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EZ1&2™ Virus Handbook

EZ1&2 Virus Mini Kit v2.0

For automated, simultaneous purification of viral DNA and RNA, and bacterial DNA using EZ1® instruments

QIAGEN

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 5 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 EZ1 Advanced and EZ1 Advanced XL 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.

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 	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 EZ1 Advanced, EZ1 Advanced XL, or BioRobot® EZ1, clean the affected area first with laboratory detergent and water, and then with disinfectants and detergents compatible with metallic surfaces as listed in the EZ1 Advanced 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 EZ1 instruments. For usage of EZ1&2 Virus Mini Kit v2.0 with EZ2 instruments, please refer to the handbook (**www.qiagen.com/HB-2978-001**) and quick-start protocol (www.qiagen.com/HB-2977-001).

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 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 instrument. Up to 6 samples are processed in a single run using the BioRobot EZ1 or EZ1 Advanced, or up to 14 samples are processed in a single run using the EZ1 Advanced XL.

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 flowchart, page 9).

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.

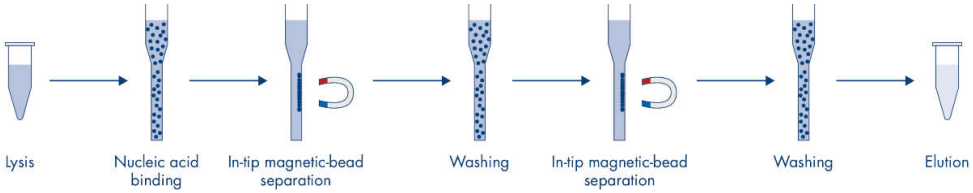

Figure 1. EZ1 Virus Mini Procedure

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)
- Vortexer

- Thermoshaker or 70°C water bath*

For pretreatment of dried swabs

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

For EZ1 Advanced users

- EZ1 Advanced instrument
- EZ1 Advanced Virus Card v2.0 (cat. no. 9018303)

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument
- EZ1 Advanced XL Virus Card v2.0 (cat. no. 9018708)

For EZ1 Advanced and EZ1 Advanced XL users

- For documentation purposes, one of the following is required:
- EZ1 Advanced Communicator Software (supplied with EZ1 Advanced and EZ1 Advanced XL instruments), PC (can be connected with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments), and monitor
- EZ1 Advanced Communicator Software (supplied with EZ1 Advanced and EZ1 Advanced XL instruments) and your own PC and monitor (connection with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments not recommended)
- Printer and accessory package for printer

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

For BioRobot EZ1 users

- BioRobot EZ1 instrument
- EZ1 Virus Card v2.0 (cat. no. 9017330)

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, CDPA (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 or –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. Incubate the bottles at 50°C for 15 minutes, and shake 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 prelysis step may be performed prior to purification on the EZ1

instrument (see page 30 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 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 48 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 39, 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 to –65°C (preferred) or at –30 to –15°C.

Working with EZ1 instruments

The main features of EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 or 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of EZ1 instruments
- Complete automation of nucleic acid purification from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps

Additional features of the EZ1 Advanced and EZ1 Advanced XL include:

- Bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV lamp to help eliminate sample carryover from run-to-run and to allow decontamination of the worktable surfaces

Note: UV decontamination helps to reduce possible pathogen contamination of the EZ1 Advanced and EZ1 Advanced XL 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.

EZ1 Cards, EZ1 Advanced Cards, and EZ1 Advanced XL Cards

Protocols for nucleic acid purification are stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Advanced XL Card into the EZ1 Advanced XL, an EZ1 Advanced Card into the EZ1 Advanced, or an EZ1 Card into the BioRobot EZ1, and the instrument is then ready to run a protocol (Figure 1). The availability of various protocols increases the flexibility of EZ1 instruments.

Figure 2. Ease of protocol setup using EZ1 Cards. Inserting an EZ1 Card, containing a protocol, into an EZ1 instrument. The instrument should only be switched on after an EZ1 Card is inserted. EZ1 Cards should not be exchanged while the instrument is switched on.

The EZ1&2 Virus Mini Kit v2.0 requires use of the EZ1 Advanced XL Virus Card v2.0 with the EZ1 Advanced XL, or use of the EZ1 Advanced Virus Card v2.0 with the EZ1 Advanced, or use of the EZ1 Virus Card v2.0 with the BioRobot EZ1. These EZ1 Cards contain protocols for purifying viral nucleic acids and bacterial DNA from various sample types.

EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 2), otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

Figure 3. Complete insertion of EZ1 Card. The EZ1 Card must be completely inserted before the EZ1 instrument is switched on.

Reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 3). 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.

