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EZ1® DSP Virus Kit Handbook

Σ 48

Version 4

IVD

For in vitro diagnostic use.

CE

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Contents

Intended Use

The EZ1 DSP Virus Kit utilizes magnetic particle technology for automated isolation and purification of viral nucleic acids and bacterial DNA from biological specimens.

The product is intended to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

The EZ1 DSP Virus system is intended for in vitro diagnostic use.

Summary and Explanation

The EZ1 DSP Virus Kit provides a fully automated procedure for simultaneous purification of viral nucleic acids and bacterial DNA from the following sample materials using EZ1 instruments:

- Serum and 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, as well as DNA from bacteria. However, kit performance is not guaranteed for each pathogen species extracted from any of the sample materials 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. The EZ1 instrument performs all steps of the sample preparation procedure for up to 6 samples (using the EZ1 Advanced or the BioRobot EZ1 DSP*) or for up to 14 samples (using the EZ1 Advanced XL) in a single run.

* Not available in the US or Canada.

Principles of the 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). Pretreatment of the sample is important for urine, whole blood, stool, respiratory samples and dried swabs. Refer to the pretreatment protocol for the respective sample material.

Lysis with proteinase K

Proteolysis of 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 nucleases.

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 protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not bound to the magnetic particles.

Washing of bound nucleic acids

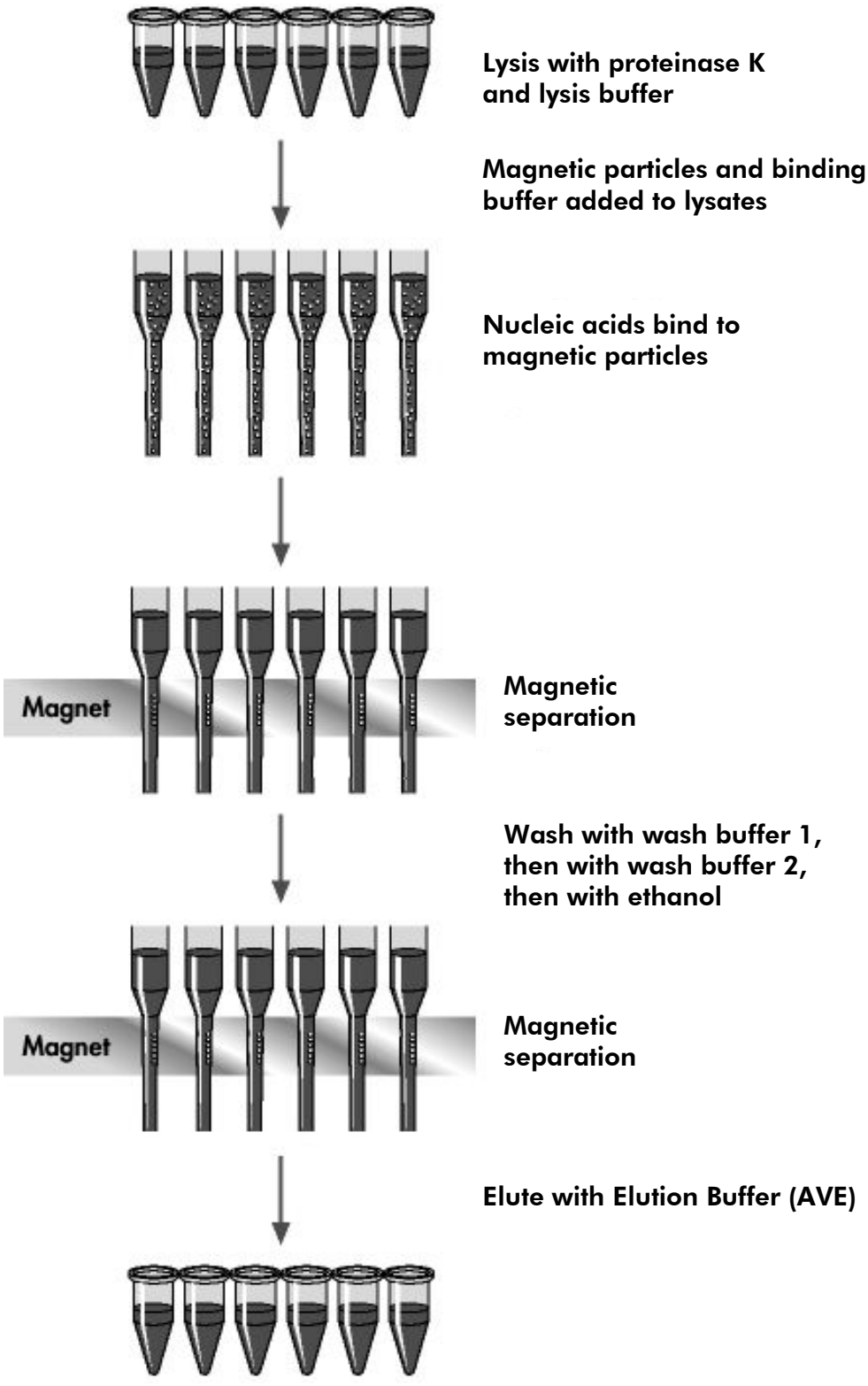
While viral nucleic acids and bacterial DNA remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps using first wash buffer 1, then wash buffer 2, and then ethanol.

