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QIAmini Pathogen Kit Instructions for Use

For automated, simultaneous purification of viral DNA and RNA, and bacterial DNA from plasma and body fluids using QIAmini

Sample to Insight

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Kit Contents

QIAmini Pathogen Kit Catalog no. Number of reactions	(48) 590205 48
Carrier RNA	310 µg
RNase-free water	10 mL
Proteinase K	2 mL
Buffer ACL*	25 mL
QIAmini Pathogen Cartridges*	48
QIAmini Rod Covers	12
Quick-Start Protocol	1

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. See Safety Information.

Shipping and Storage

The QIAmini Pathogen Kit is shipped at ambient temperature. Upon receipt, store kit components dry at room temperature (15–25°C).

When stored properly, buffers and reagent cartridges are stable until the expiration date on the kit label.

Intended Use

The QIAmini Pathogen Kit is intended for research use only. 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 at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION Do not use bleach, or acidic solutions directly to the sample preparation waste.

Buffer ACL and buffers in the QIAmini Pathogen Cartridges contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If liquid containing potentially infectious agents is spilled on QIAmini, refer to the instrument user manual for decontamination instructions.

Quality Control

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

Introduction

This instructions for use describes the use of QIAmini Pathogen Kit with the QIAmini instrument. For detailed description of QIAmini, refer to the *QIAmini User Manual* (**www.qiagen.com/HB-3799**) and the *QIAmini Suite User Guide* (**www.qiagen.com/HB-3800**).

QIAmini Pathogen Kit enables automated purification of DNA from the following sample materials using QIAmini.

- Serum
- Plasma
- Whole blood
- Urine
- Transport media
- Swabs
- Respiratory samples

QIAmini Pathogen Kit can be used to purify nucleic acids from a broad range of DNA and RNA viruses and bacteria. However, kit performance is not guaranteed for each pathogen species and must be validated by the user. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready to use for highly sensitive detection in downstream assays, such as amplification, or other enzymatic reactions. For the purification of nucleic acids from urine, respiratory samples, and difficult-to-lyse bacteria, additional pretreatment protocols have been developed to be used before the purification procedure on QIAmini. The QIAmini instrument can process up to eight samples per run.

Principle and procedure

QIAmini Pathogen Kit combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure reproducible handling of samples and comprises the steps: bind, wash, and elute, while preparation of the respective samples is carried out before loading QIAmini.

Sample lysis

Lysis of samples to be processed with the QIAmini Pathogen Kit is performed before loading onto the QIAmini instrument. The kit uses one automated protocol for purification of viral nucleic acids and bacterial DNA. Samples are lysed with Proteinase K and lysis buffer, which together support digestion of viral coat proteins and bacterial membrane proteins, as well as inactivation of RNases. Difficult-to-lyse pathogens may require additional upfront pretreatment, such as mechanical disruption or extended homogenization, to support efficient lysis. After lysis, the prepared sample is transferred to the QIAmini Pathogen Cartridge, where nucleic acids bind to the silica surface of the magnetic particles under optimized binding conditions.

Washing of bound nucleic acids

While viral and bacterial nucleic acids remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps.

Elution of purified nucleic acids

In a single step, purified viral nucleic acids and bacterial DNA are eluted in RNase-free water. Protocols for the Pathogen application include 2 elution options. To achieve a target eluate volume of 100 µL, 125 µL RNase-free water is loaded for elution. To achieve a target eluate volume of 50 µL, 75 µL RNase-free water is loaded for elution. The increased input volume compensates for the inherent dead volume associated with magnetic particles and helps ensure recovery of the intended eluate volume. The purified nucleic acids can be used immediately in downstream applications or stored for future use.

Equipment and Reagents 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. These are available online in convenient and compact PDF at **www.qiagen.com/safety** where you can find, view, and print the SDS kit for each QIAGEN kit and kit component.

The following materials are not included in the kit, and are to be supplied by the user:

For all protocols

- Pipettes and sterile, RNase-free pipette tips
- Disposable gloves
- Thermal shaker or heated water bath
- Microcentrifuge (with rotor for 2 mL tubes)
- 1.5 mL tubes
- **Optional**: Vortexer

For pretreatment of difficult-to-lyse bacteria (mechanical disruption)

- Pathogen Lysis Tubes S (cat. no. 19091, including Reagent DX) or Pathogen Lysis Tubes L (cat. no. 19092, including Reagent DX)
- Alternatively, the PowerBead Pro Tube (cat. no. 19301) or the PowerBead Pro Plate (cat. no. 19311) can be used.