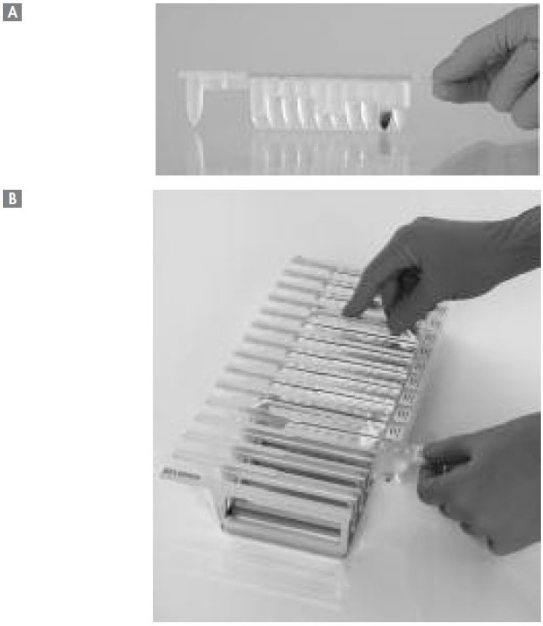

Figure 4. Ease of setup using reagent cartridges. (A) A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. (B) Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

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.

Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1&2 Virus Mini Kit v2.0 (Figure 4).

Details on worktable setup are provided in the protocol in this handbook, and are also displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced and EZ1 Advanced

XL or the liquid-crystal display (LCD) of the BioRobot EZ1 control panel when the user starts worktable setup.

The display also shows protocol status during the automated purification procedure.

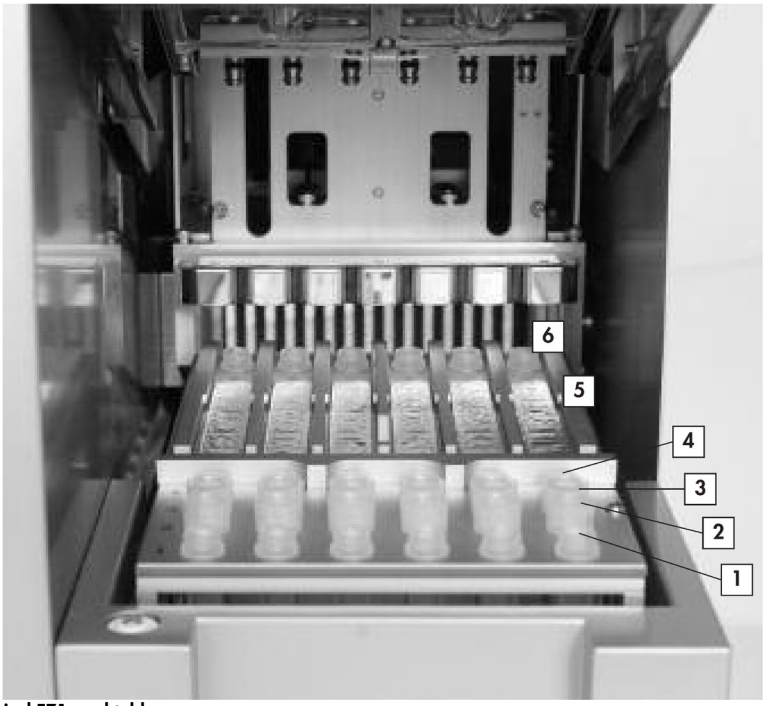

Figure 5. Typical EZ1 worktable.

1. First row: In the EZ1 Virus protocol, elution tubes (1.5 ml) are loaded here.
2. Second row: In the EZ1 Virus protocol, tip holders containing filter-tips are loaded here.
3. Third row: In the EZ1 Virus protocol, tubes (1.5 ml) containing carrier RNA and internal control (if used) in Buffer AVE are loaded here.
4. Fourth row: In the EZ1 Virus protocol, sample tubes (2 ml) are loaded here.
5. Reagent cartridges loaded into the cartridge rack.
6. Heating block with 2 ml tubes in the reagent cartridges for lysis (nonskirted 2 ml tubes provided with the EZ1&2 Virus Mini Kit v2.0 must be used).

Data tracking with the EZ1 Advanced and EZ1 Advanced XL

The EZ1 Advanced and EZ1 Advanced XL enable complete tracking of a variety of data for increased process control and reliability. The EZ1 Kit lot number and expiration date are entered at the start of the protocol using the Q-Card bar code. A user ID and the Q-Card bar code can be entered manually using the keypad or by scanning bar codes using the handheld bar code reader. Sample and assay information can also be optionally entered at the start of the protocol. At the end of the protocol run, a report file is automatically generated. The EZ1 Advanced and EZ1 Advanced XL can store up to 10 report files, and the data can be transferred to a PC or directly printed on a printer (for ordering information, see "Equipment and Reagents to Be Supplied by User" on page 10).