Elution of pure nucleic acids

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

EZ1 DSP Virus Procedure

Serum, plasma, CSF, transport media or pretreated urine, whole blood, stool, respiratory samples, or dried swabs



Lysis with proteinase K and lysis buffer

Magnetic particles and binding buffer added to lysates

Nucleic acids bind to magnetic particles

Magnetic separation

Wash with wash buffer 1, then with wash buffer 2, then with ethanol

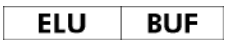
Magnetic separation

Elute with Elution Buffer (AVE)

Purified, high-quality viral nucleic acids and/or bacterial DNA

Materials Provided

Kit contents

EZ1 DSP Virus Kit			**(48)**
Catalog no.			**62724**
Number of preps			**48**
RCV	Reagent Cartridges, Virus*†		48
DTH	Disposable Tip Holders		50
DFT	Disposable Filter-Tips		50
ST	Sample Tubes (2 ml)		100
ET	Elution Tubes (1.5 ml)		100
CARRIER	Carrier RNA		310 µg
AVE	Elution Buffer†		3 x 2 ml
	Q-Card‡		1
	Handbook		1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 11 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 EZ1Advanced and EZ1 Advanced XL instruments.

Materials Required but not Provided

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

- Pipets* and sterile, RNase-free pipet tips
- Soft paper tissue
- Water
- 70% ethanol
- Optional: Vortexer* (if frozen samples need to be mixed)

For pretreatment of urine and whole blood

- ATL (cat. no. 939016)

For pretreatment of stool

- Buffer ASL (cat. no. 19082)
- Vortexer
- Thermoshaker* or 70°C water bath*

For pretreatment of dried swabs

- ATL (cat. no. 939016)
- Thermoshaker (56°C)*

For pretreatment of viscous respiratory samples

- Sputasol (Oxoid Limited; www.oxoid.com)
- Thermoshaker* or 37°C water bath*

For isolation of genomic DNA of Gram positive bacteria

- Lysozyme, Tris-HCl, EDTA, Triton X-100
- Thermoshaker* or 37°C water bath*

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

For BioRobot EZ1 users

- BioRobot EZ1 DSP instrument*† (cat. no. 9001360)
- EZ1 DSP Virus Card† (cat. no. 9017707)

For EZ1 Advanced users

- EZ1 Advanced instrument* (cat. no. 9001411)
- EZ1 Advanced DSP Virus Card (cat. no. 9018306)

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument* (cat. no. 9001492)
- EZ1 Advanced XL DSP Virus Card (cat. no. 9018703)

For EZ1 Advanced and EZ1 Advanced XL users

For sample tracking, one of the following is required:

- PC and TFT monitor, 17" (QIAGEN cat. no. 9016643), (or your own PC and monitor) with EZ1 Advanced Communicator Software (software supplied with EZ1 Advanced and EZ1 Advanced XL instruments)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

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

† Not available in the US or Canada.

Warnings and Precautions

For In Vitro Diagnostic Use.

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 (RCV) contain guanidine hydrochloride or guanidine isothiocyanate, 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 liquid containing potentially infectious agents is spilt on an EZ1 instrument, disinfect the instrument using reagents described in the user manual supplied with your EZ1 instrument.

