March 2023

EZ1&2™ Virus Handbook

EZ1&2 Virus Mini Kit v2.0
For automated, simultaneous purification of viral DNA and RNA, and bacterial DNA using EZ2® Connect instruments

Sample to Insight

Contents

Kit Contents

EZ1&2 Virus Mini Kit v2.0	**(48)**
Catalog no.	**955134**
Number of preps	**48**
Reagent Cartridges, Virus Mini v2.0*†	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Sample Tubes (2 mL)	100
Elution Tubes (1.5 mL)	100
Carrier RNA	310 µg
Buffer AVE†	3 x 2 mL
Q-Card‡	1
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for Safety Information.

† Contains sodium azide as a preservative.

‡ The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ2 Connect instruments.

Shipping and Storage

The EZ1&2 Virus Mini Kit v2.0 is shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, the reagent cartridges are stable until the expiration date on the Q-Card. Lyophilized carrier RNA is stable until the expiration date on the Q-Card when stored at room temperature.

Intended Use

The EZ1&2 Virus Mini Kit v2.0 is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The EZ1&2 Virus Mini Kit v2.0 is intended to be used with EZ1® or EZ2 Connect instruments from QIAGEN.

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.

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

Some buffers in the reagent cartridges contain guanidine hydrochloride or guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the EZ2 Connect, clean the affected area first with laboratory detergent and water, and then with disinfectants and detergents compatible with metallic surfaces as listed in the *EZ2 Connect and EZ2 Connect Fx User Manual*.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1&2 Virus Mini Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

This handbook describes processing of the EZ1&2 Virus Mini Kit v2.0 with EZ2 Connect instruments. For usage of EZ1&2 Virus Mini Kit v2.0 with EZ1 instruments, please refer to the respective handbook (**www.qiagen.com/HB-0113**) and quick-start protocol (**www.qiagen.com/HB-0787**).

The EZ1&2 Virus Mini Kit v2.0 provides a fully automated procedure for simultaneous purification of viral DNA and RNA, and bacterial DNA from the following sample materials using EZ1 or EZ2 Connect instruments:

- Serum, plasma, cerebrospinal fluid (CSF)
- Urine
- Whole blood
- Stool
- Transport media
- Respiratory samples
- Dried swabs

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. EZ1 and EZ2 Connect instruments perform all steps of the purification procedure. For the purification of nucleic acids from urine, respiratory samples, whole blood, stool, and dried swabs, additional pretreatment protocols have been developed to be used prior to the purification procedure on the EZ1 and EZ2 Connect instruments. Up to 24 samples are processed in a single run using the EZ2 Connect.

Principle and procedure

Magnetic-particle technology 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 4 steps: lyse, bind, wash, and elute (see below, and Figure 1).

Lysis with proteinase K

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

Binding to magnetic particles

Binding buffer is added to the lysed samples to adjust binding conditions. Lysates are thoroughly mixed with magnetic particles to allow optimal adsorption of viral nucleic acids and bacterial DNA to the silica surface. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not bound to the magnetic particles.

Washing of bound nucleic acids

While viral nucleic acids and bacterial DNA remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps using first wash buffer 1, then wash buffer 2, and then ethanol.

Elution of purified nucleic acids

In a single step, highly purified viral nucleic acids and bacterial DNA are eluted in Buffer AVE. The purified nucleic acids can be either used immediately in downstream applications or stored for future use.

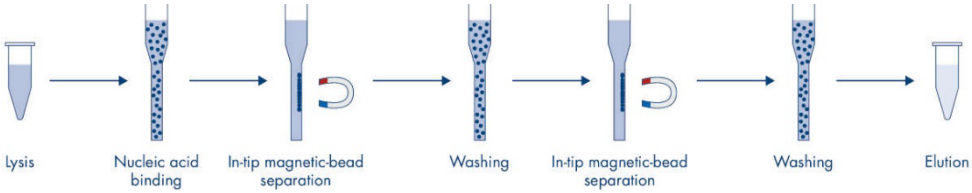

Figure 1. EZ1&2 Virus Mini Procedure.