  Note: The choice of Pathogen Lysis Tube variant/PowerBead Pro Tube or Plate depends on the pathogen target.
- Buffer ATL (cat. no. 19076 or 939011)
- Vortexer with Microtube Foam Insert or TissueLyser III (cat. no. 9003240)

For pretreatment of difficult-to-lyse bacteria (extended protocol)

- Buffer ATL (cat. no. 19076 or 939011)

For pretreatment of urine

- Buffer ATL (cat. no. 19076 or 939011)

For pretreatment of respiratory samples

- Sputasol (Oxoid Limited; **www.oxoid.com**) and 37°C water bath,* or
- NAC buffer (10 g N-acetylcysteine per liter of 0.9% NaCl solution), or
- PBS or Buffer AE (cat. no. 19077), DTT, and 37°C water bath

* Ensure that the instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Important Notes

Starting material

The purification procedure is optimized for the extraction of viral nucleic acids and bacterial DNA from a 200 µL sample volume. Prevent formation of foam in or on the samples. Depending on the starting material, sample pretreatment may be required. Samples should be equilibrated to room temperature (15–25°C) before starting the extraction.

Blood and plasma samples

Blood samples treated with EDTA, CPDA (citrate phosphate dextrose anticoagulant), or citrate as anticoagulant can be used for plasma preparation or directly as sample material.* Samples can be either fresh or frozen, provided that they have not been refrozen after thawing.

After collection (and centrifugation in the case of serum or plasma), samples can be stored at 2–8°C for up to 6 h. For longer storage, we recommend freezing aliquots at –20°C to –80°C. Whole blood should be processed as fresh samples. If storage is required, we recommend storage at 2–8°C for up to 2 days. Thaw samples at room temperature. Do not refreeze the aliquots after thawing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral and bacterial titers and, therefore, reduced yields of viral and bacterial nucleic acids. If cryoprecipitates are visible in serum or plasma, centrifuge at 6800 × *g*, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately. This step does not reduce viral titers but bacterial titers can be affected.

* Blood samples treated with heparin should not be used because heparin can interfere with downstream applications.

Swabs

Swabs should be placed immediately into a sterile transport tube containing UTM (universal transport medium), VTM (viral transport medium), or sterile 0.9% saline.

Preparing lysis buffer

Precipitates may form in pretreatment Buffer ATL or lysis Buffer ACL during storage at room temperature or at 4°C. Dissolve the precipitates by incubating the bottles at 50°C for 15 min and shaking them manually twice within this incubation period.

Preparing carrier RNA

Carrier RNA enhances viral nucleic acid binding to the silica surface of the magnetic particles, especially if the sample contains very few target molecules. The addition of large amounts of carrier RNA also reduces the risk of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergent in the lysis buffer. If carrier RNA is not added to the reaction, recovery of viral DNA or RNA may be reduced.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain viral and bacterial nucleic acids and carrier RNA, and in each eluate, the amount of carrier RNA will greatly exceed the amount of viral and/or bacterial nucleic acids. The amount of eluate added to downstream amplification reactions should therefore be based on the amount of carrier RNA in the eluate. To obtain the highest level of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA solution added.

Dissolve the lyophilized carrier RNA thoroughly in RNase-free water (final concentration of 1 µg/µL), divide it into conveniently sized aliquots, and store at –30°C to –15°C. Do not freeze–thaw the aliquots more than 3 times.

Using internal control

Using QIAmini Pathogen Kit, in combination with commercially available amplification systems, may require introducing an internal control into the purification procedure to monitor the efficiency of sample preparation.

Internal control DNA or RNA can be added to the lysis and sample mixture. If the internal control is stable in plasma, serum, urine, respiratory samples, whole blood, transport media, or dried swabs (e.g., armored RNA), it can alternatively be added to the sample shortly before beginning the sample preparation procedure.

Refer to the manufacturer's instructions to determine the optimal concentration. Using a different concentration than recommended may reduce the amplification efficiency.

Note: Internal controls are not provided in the QIAmini Pathogen Kit.