Broken or leaky reagent cartridges (RCV) must be handled and discarded according to local safety regulations. Do not use damaged reagent cartridges (RCV) or other kit components, since their use may lead to poor kit performance.

QIAGEN has not tested the liquid waste generated by the EZ1 DSP Virus procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, residual liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

The following hazard and precautionary statements apply to the components of the EZ1 DSP Virus Kit:

Reagent Cartridge, Virus Mini, v2.0 CE

Contains: ethanol; guanidine thiocyanate; Isopropanol. Danger! Causes severe skin burns and eye damage. Highly flammable liquid and vapor. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Rinse skin with water/ shower. Immediately call a POISON CENTER or doctor/ physician. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Store in a well ventilated place. Keep cool. Wear protective gloves/protective clothing/eye protection/face protection.

Reagent Storage and Handling

Store the reagent cartridges (RCV) upright at room temperature (15–25°C). The magnetic particles in the reagent cartridges (RCV) remain active when stored at this temperature. **Do not freeze the reagent cartridges (RCV).** When stored properly, the reagent cartridges (RCV) are stable until the expiration date on the Q-Card and kit box.

Lyophilized carrier RNA (CARRIER) is stable until the expiration date on the kit box when stored at room temperature.

Precipitates may form in the pretreatment buffers ATL or ASL during storage at room temperature or at 2–8°C. Incubate the bottles at 50–56°C for 15–20 minutes and shake bottles manually twice within this incubation period.

Specimen Handling and Storage

During the pretreatment procedure, samples must be handled appropriately to exclude sample mix-up.

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 extraction of viral or bacterial nucleic acids from stool. Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation. Plasma samples can be either fresh or frozen, provided that they have not been refrozen after thawing.

Whole blood should be processed as fresh samples. If storage is required, we recommend storage of whole blood samples at 2–8°C for up to 2 days.

After collection (and centrifugation in the case of plasma and serum), samples can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots of samples other than whole blood at –80°C to –20°C. Thaw frozen 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 nucleic acids and bacterial DNA. If cryoprecipitates are visible in the samples, centrifuge at 6800 x *g* for 3 minutes ± 30 seconds, 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.

For the extraction of difficult-to-lyse Gram positive bacteria, an additional prelysis step comprising lysozyme digestion may be performed prior to extraction on the EZ1 instrument (see page 28 for “Protocol: Pretreatment for Isolation of Genomic DNA of Gram Positive Bacteria“).

Procedure

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 saving laboratory space
- Preprogrammed EZ1 DSP Cards* containing ready-to-use protocols
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup
- Complete automation of nucleic acid purification

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 DSP Cards,* EZ1 Advanced DSP Cards, and EZ1 Advanced XL DSP Cards

The protocols for purification of viral nucleic acids and bacterial DNA are stored on the preprogrammed EZ1 Cards. The user simply inserts an EZ1 Advanced XL DSP Card into the EZ1 Advanced XL, an EZ1 Advanced DSP Card in to the EZ1 Advanced, or an EZ1 DSP Card* into the BioRobot EZ1 DSP instrument*, and the instrument is then ready to run a protocol (Figures 1 and 2).

* Not available in the US or Canada.

Figure 1. Ease of protocol setup using EZ1 DSP Cards. Inserting an EZ1 Card, preprogrammed with the protocol, into the EZ1 instrument.

Note: The instrument should only be switched on after the applicable EZ1 DSP Card is inserted. Make sure that the applicable EZ1 DSP Card is completely inserted! Otherwise essential instrument data could be lost, leading to a memory error. The applicable EZ1 DSP Card should not be exchanged while the instrument is switched on.

Figure 2. Card completely inserted into EZ1 Card slot.

The EZ1 DSP Virus Kit requires use of the EZ1 DSP Virus Card,* EZ1 Advanced DSP Virus Card, or EZ1 Advanced XL DSP Virus Card. The cards contain protocols for purifying viral nucleic acids and bacterial DNA from serum, plasma, CSF, urine, whole blood, stool, transport media, dried swabs and respiratory samples.