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
- Soft paper tissue
- Water
- 70% ethanol
- Optional (if working with frozen serum or plasma samples that need to be mixed): vortexer

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

For pretreatment of urine and whole blood

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

For pretreatment of stool

- Buffer ASL (cat. no. 19082)

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

- Vortexer
- Thermoshaker* or 70°C water bath

For pretreatment of dried swabs

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

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

Important Notes

Preparing samples

The purification procedure is optimized for use with 100 µL, 200 µL, or 400 µL sample volumes. A sample volume of 200 µL is recommended for purification of pathogen nucleic acids from stool. Blood samples treated with EDTA, CPDA (Citrate Phosphate Dextrose Anticoagulant), or citrate as anticoagulant can be used for plasma preparation or as sample material when used with the respective/appropriate pretreatment. (Blood samples treated with heparin should not be used, as heparin can interfere with downstream applications.) 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 and 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 (15–25°C), and process the samples immediately when they have equilibrated to 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, plasma, or CSF samples, centrifuge at 6800 x *g* for 3 minutes, 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.

Precipitates may form in the pretreatment buffers ATL or ASL during storage at room temperature or at 4°C. Dissolve the precipitates by incubating the bottles at 50°C for 15 minutes, and shaking bottles manually twice within this incubation period.

For the purification of nucleic acids from difficult-to-lyse gram-positive bacteria in respiratory samples, an additional pre-lysis step may be performed prior to purification on the EZ2 Connect instrument (see page 26 for "Protocol: Pretreatment for Isolation of Genomic DNA of Gram Positive Bacteria").

Preparing carrier RNA

Carrier RNA serves 2 purposes during the purification procedure. First, it enhances binding of viral nucleic acids to the silica surface of the magnetic particles, especially if the sample contains very few target molecules. Second, the addition of large amounts of carrier RNA reduces the chances 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.

The lyophilized carrier RNA provided with the kit is sufficient for 48 sample preparations. The concentration of carrier RNA used in the purification procedure allows the EZ1&2 Virus Mini Kit v2.0 to be used as a generic purification system that is compatible with many different amplification systems and is suitable for purifying nucleic acids from a wide range of bacteria and DNA and RNA viruses. However, amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates obtained using this kit contain viral and bacterial nucleic acids and carrier RNA, and in each eluate, the amount of carrier RNA greatly exceeds the amount of viral and bacterial nucleic acids. The amount of eluate to add to downstream amplification reactions should therefore be based on the amount of carrier RNA in the eluate. To obtain the highest levels 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 310 µL Buffer AVE, divide it into conveniently sized aliquots, and store at –30°C to –15°C. Thawed aliquots of this stock solution can be stored at 2–8°C for up to 6 weeks. Do not freeze–thaw the aliquots more than 3 times.

For each sample processed, dilute 3.6 µL of carrier RNA stock solution in a total volume of 60 µL using Buffer AVE (and/or an internal control solution). A 50 µL volume of this carrier RNA–Buffer AVE solution is transferred to the lysis mix, corresponding to 3 µg carrier RNA.

If you want to use an internal control, see "Using an internal control" below.

> **Note**: The purification procedure is optimized so that 3 µg carrier RNA is added per sample. If a different amount of carrier RNA has been shown to be better for a specific amplification system, change the volume of carrier RNA stock solution mixed with Buffer AVE or use a different concentration of stock solution. The total volume of carrier RNA–Buffer AVE solution should be 60 µL, of which 50 µL is transferred to the lysis mix. Use of different amounts of carrier RNA must be validated for each particular sample type and downstream assay. Carrier RNA is also available separately from QIAGEN if larger amounts are required (see page 39 for Ordering Information).

Using an internal control

Using the EZ1&2 Virus Mini Kit v2.0 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 should be combined with carrier RNA stock solution (3.6 µL) in one mixture. For each sample, the carrier RNA–internal control mixture should have a volume of 60 µL, of which 50 µL will be transferred to the lysis mix. This amount corresponds to 3 µL carrier RNA stock solution plus 47 µL Buffer AVE and/or internal control solution.