Elution volumes and eluate handling

In the final step of the purification procedure, viral and bacterial nucleic acids are eluted in RNase-free water. The protocol is optimized for target eluate volumes of 50 or 100 µL and requires the addition of 1 µL elution buffer per 1 µL magnetic particles to compensate for the dead volume* of the magnetic particles. Therefore, for target eluate volumes of 50 or 100 µL, the recommended elution buffer volumes are 75 or 125 µL, respectively.

Yields of viral nucleic acids and bacterial DNA

The yields of viral nucleic acids and bacterial DNA obtained in the purification procedure are normally lower than 1 µg and therefore are difficult to quantify using a spectrophotometer. We recommend using quantitative amplification methods to determine yields. Remember that

* Dead volume is the amount of liquid that cannot be recovered due to the inherent features of the magnetic particles.

the purified nucleic acids contain much more carrier RNA than viral nucleic acids or bacterial DNA.

Storing viral nucleic acids and bacterial DNA

For short-term storage of up to 24 h, we recommend storing the purified viral DNA and RNA, or bacterial DNA at 2–8°C. For long-term storage of over 24 h, we recommend storage at –90°C to –65°C (preferred) or at –30°C to –15°C.

Working with QIAmini

The main features of QIAmini include the following:

- Purification of high-quality nucleic acids from a wide variety of sample types
- Flexible throughput, allowing processing of up to eight samples per run
- Simple, low-complexity automation of magnetic, particle-based bind–wash–elute workflows
- Pre-filled reagent cartridges enabling convenient load-and-go operation
- Compact footprint, designed to fit on any laboratory bench and allow rapid implementation
- User-friendly operation, making automated nucleic acid purification accessible even for occasional users
- Browser-based application for protocol management
- UV decontamination to help reduce sample carryover from run-to-run and allow pathogen decontamination on worktable surface

Note: UV decontamination helps reduce possible pathogen contamination on QIAmini worktable surfaces. The efficiency of inactivation must be determined for each specific

organism and depends on factors such as sample type and contaminant load. QIAGEN cannot guarantee complete eradication of specific pathogens.

Pretreatment of samples

The standard protocol of QIAmini Pathogen Kit enables direct and fast extraction of viral DNA, viral RNA, and bacterial DNA from a 200 µL starting volume of plasma, serum, blood, urine, transport media, swabs, or respiratory samples. Depending on the sample material or target pathogen, additional pretreatment may be required. The QIAmini Pathogen Kit provides pretreatment options for highly inhibitory urine samples, viscous respiratory samples, and difficult-to-lyse bacteria. For difficult-to-lyse bacteria, 2 pretreatment approaches are available: a mechanical disruption protocol and an extended protocol. The extended protocol incorporates a centrifugation step and additional lysis to support the extraction of bacterial DNA. Simultaneous extraction of viral RNA and DNA remains possible by using the supernatant obtained from the first centrifugation step as input for the standard purification workflow.

Protocol: Pretreatment of Urine

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB-3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- This protocol provides guidelines for urine pretreatment prior to nucleic acid purification (see "Standard Protocol: Purification of Viral DNA and RNA, and Bacterial DNA" on page 24). Urine is suitable as a direct input material. For samples with higher concentrations of inhibitors, the following pretreatment steps may offer improved outcomes.

Procedure

1. Add 150 µL urine to 50 µL Buffer ATL to a final volume of 200 µL.
2. Mix the solution carefully.
3. Proceed to the purification protocol (see page 24).

Protocol: Pretreatment of Respiratory Samples

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB- 3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- This protocol is intended for pretreatment of viscous samples prior to nucleic acid purification. Non-viscous respiratory samples require no pretreatment and can be used directly as starting material in the purification protocol (see page 24).

Procedure

1. Liquefy the sample according to step 1a, 1b, or 1c.
    a. Add 1 volume of Sputasol solution to 1 volume of sample and shake well. Place in a 37°C water bath and incubate with periodic shaking until the sample is completely liquefied.

b. Mix 1 volume of sample with 1 volume of NAC buffer (10 g N-acetylcysteine per liter of 0.9% NaCl solution).

Note: If the sample is very viscous, or solid (e.g., when working with lower respiratory samples), try to disrupt the sample mechanically by pipetting up and down. Incubate for 30 min at room temperature (15–25°C) with constant shaking.

