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QIAasprint[®] Pathogen Application Guide

For automated, simultaneous purification of viral DNA and RNA, and bacterial DNA from whole blood, swabs, and body fluids

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Required Kits and Components

For isolation of viral RNA, DNA, and bacterial DNA from whole blood, swabs, and body fluids, the following components are required:

Required kit/component name	Cat. no.
QIAAsprint Pathogen PrepSet (384)	585799
QIAAsprint Essential Kit A (384)	585009
QIAAsprint Prep Cover (8)	582103
QIAAsprint Prep Plate Insert (32)	582226
QIAAsprint Elution Plate (10)	583204
Optional kit/component name	Cat. no.
Buffer ATL	19076 or 939011
Pathogen Lysis Tubes S/L	19091 or 19092

Kit Contents

The QIAasprint Connect offers the possibility of a modular kit concept, and the following combination is recommended for purification of viral DNA and RNA, and bacterial DNA.

QIAasprint Pathogen PrepSet	(384)
Catalog no.	585799
Number of preps	384
Buffer ACL*	220 mL
Carrier RNA	1350 µg
Proteinase K	10 mL

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 8 for Safety Information.

QIAasprint Essential Kit A	(384)
Catalog no.	585009
Number of preps	384
Buffer AW1 * (concentrate)	95 mL
Buffer AW1 * (concentrate)	27 mL
Buffer AW2 (concentrate)	2 × 68 mL
RNase-free water	50 mL
RNase-free water	30 mL
MagG Bead Suspension	12 mL

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 8 for Safety Information.

Shipping and Storage

The QIAasprint Pathogen PrepSet and QIAasprint Essential Kit A are shipped at ambient temperature.

All buffers and reagents should be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

The QIAasprint Pathogen PrepSet 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 components.

CAUTION

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



Buffers ACL and AW1 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.

Quality Control

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

Introduction

The application guide describes processing of the QIAasprint Pathogen PrepSet with the QIAasprint Connect instrument. For a detailed description of the QIAasprint Connect instrument, refer to the respective user manual (www.qiagen.com/HB-3708) and quick-start guide (www.qiagen.com/HB-3709). The QIAasprint Pathogen application enables automated high-throughput purification of viral DNA and RNA, and bacterial DNA from the following sample materials using the QIAasprint Connect instrument:

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

The 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 the QIAasprint Connect instrument. The QIAasprint Connect can process up to 96 samples per run, and subsequent runs allow handling of up to 192 samples in a single session.

Automated purification on QIAasprint Connect

Nucleic acid purification is fully automated on the QIAasprint Connect platform. The advanced technology integrated into QIAasprint Connect ensures efficient purification of nucleic acids for a wide range of applications while offering exceptional flexibility to allow customers to customize their purification protocols according to specific requirements.

QIAasprint Connect provides comprehensive protocols and purification guides for plasmid DNA, gDNA, RNA, and viral/bacterial nucleic acids.



Figure 1. The QIAasprint Connect instrument.

Principle and procedure

The QIAprint Pathogen PrepSet 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 safe and reproducible handling of potentially infectious samples. The purification procedure comprises four steps: lyse, bind, wash, and elute.

Lysis with Proteinase K

Proteolysis of viruses and bacteria in the samples is performed under highly denaturing condition at elevated temperatures. Lysis is performed in the presence of Proteinase K and lysis buffer, which together ensure digestion of viral coat proteins, bacterial membrane proteins, and inactivation of RNases.

Binding to magnetic particles

The QIAprint Pathogen PrepSet provides a fully automated protocol for purification of easy-to-lyse viruses and pathogens, as well as an extended protocol with offboard lysis for hard-to-lyse viruses and pathogens. In case of the easy-to-lyse protocol, a lysis and binding mixture is added directly to the sample and thoroughly mixed to ensure optimal adsorption of viral nucleic acids and bacterial DNA to the silica surface. In the offboard protocol, the binding mixture is added to the lysed sample after an incubation period.

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 using first Buffer AW1, then Buffer AW2, and then ethanol.