If the internal control is stable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on 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 amount of internal control for specific downstream applications. Using an amount other than that recommended may reduce amplification efficiency. To determine the amount of internal control needed for the EZ1&2 Virus Mini v2.0 protocol, the sample input volume and the volume of the eluate need to be taken into account. See "Appendix A: Calculating the Amount of Internal Control", page 33, for detailed instructions on how to calculate the correct volume of internal control.

Internal controls are not provided in the EZ1&2 Virus Mini Kit v2.0.

Elution volumes and eluate handling

The final step of the purification procedure is elution of viral nucleic acids and bacterial DNA in a final volume of 60 µL, 90 µL, 120 µL, or 150 µL. If the sample material is stool, we recommend an elution volume of 120–150 µL.

If eluates obtained from stool are turbid, centrifuge at full speed (20,000 x *g*) for 3 minutes to clear the eluates. This treatment will not have a negative impact on clear eluates but will improve performance of turbid eluates in downstream applications.

Yields of viral nucleic acids and bacterial DNA

The yields of viral nucleic acids obtained in the purification procedure are normally below 1 µg and therefore 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.

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.

Working with EZ2 Connect instruments

The main features of EZ2 Connect instruments include:

- Small footprint to save laboratory space
- Preprogrammed protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast run setup
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps
- Optional bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV LED to help eliminate sample carryover from run-to-run and to allow pathogen decontamination on the worktable surfaces

Note: UV decontamination helps to reduce possible pathogen contamination of the EZ2 Connect worktable surfaces. The efficiency of inactivation has to be determined for each specific organism and depends, for example, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

Reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 2). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or RNase-free elution buffer. Since each well

contains only the required amount of reagent, generation of additional waste due to leftover reagent at the end of the purification procedure is avoided.

Figure 2. Ease of worktable setup using reagent cartridges. (A) A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. (B) Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded. Each reagent cartridge contains 12 individual positions.

The reagent cartridges supplied with the EZ1&2 Virus Mini Kit v2.0 are prefilled with all the necessary reagents for purification of viral DNA and RNA, and bacterial DNA, except carrier RNA. Carrier RNA and internal controls (optional) are added in a tube outside the reagent cartridge.

EZ2 Connect tip racks

The EZ2 Connect tip racks hold tips inserted into tip holders and tubes for samples or elution. Details on how to equip the tip racks are displayed during the run setup on the LED display of the EZ2 Connect.

Figure 3. The EZ2 Connect Tip Rack (A) has 4 positions labeled A–D by engravings. It is designed to hold sample and elution tubes as well as tips in their respective tip holders (B).

Worktable

The worktable of EZ2 Connect instruments is where the user loads equipped cartridge and tip racks (Figure 4).

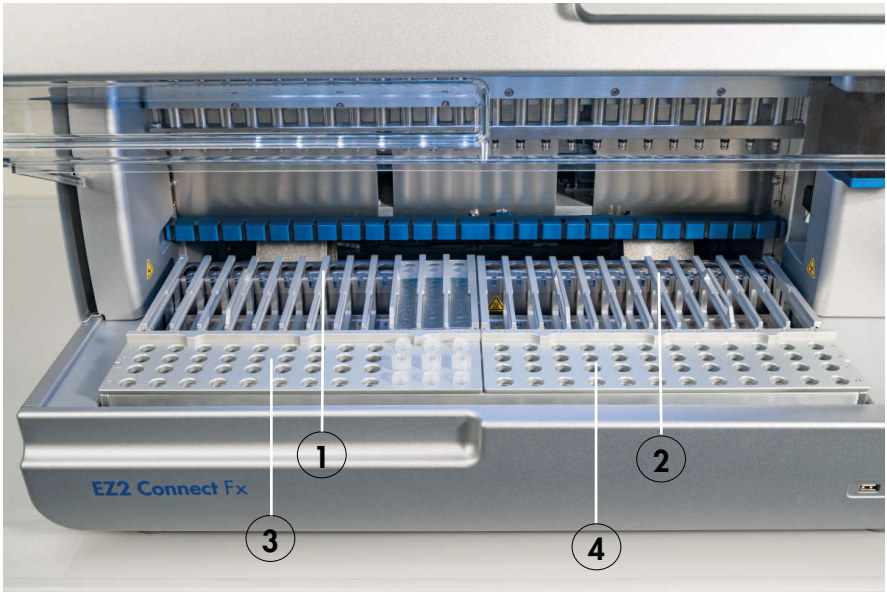

Figure 4. EZ2 Connect Worktable.