Note: For easier pipetting, it may be necessary to cut off the end of the pipette tip. If the sample is solid, the incubation time needs to be increased to completely liquefy the sample.

c. Mix 1 volume of sample with 1 volume of 1 × PBS or Buffer AE. Add freshly prepared DTT to a final concentration of 0.15% (w/v). Incubate the sample at 37°C until the sample is completely liquefied.

2. Centrifuge the liquefied sample to pellet debris, then transfer the clear supernatant to a clean tube.
3. Proceed to the purification protocol (see page 24).

Protocol: Pretreatment of Difficult-to-Lyse Bacteria (Mechanical Disruption)

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB-3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- This pretreatment is for the extraction of DNA of difficult-to-lyse bacteria from body fluids.
- Buffer ATL and Pathogen Lysis Tubes L or S with glass beads (including Reagent DX) must be ordered separately (see Ordering Information on page 30).
- Alternatively, the PowerBead Pro Tube (cat. no. 19301) or the PowerBead Pro Plate (cat. no. 19311) can be used.

  Note: The choice of Pathogen Lysis Tube variant/PowerBead Pro Tube or Plate depends on the pathogen target.

Things to do before starting

- Before use, add 100 µL Reagent DX to 15 mL Buffer ATL. If smaller amounts are needed, transfer 1.5 mL of Buffer ATL into a sterile 2 mL vial and add 10 µL Reagent DX. Mix well after addition of 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.
2. Add 400 µL blood or other sample fluids.

   If using less starting material, adjust the volume to 400 µL with PBS or 0.9% NaCl.
3. Place the Pathogen Lysis Tube on a vortexer with a Microtube Foam Insert and vortex for 10 min at maximum speed.

   Alternatively, use the Vortex-Genie 2 and QIAGEN Vortex Adapter for 24 (1.5–2.0 mL) tubes (cat. no. 13000-V1-24).

   For higher throughput, the Pathogen Lysis Tube can be vortexed for 2 × 5 min at 25 Hz on TissueLyser LT or, alternatively, on the TissueLyser III (cat. no. 9003240) using adapter sets for the Pathogen Lysis Tube (TissueLyser Adapter Set 2 × 24 [cat. no. 69982] or 2 mL Tube Holder [cat. no. 11993] in conjunction with Plate Adapter Set [cat. no. 11990]).
4. Remove the Pathogen Lysis Tube from the vortexer and centrifuge the tube to remove drops from the inside of the lid.

   Use 200 µL of the supernatant as starting material for “Standard Protocol: Purification of Viral DNA and RNA, and Bacterial DNA“ on page 24.

Protocol: Pretreatment of Difficult-to-Lyse Bacteria (Extended Lysis)

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB-3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- This pretreatment is for the simultaneous extraction of DNA from difficult-to-lyse bacteria, viral DNA, and/or viral RNA from body fluids. After centrifugation, the sample is split into separate fractions for bacterial and viral nucleic acid extraction, requiring 2 cartridges for one starting sample.

  Note: The QIAmini Pathogen Kit contains 48 single-use cartridges for 48 standard samples; therefore, customers should be aware that one kit will not cover 48 samples when using this extended protocol.
- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- If a precipitate has formed in Buffer ATL, dissolve by incubating at 56°C.

Procedure

1. Pellet bacteria by centrifugation for 10 min at 5000 × *g* (7500 rpm).

   Note: Depending on the bacterial target, a higher centrifugation speed may be required (>14,000 × *g*).

2. a. Use the supernatant for viral nucleic acid extraction with the "Standard Protocol: Purification of Viral DNA and RNA, and Bacterial DNA" on page 24.

   b. Resuspend the bacterial pellet in 180 µL Buffer ATL.

3. Add 20 µL Proteinase K, mix by vortexing, and incubate at 56°C for 10 min at 800 rpm.

4. Add 240 µL Buffer ACL and mix thoroughly either by pulse vortexing for 20 s or by pipetting up and down at least 10 times. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

5. Add 1 µL reconstituted carrier RNA.

6. Incubate at room temperature for 15 min with shaking at 800 rpm (e.g., thermal shaker).

Cartridge preparation

7. Invert pre-filled cartridge(s) to resuspend magnetic beads. Flick down to remove reagents and bead suspension from under the foil.

8. Place cartridge(s) into the cartridge frame. Make sure to keep the pull tab accessible. Ensure cartridge(s) are fully inserted into the cartridge frame.