To receive report files on a PC, the EZ1 Advanced Communicator software needs to be installed. The software receives the report file and stores it in a folder that you define. After the PC has received the report file, you can use and process the file with a LIMS (Laboratory Information Management System) or other programs. An example of a report file is shown in Appendix C (page 45). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A to F or the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

When scanning a user ID or Q-Card bar code with the bar code reader, a beep confirms data input. After the information is displayed for 2 seconds, it is automatically stored, and the next display message is shown. When scanning sample ID, assay kit ID, or notes, a beep confirms data input, the information is displayed, and a message prompts you to enter the next item of information. After scanning sample ID, assay kit ID, and notes, press **ENT** once to confirm that the information entered is correct. If, for example, a wrong bar code was scanned for one of the samples, press **ESC** and then rescan all sample bar codes according to the onscreen instructions. For user ID and notes, you can enter the numbers using the keypad, or you can easily generate your own bar codes to encode these numbers.

For details about data tracking and using EZ1 Advanced Communicator software, see the *EZ1 Advanced User Manual* or the *EZ1 Advanced XL User Manual*.

Workflow of EZ1&2 Virus operation

Insert EZ1 Card into the EZ1 Card slot

↓

Switch on the EZ1 instrument

↓

Follow onscreen messages for data tracking*

↓

Follow onscreen messages for worktable setup

↓

Start the protocol

↓

Collect purified nucleic acids

↓

UV decontamination*

* EZ1 Advanced and EZ1 Advanced XL only.

Protocol: Pretreatment of Urine

This protocol is intended for pretreatment of urine prior to nucleic acid purification (page 31).

Procedure

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 48.

2. Mix the solution carefully.
3. Proceed to the purification protocol (page 31).

Protocol: Pretreatment of Whole Blood

This protocol is intended for pretreatment of whole blood prior to nucleic acid purification (page 31).

Procedure

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 48.

2. Mix the solution carefully.
3. Proceed to the purification protocol (page 31).

Protocol: Pretreatment of Stool

This protocol is intended for pretreatment of solid as well as liquid stool samples prior to nucleic acid purification (page 31).

Procedure

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 EZ1 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 EZ1 instrument. Large stool particles in the sample may lead to clogging of the filter tip of the EZ1 instrument.

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

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

6. Proceed to the purification protocol (page 31).

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 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 31).

Procedure

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

   Note: The volume is adjusted depending on the swab type. A volume of 400 µl must 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 31).

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 31).

Procedure

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 (cat. no. 19077). 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 31).

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

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 EZ1 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.

Procedure:

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 31).

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 13).
- 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 5). Do not use damaged kit components, since their use may lead to poor kit performance or contamination of the EZ1 instrument.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 5 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two choices can be made. Choose ▲ if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● if using the BioRobot EZ1.

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 13. 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 without pretreatment for purification of viral DNA and RNA, and bacterial DNA, with the EZ1&2 Virus Mini Kit v2.0.
- 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 14–15.

Procedure

1. For each sample, prepare a 60 µl solution containing 3.6 µl dissolved carrier RNA (with optional internal control) in a 1.5 ml tube (supplied). Mix gently by pipetting the solution 10 times. Do not vortex.

   The 1.5 ml tube is loaded into the third row in step 8.

   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 EZ1 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.

   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 (extra Buffer AVE available separately; see page 48 for ordering information).

   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. Insert ▲ the EZ1 Advanced Virus Card v2.0 completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL Virus Card v2.0 completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 Virus Card v2.0 completely into the EZ1 Card slot of the BioRobot EZ1.

4. Switch on the EZ1 instrument.

   The power switch is located at the rear of the instrument.

5. Press **START** to start worktable setup of the EZ1 Virus protocol.
6. Open the instrument door.
7. Invert reagent cartridges 3 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents to the bottom of their wells.
8. Follow the onscreen instructions for worktable setup, protocol variable selection, and ▲ data tracking.

   Note: After sliding a reagent cartridge into the cartridge rack, press down on the cartridge until it clicks into place.

   If there are fewer than 6 (BioRobot EZ1, EZ1 Advanced), or 14 (EZ1 Advanced XL) reagent cartridges, they can be loaded in any order on the rack. However, when loading the other labware, ensure that they also follow the same order.

   Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.

9. Close the instrument door.
10. Press **START** to start the protocol.
11. When the protocol ends, the display shows "Protocol finished". ▲ Press **ENT** to generate the report file.