1. EZ2 Connect Cartridge Rack – left
2. EZ2 Connect Cartridge Rack – right
3. EZ2 Connect Tip Rack – left
4. EZ2 Connect Tip Rack – right

Operation of the EZ2 Connect

The EZ2 Connect provides various features to support the sample preparation workflow. These include functions for remote access via QIAsphere®, data input via bar code reading, data storage and transfer, report generation, and guided instrument maintenance. For more information about these features, please refer to the *EZ2 Connect and EZ2 Connect Fx User Manual*.

Protocol: Pretreatment of Urine

This protocol is intended for pretreatment of urine prior to nucleic acid purification (page 27).

1. Add urine to Buffer ATL to a final volume of 100 µL, 200 µL, or 400 µL, according to the table.

Table 1. Urine and Buffer ATL volumes

Urine (µL)	Buffer ATL (µL)	Final sample volume (µL)
75	25	100
150	50	200
300	100	400

Buffer ATL should be ordered separately. See "Ordering Information", page 39.

2. Mix the solution carefully.
3. Proceed to the purification protocol (page 27).

Protocol: Pretreatment of Whole Blood

This protocol is intended for pretreatment of whole blood prior to nucleic acid purification (page 27).

1. Add whole blood to Buffer ATL to a final volume of 100 µL, 200 µL, or 400 µL, according to the table.

Table 2. Urine and Buffer ATL volumes

Whole blood (µL)	Buffer ATL (µL)	Final sample volume (µL)
50	50	100
100	100	200
200	200	400

Buffer ATL should be ordered separately. See "Ordering Information", page 39.

2. Mix the solution carefully.
3. Proceed to the purification protocol (page 27).

Protocol: Pretreatment of Stool

This protocol is intended for pretreatment of solid as well as liquid stool samples prior to nucleic acid purification (page 27).

1. Resuspend 100 mg of solid or liquid stool in 900 µL Buffer ASL.

   Note: If less or more stool is used, the amount of Buffer ASL needs to be adjusted to maintain a dilution ratio of 1:10 (w/v). Use of 30 mg stool is a minimum requirement to obtain at least 200 µL sample volume after the pretreatment procedure for purification with the EZ2 Connect instrument.

2. Vortex the sample vigorously for 1 min or until the suspension is homogenous.

   Note: If working with highly solid stool, the resuspension procedure may be extended, or try to disrupt the sample by pipetting up and down. For easier pipetting, it may be necessary to cut off the end of the pipette tip. Some particles will remain insoluble and will be removed during the next step.

3. Incubate the sample for 10 min at room temperature on the bench to allow sedimentation of large stool particles.

4. Transfer at least 400 µL supernatant from the top of the suspension to a fresh 1.5 mL screw cap tube without carryover of large stool particles.

   Note: Ensure that no solid particles are transferred with the supernatant to the EZ2 Connect instrument. Large stool particles in the sample may lead to clogging of the filter tip of the EZ2 Connect instrument.

5. Incubate the sample for 10 min at 70°C in a water bath or thermoshaker.

6. Proceed to the purification protocol (page 27).

Note: For stool samples, we recommend using 200 µL sample volume for purification and 120–150 µL volume for elution. Higher sample volumes and lower elution volumes may lead to reduced sensitivity of downstream applications.

Note: If eluates obtained from stool are turbid, we recommend centrifugation at full speed (20,000 x *g*) for 3 min in order to clear eluates. This will not have a negative impact on clear eluates but will improve the performance of turbid eluates in downstream applications.

Protocol: Pretreatment of Dried Swabs

This protocol is intended for pretreatment of dried swabs to release dried sample material from swabs prior to nucleic acid purification (page 27).