   Important: Carefully remove the sealing foil. Avoid splashing of reagents.

9. Pipet RNase-free water for the required elution volume (75 µL for 50 µL eluate or 125 µL for 100 µL eluate) into well 5 of the cartridge.

10. Transfer the lysate from step 6 into well 1 of the cartridge.

    Note: At times, precipitation may form after this step; this does not affect the performance.

Standard Protocol: Purification of Viral DNA and RNA, and Bacterial DNA

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB- 3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- This protocol is intended for the purification of viral DNA, viral RNA, and bacterial DNA from plasma, serum, whole blood, urine, transport media, swabs, and respiratory samples. The starting sample volume for this protocol is 200 µL.
- If using the QIAmini Pathogen Kit for the first time, read "Important Notes".
- After receiving the QIAmini Pathogen Kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Service or your local distributor. In case of liquid spillage, refer to Safety Information. Do not use damaged kit components because their use may lead to poor performance.
- Buffer ACL and the QIAmini Pathogen Cartridges contain guanidine and salt and are therefore not compatible with disinfecting reagents containing bleach.

Procedure

1. Pipet 20 µL QIAGEN Proteinase K into the bottom of a microcentrifuge tube.

   Note: Do not add QIAGEN Proteinase K directly to the Buffer ACL.

   Important: It is possible to add QIAGEN Proteinase K after dispensing samples, but it is best added beforehand.

2. Add 200 µL of the sample.
3. Add 240 µL Buffer ACL and mix thoroughly either by pulse vortexing for 20 s or by pipetting up and down at least 10 times. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.
4. Add 1 µL reconstituted carrier RNA.
5. Incubate at room temperature for 15 min with shaking at 800 rpm (e.g., thermal shaker).

Cartridge preparation

6. Invert pre-filled cartridge(s) to resuspend magnetic beads. Flick down to remove reagents and bead suspension from under the foil.
7. Place cartridge(s) into the cartridge frame.
8. **Important**: Carefully remove the sealing foil. Avoid splashing of reagents.
9. Pipet RNase-free water for the required elution volume (75 µL for 50 µL eluate or 125 µL for 100 µL eluate) into well 5 of the cartridge.
10. Transfer the lysate from step 5 into well 1 of the cartridge.

    Note: At times, precipitation may form after this step; this does not affect the performance.

Protocol: Large Volume Plasma Purification

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB-3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- This protocol is intended for the purification of nucleic acids from 300 µL plasma samples, providing a larger input volume than the 200 µL used in the standard protocol.
- After receiving the QIAmini Pathogen Kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Service or your local distributor. In case of liquid spillage, refer to Safety Information. Do not use damaged kit components because their use may lead to poor performance.
- Buffer ACL and the QIAmini Pathogen Cartridges contain guanidine and salt and are therefore not compatible with disinfecting reagents containing bleach.

Procedure

1. Pipet 20 µL QIAGEN Proteinase K into the bottom of a microcentrifuge tube.

   Note: Do not add QIAGEN Proteinase K directly to the Buffer ACL.

   Important: It is possible to add QIAGEN Proteinase K after dispensing samples, but it is best added beforehand.

2. Add 300 µL of the sample.
3. Add 240 µL Buffer ACL and mix thoroughly either by pulse vortexing for 20 s or by pipetting up and down at least 10 times. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.
4. Add 1 µL reconstituted carrier RNA.
5. Incubate at room temperature for 15 min with shaking at 800 rpm (e.g., thermal shaker).

Cartridge preparation

6. Invert pre-filled cartridge(s) to resuspend magnetic beads. Flick down to remove reagents and bead suspension from under the foil.
7. Place cartridge(s) into the cartridge frame. Make sure to keep the pull tab accessible. Ensure cartridge(s) are fully inserted into the cartridge frame.

   Important: Carefully remove the sealing foil. Avoid splashing of reagents.

8. Pipet RNase-free water for the required elution volume (75 µL for 50 µL eluate or 125 µL for 100 µL eluate) into well 5 of the cartridge
9. Transfer the lysate from step 5 into well 1 of the cartridge.

   Note: At times, precipitation may form after this step; this does not affect the performance.

Procedure on QIAmini

1. Turn on QIAmini.
2. Select the desired protocol.

   Note: A list of all imported protocols is available in the Run menu list view.