    The EZ1 Advanced and EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

12. Open the instrument door.
13. Remove the elution tubes containing the purified viral nucleic acids and/or bacterial DNA from the first row. Discard the sample-preparation waste.*

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 5 for safety information.

14. ▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
15. Carry out the regular maintenance procedure as described in the user manual supplied with your EZ1 instrument.

    Regular maintenance must be carried out at the end of each protocol run. It consists of cleaning the piercing unit and the worktable surfaces.

    Note: The piercing unit is sharp! Use of double gloves is recommended.
16. To run another protocol, press **START**, carry out steps 1 and 2 of the protocol, and then follow the protocol from step 5. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

    Steps 3–4 are not necessary when running another protocol. Skip these steps.

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 EZ1 instrument.
b)	Report file not printed	Check whether the printer is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a printer.
c)	Report file not sent to the PC	Check whether the PC is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a PC.
d)	Wrong Q-Card ID entered	If the wrong ID was entered instead of the Q-Card ID, the EZ1 Advanced or EZ1 Advanced XL will not accept the ID and will prompt for the Q-Card ID until the correct ID is entered. Press **STOP** twice to go to the main menu.
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 14–15. Repeat the purification procedure with new samples.

		Comments and suggestions
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.
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", page 36, 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 (ICRXN) and 20 µl of eluate per reaction (ELRXN). User 2 follows the EZ1&2 Virus Mini v2.0 protocol, and a 60 µl elution volume (ELSAM) 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 EZ1 worktable, but during the sample preparation process of the EZ1&2 Virus Mini v2.0 protocol the EZ1 instrument will only transfer 50 µl of diluted internal control (ICSAM) from well 3 to the binding reaction. For 6 samples being processed in one run, the total volume of diluted internal control (ICTOT) 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 (ICAVE) 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 EZ1 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-

* 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.

free water (see "Solutions", page 43). 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 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 CO2.When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer.

* 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.

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.

Appendix C: Example of an EZ1 Advanced Report File

This appendix shows a typical report file generated on the EZ1 Advanced. The values for each parameter will differ from the report file generated on your EZ1 Advanced. Please note that "User ID" is allowed a maximum of 9 characters, and that "Assay kit ID" and "Note" are allowed a maximum of 14 characters. The EZ1 Advanced XL generates a similar report file containing instrument and protocol information relevant to the EZ1 Advanced XL and information for channels 1–14.

REPORT - FILE EZ1 Advanced:

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Serial no. EZ1 Advanced: _ _ _ _ _ _ _ _ _ _ _ SN 0001
User ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9876543210
Firmware version: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ V 1.0.0
Installation date of instrument: _ _ _ _ _ _ Jan 05, 2008
Weekly maintenance done on: _ _ _ _ _ Jun 15, 2008
Yearly maintenance done on: _ _ _ _ _ _ Jan 10, 2008
Date of last UV-run: _ _ _ _ _ _ _ _ _ _ _ Mar13, 2008
Start of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:06
End of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:26
Status UV-run: _ o.k.

Protocol name: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Virus 2.0

_ _

Date of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Jul 25, 2008
Start of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 12:57
End of run: _ 13:50
Status run: _ o.k.
Error code: _
Sample input volume [ul]: _ _ _ _ _ _ _ _ _ _ _ _ _ 400
Elution volume [ul]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 150

Channel A:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 123456789
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 1181234567
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 848373922
Note: _ 2000

Channel B:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 234567890
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 1181234567
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 836266738
Note: _

Channel C:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 345678901
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 1181234567
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 883727832
Note: _ 1000

Channel D:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 456789012
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 1181234567
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 763684837
Note: _

Channel E:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 567890123

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 4387728002

Note: _

Channel F:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 678901234

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 509389403

Note: _ 50

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
EZ1 Advanced Virus Card v2.0	Preprogrammed card for EZ1 Virus purification protocols on the EZ1 Advanced	9018303
EZ1 Advanced XL Virus Card v2.0	Preprogrammed card for EZ1 Virus purification protocols on the EZ1 Advanced XL	9018708
EZ1 Virus Card v2.0	Preprogrammed card for EZ1 Virus purification protocols on the BioRobot EZ1	9017330
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, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1 Kits	994900
Buffer AVE (108 x 2 ml)	108 tubes containing 2 ml Buffer AVE	1020953

Product	Contents	Cat. no.
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

Date	Changes
11/2021	Rebranded the name of the kit from “EZ1” to “EZ1&2”. Added the EZ2 Connect Instrument to the “Ordering Information” section. Editorial and layout changes.

Notes

Limited License Agreement for EZ1 Virus Mini Kit v2.0

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

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