1. Add 600 µL of Buffer ATL to the dried swab.

   Note: The volume obtained after lysis might differ depending on the swab type. Optimally, a volume of 400 µL should be available for the purification.
2. Incubate the swab for 15 min at 56°C with vigorous shaking.
3. Transfer 100 µL, 200 µL, or 400 µL of the liquid to a new screw cap tube, depending on the sample volume chosen.
4. Proceed to the purification protocol (page 27).

Protocol: Pretreatment of Respiratory Samples

This protocol is intended for pretreatment of viscous respiratory samples prior to nucleic acid purification. Nonviscous respiratory samples require no pretreatment and can be used directly as starting material in the purification protocol (page 27).

1. Liquefy the sample according to step 1a, 1b, or 1c.
   1a. 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.
   1b. 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.
   1c. Mix 1 volume of sample with 1 volume of 1 x 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 (page 27).

Protocol: Pretreatment for Isolation of Genomic DNA of Gram-Positive Bacteria

DNA purification can be improved for some gram-positive bacteria by enzymatic pretreatment before transferring the sample to the EZ2 Connect instrument. If samples show high viscosity, like sputum, then liquefaction according to the protocol for respiratory samples is recommended prior to starting this protocol. This protocol is not intended for use with stool or whole blood samples.

1. Pellet bacteria by centrifugation for 10 min at 5000 x *g* (7500 rpm in a microcentrifuge).
2. Suspend bacterial pellet in 180 µL of the appropriate enzyme solution (20 mg/mL lysozyme or 200 µg/mL lysostaphin; 20 mM Tris·HCl, pH 8.0; 2 mM EDTA; 1.2% Triton X-100) in a 2 mL screw cap tube.
3. Incubate for at least 30 min at 37°C.
4. Briefly centrifuge the tube to remove drops from the inside of the lid.
5. Proceed to the purification protocol (page 27).

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

Important points before starting

- If using the EZ1&2 Virus Mini Kit v2.0 for the first time, read "Important Notes" (page 12).
- After receiving the kit, 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 "Safety Information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance or contamination of the EZ2 Connect instrument.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).
- Prepare serum, plasma, or CSF samples as described in "Preparing samples", page 12. If cryoprecipitates are visible in the thawed samples, centrifuge at 6800 x *g* for 3 minutes, 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 may be affected.
- Prepare viscous respiratory samples, urine, whole blood, stool, or dried swabs as described in the pretreatment protocols. Nonviscous respiratory samples do not require pretreatment.

- The liquid of samples stored in transport media can be used for purification of viral DNA and RNA, and bacterial DNA, with the EZ1&2 Virus Mini Kit v2.0 without pretreatment.
- Prepare a carrier RNA stock solution (with optional internal control) before using it for the first time. Dissolve the lyophilized carrier RNA in 310 µL Buffer AVE (provided in the kit), and mix it with the internal control (optional) as described in "Preparing carrier RNA" and "Using an internal control", pages 13 and 14, respectively.

Procedure

1. For each sample, prepare a 60 µL solution containing 3.6 µL carrier RNA dissolved in Buffer AVE (with optional internal control) in a 1.5 mL tube (supplied). Mix gently by pipetting the solution 10 times. Do not vortex. For detailed description see "Preparing carrier RNA" on page 13.

   Note: Make sure that the carrier RNA solution is at the bottom of the 1.5 mL tube. This ensures that the appropriate amount can be transferred by the EZ2 Connect instrument.

2. Transfer 100 µL, 200 µL, or 400 µL sample into 2 mL sample tubes (supplied), and equilibrate to room temperature before loading on the worktable. If using frozen samples, thaw and equilibrate at room temperature, and mix well by vortexing.

   Note: for optimal performance, it is essential to use the nonskirted 2 mL tubes provided with the kit.

   Note: do not refreeze thawed samples or store samples for over 6 h at 2–8°C, as this leads to significantly reduced yields of viral nucleic acids and bacterial DNA.

   Note: We recommend using 100 µL, 200 µL, or 400 µL of plasma, serum, CSF, transport media, and pretreated respiratory samples, urine, whole blood, and dried swabs. For stool samples, we recommend 200 µL sample volumes. Higher sample volumes may lead to reduced sensitivity of downstream applications. If you want to use less sample, bring the volume up to 100 µL, 200 µL, or 400 µL with the appropriate amount of Buffer AVE.