   Note: For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB- 3870**), available for download separately.
3. Open the instrument door. Load the cartridge frame holding the cartridge(s) into the instrument.

   Note: Ensure that the cartridge frame is placed in correct orientation. Press the cartridges down to fully engage with the heater block.
4. Slide rod cover(s) into the rod cover holder.

   Note: Press the clip down to allow full insertion of the rod cover holder.
5. Close the instrument door, press ▶, and confirm that consumables have been installed correctly to initiate the protocol run.
6. The display shows "End of run" when the run is completed. Select **Confirm** and press ⏎.
7. Open the instrument door. Remove and discard the rod cover(s). Then remove the cartridge frame holding the cartridge(s) and transfer the purified nucleic acids from well 5 of each cartridge into a new reaction tube (e.g., 1.5 mL tube). Discard the cartridge(s).

   Recommended: Clean the instrument and perform a UV decontamination of the instrument chamber.

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** (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
General handling	
Error message in instrument	Refer to the *QIAmini User Manual* (**www.qiagen.com/HB-3799**).
Low yield of viral DNA and RNA or bacterial DNA	
Magnetic particles not completely resuspended	Ensure to invert QIAmini Pathogen Cartridge before use.
Insufficient sample lysis	Make sure to mix thoroughly after adding Buffer ACL, either by vortexing or by pipetting up and down.
Carrier RNA not added	Reconstitute the lyophilized carrier RNA as described in "Preparing carrier RNA". Repeat the purification procedure with new samples
Sample not equilibrated to room temperature	Using cold samples can lower the lysis temperature, leading to incomplete sample lysis.
Precipitate in buffers	
Precipitate in Buffer ATL (required for pretreatments) and Buffer ACL	Precipitate may form after storage at low temperature or after prolonged storage. To dissolve precipitate, incubate Buffer ATL and Buffer ACL at 37°C with occasional shaking.
Beads in eluate	
Too much input material used	Adhere to the instructions regarding sample material, and carry out initial tests with lower amounts. Too much material may increase viscosity of the sample that can impact bead handling throughout the protocol.

Ordering Information

Product	Contents	Cat. no.
QIAmini Pathogen Kit (48)	For 48 preparations for viral DNA, viral RNA, and bacterial DNA extraction: Buffer ACL, Proteinase K, Carrier RNA, QIAmini Pathogen Cartridges, QIAmini Rod Covers and RNase-free water	590205
Related products		
QIAmini*	Benchtop instrument for automated isolation of nucleic acids; includes 1 year warranty on parts and labor	9003607
QIAmini Rod Covers (50)	Set of 50 QIAmini Rod Covers; for use with QIAmini	590025
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes with large beads, 1 vial of Reagent DX	19092
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes with small beads, 1 vial of Reagent DX	19091
Buffer ATL (4 × 50 mL)	4 × 50 mL Tissue Lysis Buffer for use in purification of nucleic acids	939011
Buffer ATL (200 mL)	1 × 200 mL Tissue Lysis Buffer for use in purification nucleic acids	19076
PowerBead Pro Tubes (50)	Bead tubes ready for rapid and reliable biological sample lysis from a wide variety of starting materials, 2 mL	19301
RNase-Free DNase Set (50)	For the removal of genomic DNA contamination in RNA preparations	79254
RNAse A (17,500 U)	2.5 mL solution for removal of RNA	19101
TissueLyser III†	Bead mill, 100–120/220–240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 × 24 or TissueLyser Adapter Set 2 × 96	9003240
TissueLyser Adapter Set 2 × 24	Two sets of adapter plates and two racks for use with 2 × 24 samples in 2 mL microcentrifuge tubes on the TissueLyser III	69982
Plate Adapter Set	Two sets of adapter plates and two racks for use with two 96-well plates on the TissueLyser III	11990
2 mL Tube Holder Set	Only operational in conjunction with Plate Adapter Set	11993
Reagent DX (1 mL)	1 mL antifoaming reagent for QIAGEN Lysis Buffers	19088

* Contact your QIAGEN sales team or local distributor for the instrument available in your country.

† The TissueLyser III must be used in combination with TissueLyser adapters.

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

Document Revision History

Date	Description
08/2026	Initial release

Limited License Agreement for QIAmini Pathogen Kit

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

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