Elution of purified nucleic acids

In a single step, purified viral nucleic acids and bacterial DNA are eluted in RNase-free water. The purified nucleic acid can be either used immediately in downstream applications or stored for future use.

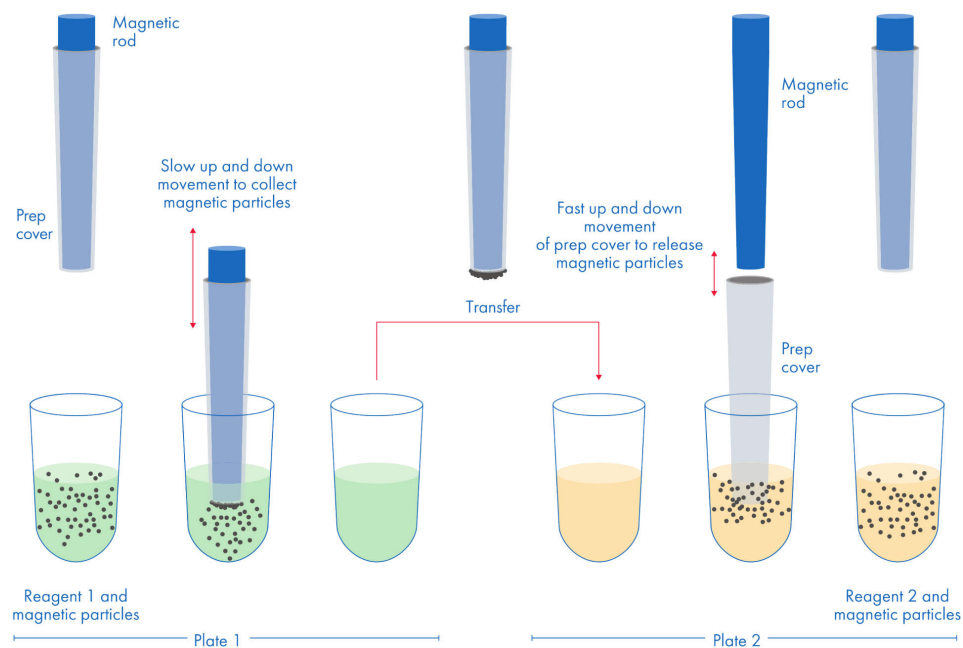


Figure 2. Handling of magnetic particles by the QIAprint Connect instrument.

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.

All protocols

- Pipettes and sterile, RNase-free pipette tips
- Disposable gloves
- 96–100% Ethanol*
- Isopropanol
- **For samples <200 µL:** PBS or 0.9% NaCl solution
- **Optional:** Vortexer
- **Optional:** Thermal shaker (for offboard lysis protocol)
- **Optional:** QIASprint Frame Shield (100) (cat. no. 580010)

Note: If desired, use QIASprint Frame Shield to minimize adhesions of beads to the QIASprint Prep Cover Frame (cat. no. 582111). For this, place QIASprint Frame Shield into the QIASprint Prep Cover Frame before loading the QIASprint Prep Cover. After the run, discard the QIASprint Frame Shield.

- QIASprint Connect consumables (see Table 1)

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

Table 1. QIAprint Connect consumables

Item	Cat. no.	Pieces required for 4 × 96 samples
QIAprint Prep Cover (8)	582103	4
QIAprint Prep Plate Insert (32)	582226	16
QIAprint Elution Plate (10)	583204	4

For pretreatment of difficult-to-lyse bacteria

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

For offboard lysis protocol

- Microcentrifuge tubes or 96-well plate for offboard lysis
- Thermal shaker

Important Notes

Preparing samples

The purification procedure is optimized for the extraction of viral nucleic acids and bacterial DNA from 200 µL sample volume.

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 hours. 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 will not reduce viral titers but bacterial titers can be affected.

Swabs

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

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

Preparing lysis buffer

Precipitates may form in pretreatment Buffer ATL or 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 to add to downstream amplification reaction should therefore be based on the amount of carrier RNA in the eluate. To obtain 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 three times.