   Note: Do not use sample volumes greater than 100 µL, 200 µL, or 400 µL. After lysis and binding of viral nucleic acids and bacterial DNA to the magnetic particles, a portion of

the lysate is transferred to the sample tube to inactivate residual viruses. Any sample left in the sample tube after sample transfer will therefore be lost.

3. Turn on the EZ2 Connect instrument.
4. Tap Virus on the Applications panel, and then select **Virus Mini Kit v2.0** and press **Next**.
5. Choose the **Virus** protocol and press **Next**.
6. Choose sample volume and elution volume and press **Next**.
7. Select positions on the work deck according to the number of samples to be processed and press **Next**.
8. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
9. Load the EZ1&2 Virus reagent cartridges into respective positions of the EZ2 Connect Cartridge Racks, as selected in step 7.
10. Place an empty non-skirted tube into position 11 of the cartridges (Figure 2).
11. Open the instrument hood. Load the EZ2 Connect Cartridge Racks into the EZ2 Connect instrument.
12. Remove caps of all sample and elution tubes to be used and prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: 2.0 mL sample tube from step 2
    - Position B: 1.5 mL tube with Carrier RNA from step 1
    - Position C: Tip holder with Filter Tip
    - Position D: 1.5 mL empty elution tube

    Press **Next**.
13. Place the EZ2 Connect Tip Racks into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
14. The display will show **Protocol finished** when the run is completed. Select **Finish**.
15. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position D of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

    Optional: follow onscreen instructions for UV decontamination of worktable surfaces.
16. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

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	
General handling		
a)	Error message in instrument	Refer to the user manual supplied with your display EZ2 Connect instrument.
Low yield of viral DNA and RNA or bacterial DNA		
a)	Magnetic particles not completely resuspended	Ensure that you resuspend the magnetic particles thoroughly before loading the reagent cartridges into the holder.
b)	Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, ensure that you tap the cartridges to deposit the reagents at the bottom of the wells.
c)	Reagents loaded onto worktable in wrong order	Ensure that all tubes and the tip holders with the tips are loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.
d)	Carrier RNA not added	Reconstitute the lyophilized carrier RNA in 310 µL Buffer AVE. For each sample, use 3.6 µL of this carrier RNA stock solution, mixed with internal control (optional) and additional Buffer AVE to a final volume of 60 µL, as described in "Preparing carrier RNA" and "Using an internal control", pages 13–14. Repeat the purification procedure with new samples.
e)	Carrier RNA and Buffer AVE not sufficiently mixed	Mix carrier RNA, internal control (optional), and Buffer AVE by pipetting at least 10 times.
f)	RNA degraded	RNA may have been degraded by RNases in the original plasma, serum, CSF, urine, or respiratory samples. Ensure that the samples are processed immediately after collection or removal from storage.

		Comments and suggestions
g)	Precipitates visible at the bottom of the wells of the reagent cartridges	Place the reagent cartridges into a shaker incubator and incubate at 30–40°C with mild agitation for up to 2 h. Do not use the reagent cartridges if the precipitates do not redissolve.
h)	Samples not equilibrated to room temperature	Using cold samples can lower the lysis temperature, leading to incomplete sample lysis.

RNA or DNA does not perform well in downstream enzymatic reactions

a)	Little or no nucleic acid in the eluate	See "Low yield of viral DNA and RNA or bacterial DNA", previous page, for possible reasons. Increase the amount of eluate added to the downstream enzymatic reaction, if possible.
b)	Frozen samples not mixed properly after thawing	Thaw frozen samples at room temperature (15–25°C) and mix by pulse vortexing for 15 s.
c)	Nucleic acids in samples already degraded prior to purification	This can occur if samples were refrozen after thawing once or stored at room temperature (15–25°C) for too long. Always use fresh samples or samples thawed only once. Repeat the purification procedure with new samples.
d)	Insufficient sample lysis	This can occur if reagent cartridges were stored at elevated temperatures for too long, leading to inactivation of proteinase K. Repeat the purification procedure using new samples and reagent cartridges.
e)	Salt carryover during elution	For best results, ensure that the reagent cartridges are at 20–30°C.
f)	Too much or too little carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the concentration of carrier RNA solution.
g)	Too much eluate in the amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce the volume of eluate added to the amplification reaction or increase the elution volume accordingly. A positive control can be spiked into the eluate, if desired, to determine the effect of eluate on the amplification reaction.
h)	Varying performance of purified nucleic acids in downstream assays	The salt and ethanol components of wash buffer 1 or wash buffer 2 in the cartridge may have separated due to long-term storage. Always shake the cartridges thoroughly and tap them before starting a purification procedure.