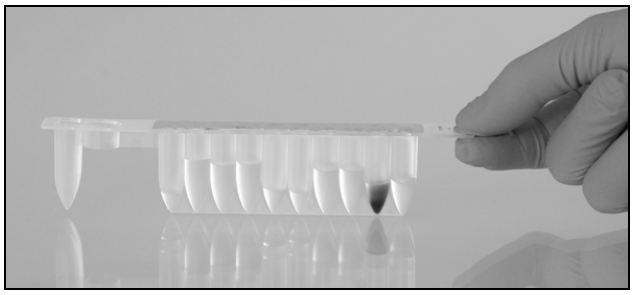
* Not available in the US or Canada.

Reagent cartridges (RCV)

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (RCV) (Figure 3). Each well of the cartridge (RCV) contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or RNase-free elution buffer (AVE). 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.

The reagent cartridges (RCV) supplied with the EZ1 DSP Virus Kit are prefilled with all the necessary reagents for purification of viral nucleic acids and bacterial DNA, except carrier RNA (CARRIER). Carrier RNA (CARRIER) and internal controls (IC) (optional) are added in a tube outside the reagent cartridge (RCV).

A



B

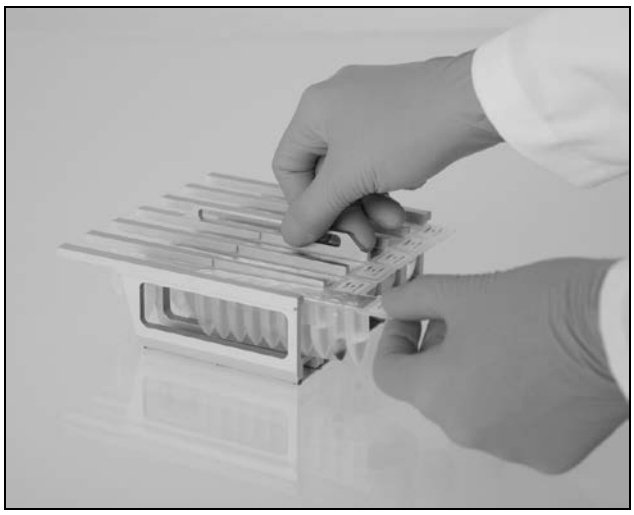


Figure 3. Ease of instrument setup using reagent cartridges (RCV). **A** A sealed, prefilled reagent cartridge (RCV). Fill levels vary, depending on the type of reagent cartridge (RCV). **B** Loading reagent cartridges (RCV) into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges (RCV) must be loaded.

Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1 DSP Virus Kit.

Details on worktable setup are 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 DSP* control panel when the user starts worktable setup.

