Sample Prep (Vacuum Protocol)".

Procedure

1. Add 40 µl Proteinase K and mix the sample by vortexing for 10 s.
2. Incubate the sample at 56°C for 10 min.
3. Add 200 µl of Buffer APL2 to the sample. Close the cap and mix by pulse-vortexing for 30 s.

   Note: To ensure efficient pathogen lysis, it is essential that the sample and Buffer APL2 are mixed thoroughly to yield a homogeneous solution.
4. Incubate at 70°C for 10 min.
5. Briefly spin the tube to remove drops from the inside of the lid.
6. Add 300 µl ethanol to the lysate. Close the cap, and mix thoroughly by pulse-vortexing for 15–30 s.
7. Carefully apply 600 µl of the mixture from step 6 to the QIAamp UCP Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

   Close each spin column to avoid aerosol formation during centrifugation.
8. Repeat step 7 by applying the remaining mixture from step 6 to the QIAamp UCP Mini spin column.

9. Carefully open the QIAamp UCP Mini spin column and add 600 µl Buffer APW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP Mini spin column in a clean 2 ml collection tube (not provided), and discard the collection tube containing the filtrate.*
10. Carefully open the QIAamp UCP Mini spin column and add 750 µl Buffer APW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
11. **Recommended**: Place the QIAamp UCP Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

    This step helps to eliminate the chance of possible Buffer APW2 carryover.
12. Place the QIAamp UCP Mini Column into a new 2 ml collection tube. Open the lid and incubate the assembly at 56°C for 3 min to dry the membrane completely.
13. Place the QIAamp UCP Mini Column in a clean 1.5 ml elution tube and discard the collection tube. Carefully apply 20–100 µl of Buffer AVE to the center of the QIAamp UCP Mini membrane. Close the lid and incubate at room temperature for 1 min.

    Important: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl) the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will up to 5 µl less than the elution volume applied onto the column.
14. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the DNA.
15. Repeat steps 13 and 14.

* Flow-through contains Buffer APL2 or Buffer APW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

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 in 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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Little or no pathogen DNA in the eluate		
a)	Primary blood tube contains an anticoagulant other than EDTA	Anticoagulants other than EDTA may lead to accelerated DNA degradation compared to EDTA blood. Repeat the purification procedure with new samples.
b)	Inefficient mechanical lysis of pathogens	Make sure that the Pathogen Lysis Tube has been vortexed for 10 min at maximal speed using a microtube foam insert of a Vortex-Genie® or using a TissueLyser LT at 50 Hz or using a FastPrep-24 instrument with 2 x 45 s with a 5 min intermission.
c)	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.
d)	Buffer APW1 or Buffer APW2 prepared incorrectly	Check that Buffer APW1 and Buffer APW2 concentrates were diluted with the correct volume of ethanol (see page 9). Repeat the purification procedure with new samples.
e)	Buffer APW1 or Buffer APW2 prepared with 70% ethanol	Check that Buffer APW1 and Buffer APW2 concentrates were diluted with 96–100% ethanol (see page 9). Repeat the purification procedure with new samples.
f)	QIAamp UCP Mini Column not incubated at room temperature (15–25°C) for 1 min	After addition of Buffer AVE the QIAamp UCP Mini Column should be incubated at room temperature for 1 min.

		Comments and suggestions
Eluted nucleic acids do not perform well in downstream reactions		
a)	Little or no DNA in the eluate	See "**Little or no pathogen DNA in the eluate**" 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 reaction. Reduce or increase the volume of eluate added to the downstream reaction accordingly. The elution volume can be adapted proportionally.
c)	Buffers not mixed thoroughly	Salt and ethanol components of wash Buffer APW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
d)	Buffers APW1 and APW2 used in the wrong order	Ensure that Buffers APW1 and APW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
e)	Reduced sensitivity of downstream reaction	Adjust the volume of eluate added as template in the downstream reaction.
f)	Residual ethanol in the eluate	Use recommended drying step in the relevant protocol.
White precipitate in Buffer ATL or Buffer APL2		
White precipitate after addition of Buffer APL2		In most cases, the precipitate formed after addition of Buffer APL2 will dissolve during incubation at 70°C. The precipitates do not interfere with the procedure or with any subsequent application.
White precipitate in Buffer ATL or Buffer APL2		
White precipitate may form after storage at low temperature or prolonged storage		Any precipitate in Buffer ATL or Buffer APL2 must be dissolved by incubation of the buffer at 56°C. The precipitate has no effect on function. Dissolving the precipitate at high temperature will not compromise yield or quality of the purified nucleic acids.

		Comments and suggestions
Clogging of Membrane		
a)	Vacuum pressure of –800 to –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 directly into the luer extensions. Insert QIAamp UCP Mini Columns into VacConnectors, 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.
b)	Inefficient proteinase digestion	If Proteinase K is subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh Proteinase K.
c)	Membrane is clogged	Close the VacValve, if used, and carefully remove the whole assembly consisting of tube extender, QIAamp UCP Mini Column, VacConnector, and VacValve from the QIAvac 24 Plus manifold. Carefully transfer the remaining sample lysate from the tube extender to a fresh 2 ml tube. Remove the QIAamp UCP Mini Column from the assembly (see above), place it in a 2 ml collection tube, and spin it at full speed for 1 minute or until sample has completely passed through the membrane. Re-assemble the QIAamp UCP Mini Column with tube extender, VacConnector and VacValve (optional). Transfer the remaining sample lysate into the tube extender, switch on the vacuum pump, open the VacValve, and pass the remaining lysate through the QIAamp UCP Mini Column. Repeat the above procedure if the QIAamp UCP Mini Column continues to clog.

Appendix: General Remarks

General handling

Proper microbiological, aseptic technique should always be used when purifying nucleic acids with the QIAamp UCP Pathogen Mini Kit. Hands and dust particles may carry bacteria and molds and are the most common sources of contamination. Always wear latex or vinyl gloves while handling reagents and consumables. Change gloves frequently and keep tubes closed whenever possible. Keep purified nucleic acids on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally free of contaminating nucleic acids as well as nucleases and do not require pretreatment.

Ordering Information

Product	Contents	Cat. no.
QIAamp UCP Pathogen Mini Kit (50)	For 50 preps: 50 QIAamp UCP Mini Columns, Collection Tubes, Tube Extenders, Elution Tubes, and Buffers	50214
Accessories		
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes and 1 vial Reagent DX	19091
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes and 1 vial Reagent DX	19092

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

Date	Changes
04/2021	Corrected the Buffer AVE in the Kit Contents section by removing "(concentrate)". Corrected the numbering of figures. Presented Figure 2 in one image. Updated the Ordering Information section.

Notes

Notes

Notes

Limited License Agreement for QIAamp UCP Pathogen Mini Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.

HB-0558-004 04/2021