Using an internal control

Using the QIAasprint Pathogen PrepSet 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 binding mixture or the binding mix in case of the extended protocol. 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 QIAasprint Pathogen PrepSet.

Elution volumes and eluate handling

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

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

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

Preparation of Buffer AW1 and Buffer AW2

Add the required volume of ethanol (96–100%) to Buffer AW1^{*} concentrate and Buffer AW2 concentrate, as described on the bottle. Tick the checkbox on the label to indicate that ethanol has been added. Store reconstituted Buffers AW1 and AW2 at room temperature (15–25°C). Reconstituted Buffers AW1 and AW2 are stable for up to 1 year when stored at room temperature, but only until the kit expiration date.

Note: Always mix reconstituted Buffers AW1 and AW2 by shaking before starting the purification procedure.

^{*} Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 8 for Safety Information.

Working with the QIAasprint Connect

Procedure on the QIAasprint Connect

1. Turn on the QIAasprint Connect instrument and log in.
 2. Navigate to the Protocols screen with the tab in the upper part of the home screen.
 3. Locate the desired protocol for the pathogen application, and press  and  **Run protocol**.
- Optional:** Enter run information in the Run details screen. This information will be displayed in the run report.
4. Close the hood and press **Confirm** to get to the loading screen.
 5. With the plate hotels located on the lab bench, insert the plates according to the screen instructions into the hotels.
 - The QIAasprint Prep Cover must be located in the metal prep cover frame.
- Note:** If desired, use the QIAasprint Frame Shield to minimize adhesions of beads to the QIAasprint Prep Cover Frame. For this, place QIAasprint Frame Shield into the QIAasprint Prep Cover Frame before loading the QIAasprint Prep Cover. After the run, discard the QIAasprint Frame Shield.
- All QIAasprint Prep Plates need to sit firmly in their metal frames.
 - The QR codes need to face the user.
6. Open the hood and place the hotels on their platforms, taking care not to mix up the left and right hotels.
 7. **Optional:** If you have heating or cooling steps during your protocol run and the thermal adapter is not already located on the heater-cooler, place the thermal adapter in one of the slots of the thermal adapter platform with the QR code facing the user. The instrument will install it automatically during the run.

8. Press **Start run** to start the run.
9. After the end of the run, remove the plate hotels from the instrument. Store the elution plate safely or use it directly in your downstream applications.

Protocol: Pretreatment of Urine

This protocol provides guidelines for urine pretreatment prior to nucleic acid purification (see page 27). 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 27).

Protocol: Pretreatment of Respiratory Samples

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 27).

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 27).

Protocol: Pretreatment of Difficult-to-Lyse Bacteria

This pretreatment is for the extraction of DNA of difficult-to-lyse bacteria from body fluids.

Important points before starting

- Buffer ATL and Pathogen Lysis Tubes L or S with glass beads (including Reagent DX) must be ordered separately (for Ordering Information, see page 36).

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 μ L 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 μ L 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 μ L Buffer ATL (containing Reagent DX) into a fresh Pathogen Lysis Tube.
2. Add 400 μ L blood or other sample fluids.
If using less starting material, adjust the volume to 400 μ L 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 “Onboard Lysis Protocol: Purification of Viral DNA and RNA, and Bacterial DNA”, on page 27.

Onboard Lysis Protocol: Purification of Viral DNA and RNA, and Bacterial DNA

Important points before starting

- If using the QIAasprint Pathogen PrepSet and QIAasprint Essential Kit A for the first time, read "Important Notes".
- After receiving the QIAasprint Pathogen PrepSet and QIAasprint Essential Kit A, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to the Safety Information. Do not use damaged PrepSet and Essential Kit components because their use may lead to poor performance.
- Buffer ACL and Buffer AW1 contain guanidine and salt and are therefore not compatible with disinfecting reagents containing bleach.

Things to do before starting

- Prepare Buffer AW1 and Buffer AW2 according to the instructions on the bottle.
- Dissolve the lyophilized carrier RNA in RNase-free water to a final concentration of 1 µg/µL.
- Ethanol (96–100%) is required in this protocol and needs to be supplied by the user.
- Isopropanol is required in this protocol and needs to be supplied by the user.
- For a 50 µL elution, add 75 µL RNase-free water; for 100 µL elution, add 125 µL. Select the appropriate protocol.