		Comments and suggestions
i)	Lack of sensitivity because of inhibitory substances	Increase the elution volume. A positive control can be spiked into the eluate, if desired, to determine the effect of elution volume on the amplification reaction. If eluates obtained from stool samples are turbid, we recommend centrifugation at full speed (20,000 x *g*) for 3 min to clear eluates. This will not have a negative impact on clear eluates, but will improve performance of turbid eluates in downstream applications.
j)	New combination of reverse transcriptase and *Taq* DNA polymerase	If the enzymes are changed, it may be necessary to readjust the amount of carrier RNA added to Buffer AVE and the amount of eluate used.
k)	Carryover of magnetic particles	Carryover of magnetic particles in the eluates will not affect most downstream applications, including RT-PCR. If the risk of magnetic-particle carryover needs to be minimized (e.g., for applications such as real-time PCR), first place the tubes containing eluate in a suitable magnet (e.g., 12-Tube Magnet [cat. no. 36912] for 1 min), and then transfer the eluates to clean tubes. If a suitable magnet is not available, centrifuge the tubes containing eluates in a microcentrifuge at full speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to clean tubes.
l)	Varying pipetting volumes	To ensure pipetting accuracy, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument User Manual. Check the fit of the filter tips regularly as described in the User Manual.

Appendix A: Calculating the Amount of Internal Control

To monitor the efficiency of sample preparation and downstream assay, an internal control may need to be added to the sample preparation process. To calculate the amount of internal control (IC) required in the EZ1&2 Virus Mini v2.0 protocol, the volume of the IC-containing buffer added per sample and the elution volume must be taken into account.

Determining how much internal control will be in downstream reactions

To determine the volume of internal control that will be present in the downstream assay, use the formula:

$$IC_{RXN} = \frac{IC_{AL} \times AL_{SAM} \times EL_{RXN}}{(AL_{TOT} + IC_{AL}) \times EL_{SAM}}$$

where:

IC_{RXN}	=	Volume of internal control per downstream reaction
IC_{AL}	=	Volume of internal control added to lysis buffer (AL)
AL_{SAM}	=	Volume of lysis buffer (AL) per sample
EL_{RXN}	=	Volume of eluate per downstream reaction
AL_{TOT}	=	Total volume of lysis buffer (AL) plus carrier RNA used in the protocol
EL_{SAM}	=	Volume of eluate per sample

As an example, User 1 has added 300 µL of internal control solution (IC_{AL}) to 22.1 mL of lysis buffer (AL_{TOT}) and 152 µL of carrier RNA. Using this procedure, 435 µL of lysis buffer will be added per sample (AL_{SAM}), and an elution volume of 75 µL (EL_{SAM}) has been selected by the user. User 1 uses 50 µL of eluate per downstream reaction (EL_{RXN}). The volume of internal control solution in each downstream reaction (IC_{RXN}) is:

$$IC_{RXN} = \frac{300\ \mu L \times 435\ \mu L \times 50\ \mu L}{(22.252\ \mu L + 300\ \mu L) \times 75\ \mu L} = 3.86\ \mu L$$

The final downstream reactions will contain 3.86 µL of internal control solution per reaction.