Procedure

Sample preparation in sample plate

1. Prepare the lysis/binding master mix (x + 10% samples) according to the table below:

Lysis/binding mix	Per sample (µL)
Buffer ACL	400
Isopropanol	200
Carrier RNA	1
MagG Bead Suspension	25

Note: Ensure sufficient resuspension of the MagG Bead Suspension before distribution by thorough vortexing.

2. Add 20 µL QIAGEN Proteinase K into the bottom of a well of a prep plate insert (Sample Plate).

Important: Even though you can add QIAGEN Proteinase K after dispensing samples into the prep plate insert, it is best to add it beforehand.

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

3. Transfer 200 µL of sample to the Sample Plate.
4. Add 626 µL of the lysis/binding mix to each well.

Plates preparation

5. Prepare the plates as described in the table below:

Plate	Content	Volume (µL)
Sample Plate	Proteinase K	20
	Sample	200
	Lysis/binding mix	626
Wash Plate 1	Buffer AW1	700
Wash Plate 2	Buffer AW2	700
Wash Plate 3	96–100% Ethanol	750
Elution Plate	RNase-free Water	75/125

Note: Residual adhesive can lead to instrument crash. If you cover plates with adhesive foil after plate preparation, ensure that no adhesive remains on the plate surface after removal of the foil.

6. To start the run, follow the instructions from “Working with the QIAprint Connect” on page 21.

Offboard Lysis Protocol: Purification of Viral DNA and RNA, and Bacterial DNA

The extended protocol allows you to perform a longer offboard lysis step, which is especially useful for pathogens that are difficult to break down or in situations where offboard lysis is necessary. This extended lysis can improve the efficiency of nucleic acid extraction by ensuring that challenging pathogens are adequately lysed before purification, resulting in higher yields of viral DNA, RNA, or bacterial DNA.

Important points before starting

- If using the QIAprint Pathogen PrepSet and QIAprint Essential Kit A for the first time, read “Important Notes”.
- After receiving the QIAprint Pathogen PrepSet and QIAprint Essential Kit A, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to the Safety Information. Do not use damaged PrepSet and Essential Kit components because their use may lead to poor performance.
- Buffer ACL and Buffer AW1 contain guanidine and salt and are therefore not compatible with disinfecting reagents containing bleach.

Things to do before starting

- Prepare Buffer AW1 and Buffer AW2 according to the instructions on the bottle.
- Dissolve the lyophilized carrier RNA in RNase-free water to a final concentration of 1 µg/µL.
- Ethanol (96–100%) is required in this protocol and needs to be supplied by the user.
- Isopropanol is required in this protocol and needs to be supplied by the user.
- For a 50 µL elution, add 75 µL RNase-free water; for 100 µL elution, add 125 µL. Select the appropriate protocol.

Procedure

Sample preparation and offboard lysis

1. Pipet 20 µL QIAGEN Proteinase K into the bottom of a well of a 96-well plate or microcentrifuge tube.

Important: Even though you can add QIAGEN Proteinase K after dispensing samples, it is best to add it beforehand.

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

2. Add 200 µL of sample to the well or microcentrifuge tube containing QIAGEN Proteinase K.
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. Incubate at 56°C for 15 min with shaking at 800 rpm (e.g., thermal shaker).

Note: If the sample fluid contains Buffer ATL (e.g., after using pre-treatment protocols), precipitates may form. After incubation at 56°C, the precipitates dissolve and do not affect purification.

5. Prepare the Binding master mix (x + 10% samples) according to the table below:

Binding mix	Per sample (µL)
Buffer ACL	160
Isopropanol	200
Carrier RNA	1
MagG Bead Suspension	25

Note: Ensure sufficient resuspension of the MagG Bead Suspension before distribution by thorough vortexing.

6. Add 386 µl of binding master mix to each well of the prep plate (Lysate Plate) .
7. Transfer the lysate from step 4 into the Lysate plate containing the binding mix.

Note: Keep MagG Bead Suspension in suspension to ensure even distribution.

Plates preparation

8. Prepare the plates as described in the table below:

Plate	Content	Volume (µL)
Sample Plate	Lysate	460
	Binding mix	386
Wash Plate 1	Buffer AW1	700
Wash Plate 2	Buffer AW2	700
Wash Plate 3	96–100% Ethanol	750
Elution Plate	RNase-free Water	75/125

Note: Residual adhesive can lead to instrument crash. If you cover plates with adhesive foil after plate preparation, ensure that no adhesive remains on the plate surface after removal of the foil.

9. To start the run, follow the instructions from “Working with the QIA sprint Connect” on page 21.

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 user manual supplied with the QIA Sprint Connect instrument.

Low yield of viral DNA and RNA or bacterial DNA

Buffer AW1 or Buffer AW2 prepared incorrectly Check that Buffer AW1 or Buffer AW2 concentrate was diluted with the correct volume of ethanol, as indicated on the bottle. Use 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methyl ethyl ketone. Repeat the purification protocol with new samples.

Insufficient sample lysis (extended protocol) Make sure to mix thoroughly after adding Buffer ACL, either by vortexing or by pipetting up and down.

Magnetic particles not completely resuspended Ensure that you resuspend the magnetic particles either by thoroughly vortexing or by shaking until no precipitate is visible at the bottom of the bottle.

Carrier RNA not added Reconstitute the lyophilized carrier RNA as described in “Preparing carrier RNA” on page 18. 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 ACL or Buffer ATL Precipitate may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer ACL or ATL for 30 min at 37°C with occasional shaking.

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or contact one of the QIAGEN Technical Service Departments or local distributors (visit support.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
QIAasprint Pathogen PrepSet (384)	For 384 preparations of viral DNA, RNA, and bacterial DNA extraction: Buffer ACL, Proteinase K, and carrier RNA	585799
QIAasprint Essential Kit A (384)	For 384 preps for purification of nucleic acids: MagG Bead Suspension, Buffer AW1, Buffer AW2, and nuclease-free water	585009
QIAasprint Prep Cover (8)	Set of 8 prep covers for use with QIAasprint Connect	582103
QIAasprint Prep Plate Insert (32)	Set of 32 prep plate inserts for use with QIAasprint Connect	582226
QIAasprint Elution Plate (10)	Set of 10 elution plates for use with QIAasprint Connect	583204
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
Related products		
QIAasprint Connect	Benchtop instrument for automated isolation of nucleic acids; includes 1 year warranty on parts and labor	9001234
QIAasprint Frame Shield (100)	Set of 100 frame shields for use with QIAasprint Connect	580010
QIAasprint Prep Cover Frame (2)	Set of 2 prep cover frames for use with QIAasprint Connect	582111
QIAasprint Prep Plate Frame (2)	Set of 2 prep plate frames for use with QIAasprint Connect	582211
Buffer ATL	200 mL tissue lysis buffer	19076
PowerBead Pro Tubes (2 mL) (50)	For disruption of stool and all soil samples in 2 mL format	19301
PowerBead Pro Plates (4)	Bead plates ready for rapid and reliable biological sample lysis from a wide variety of starting materials	19311
TissueLyser III	Bead mill, 100–120/220–240 V, 50/60 Hz; requires TissueLyser Adapters (available separately)	9003240

Product	Contents	Cat. no.
Vortex Adapter for 24 (1.5–2.0 mL) tubes	For vortexing 1.5 and 2 mL tubes using the Vortex-Genie 2 Vortex.	13000-V1-24
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
Tissuelyser Adapter Set 2 × 96	Two sets of adapter plates for use with Collection Microtubes (racked) on the Tissuelyser III	69984
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	For sample homogenization of up to 2 × 48 samples in 2 mL bead tubes on the Tissuelyser III	11993
Reagent DX (1 mL)	1 mL antifoaming reagent for QIAGEN Lysis Buffers	19088

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Document Revision History

Date	Description
03/2026	Initial release
05/2026	Updated to match the Essential Kit configuration.

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