Determining how much internal control solution to add before starting

If you know the amount of internal control that you want to have present in the downstream assay (IC_{RXN}), then you need to determine the amount of internal control to be diluted with Buffer AVE and carrier RNA (IC_{AVE}) before starting the purification. To calculate this value, use the formula:

$$IC_{AVE} = \frac{IC_{RXN} \times IC_{TOT} \times EL_{SAM}}{IC_{SAM} \times EL_{RXN}}$$

where:

IC_{RXN} = Volume of internal control per downstream reaction

IC_{AVE} = Volume of internal control diluted in Buffer AVE–carrier RNA

IC_{SAM} = Volume of diluted internal control added per sample

EL_{RXN} = Volume of eluate per downstream reaction

IC_{TOT} = Total volume of diluted internal control in Buffer AVE–carrier RNA per run

EL_{SAM} = Volume of eluate per sample

As an example, User 2 is working with an assay that is optimized for use with 1 µL of internal control solution per reaction (IC_{RXN}) and 20 µL of eluate per reaction (EL_{RXN}). User 2 follows the EZ1&2 Virus Mini v2.0 protocol, and a 60 µL elution volume (EL_{SAM}) has been selected. For each processed sample, a volume of 60 µL of diluted internal control has to be manually pipetted into the 1.5 mL tube in position 3 of the EZ2 Connect worktable, but during the sample preparation process of the EZ1&2 Virus Mini v2.0 protocol the EZ2 Connect instrument will only transfer 50 µL of diluted internal control (IC_{SAM}) from well 3 to the binding reaction. For 6 samples being processed in one run, the total volume of diluted internal control (IC_{TOT}) to be made is:

IC_{TOT} = Number of samples per run x 60 µL

= 6 x 60 µL = 360 µL

The volume of internal control solution (IC_{AVE}) that User 2 needs for 6 samples is:

$$IC_{AVE} = \frac{1\ \mu L \times 360\ \mu L \times 60\ \mu L}{(50\ \mu L + 20\ \mu L)} = 21.6\ \mu L$$

For each sample, 3.6 µL carrier RNA stock solution with 1 µg/µL has to be added to the IC dilution. For 6 samples the total volume has to be calculated:

Total volume of carrier RNA stock = 6 x 3.6 µL carrier RNA stock = 21.6 µL

For a final total volume of 360 µL of diluted internal control, the user has to add Buffer AVE:

Volume of Buffer AVE = IC_{TOT} – IC_{AVE} – Volume of carrier RNA

= 360 µL – 21.6 µL – 21.6 µL = 316.8 µL

User 2 needs to add 21.6 µL of internal control solution to 316.8 µL Buffer AVE and 21.6 µL of carrier RNA stock in order to obtain 360 µL of diluted internal control. From this diluted

internal control, 60 µL has to be manually transferred into 1.5 mL tubes in position 3 of the EZ2 Connect worktable before starting the EZ1&2 Virus Mini v2.0 protocol.

Appendix B: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA 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 RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 37). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent*, thoroughly rinsed, and oven baked at 240°C for 4 hours or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC (diethyl pyrocarbonate)*. Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS)*, thoroughly rinsed with RNase-free water, and then rinsed with ethanol** and allowed to dry.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates

* 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.

** Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

RNases by covalent modification. Add 0.1 mL DEPC to 100 mL of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers*. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2.When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: The buffers of the EZ1&2 Virus Mini Kit v2.0 are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

Ordering Information

Product	Contents	Cat. no.
EZ1&2 Virus Mini Kit v2.0 (48)	For 48 preps: Reagent Cartridges (Virus Mini v2.0), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 mL), Elution Tubes (1.5 mL), Buffer AVE, Carrier RNA	955134
EZ2 Connect	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 1-year warranty on parts and labor	9003210
Accessories		
Filter-Tips and Holders (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1, EZ1&2, and EZ2 Kits	994900
Buffer AVE (108 x 2 mL)	108 tubes containing 2 mL Buffer AVE	1020953
Carrier RNA (12 x 1350 µg)	12 tubes containing 1350 µg carrier RNA	1017647
Buffer ATL (200 mL)	200 mL Buffer ATL	19076
Buffer ATL (4 x 50 mL)	4 x 50 mL Buffer ATL	939011
Buffer ASL	560 mL Buffer ASL	19082
Buffer AE	240 mL Elution Buffer for 1000 preps	19077

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

Document Revision History

Revision	Description
March 2023	Initial revision

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