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

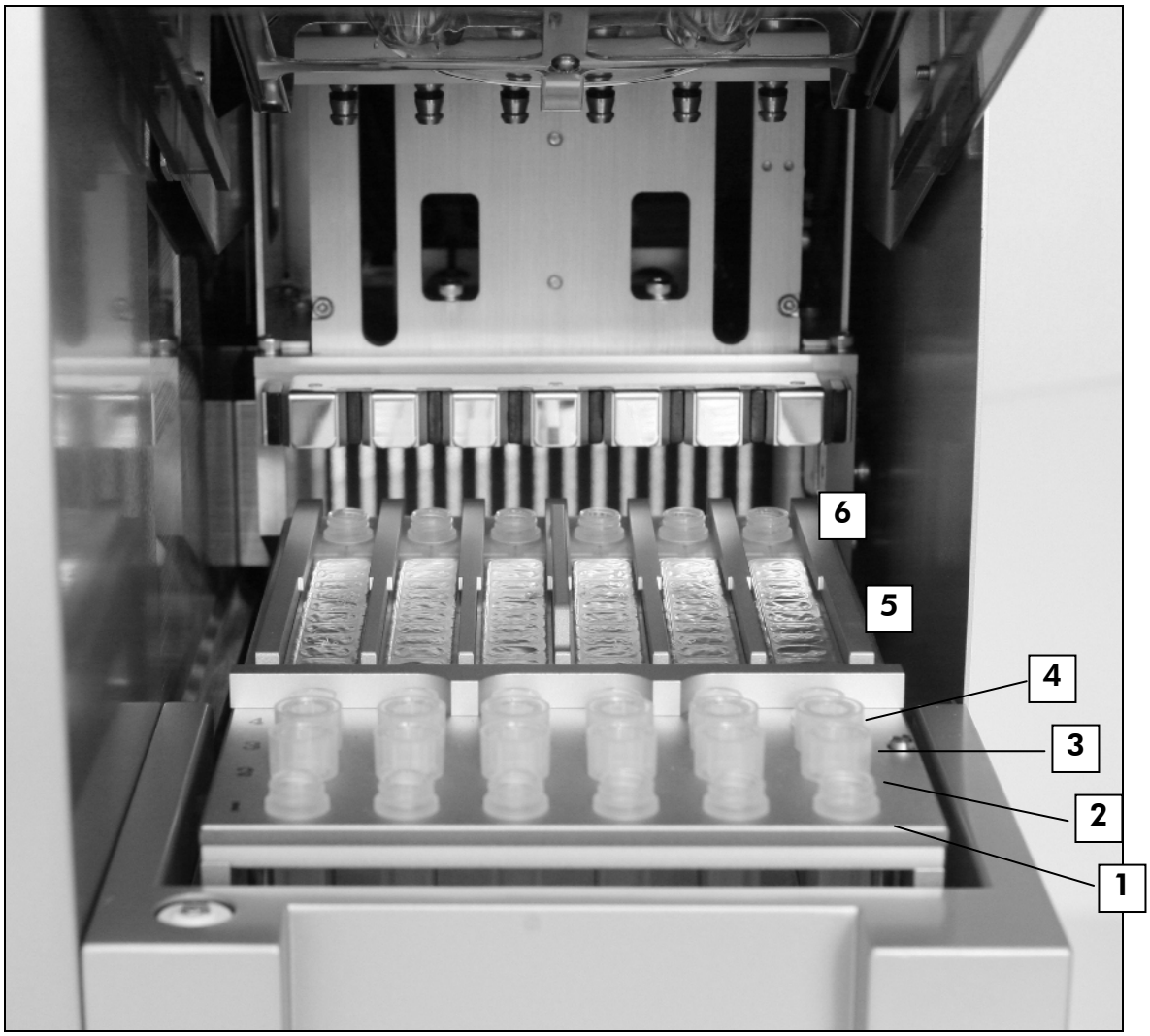


Figure 4. Worktable of an EZ1 instrument.

1. Elution tubes (ET) (1.5 ml) loaded into the first row.
2. Disposable tip holders (DTH) containing disposable filter-tips (DFT) loaded into the second row.
3. Tube (ET) (1.5 ml) containing carrier RNA (CARRIER) and internal control (IC) (if used) in elution buffer (AVE), loaded into the third row.
4. Sample tubes (ST) (2 ml) loaded into the fourth row.
5. Reagent cartridges (RCV) loaded into the cartridge rack.
6. Heating block with 2 ml tubes (ST) in the reagent cartridges for lysis.

* Not available in the US or Canada.

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 DSP 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 (see "Workflow of EZ1 DSP Virus operation", page 19).

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. 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.

Note: For data tracking, always start loading samples in position A on the EZ1 Advanced and position 1 on the EZ1 Advanced XL. Place the remaining samples consecutively into the next open positions on the worktable.

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

Workflow of EZ1 DSP Virus operation

Insert EZ1 DSP Virus 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.

Preparing carrier RNA (CARRIER)

Carrier RNA (CARRIER) serves two purposes during the purification procedure. First, it enhances binding of viral nucleic acids and bacterial DNA 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 (CARRIER) 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 (CARRIER) is not added to the reaction, recovery of viral DNA or RNA or bacterial DNA may be reduced.

The lyophilized carrier RNA (CARRIER) provided with the kit is sufficient for 48 sample preparations. The concentration of carrier RNA (CARRIER) used in the purification procedure allows the EZ1 DSP Virus Kit 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 the EZ1 DSP Virus Kit contain viral and bacterial nucleic acids and carrier RNA (CARRIER), and the amount of carrier RNA (CARRIER) in each eluate greatly exceeds the amount of viral and bacterial nucleic acids. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA (CARRIER) solution added.

Dissolve the lyophilized carrier RNA (CARRIER) thoroughly in 310 µl elution buffer (AVE), divide it into conveniently sized aliquots, and store at –20 ± 5°C. Do not freeze–thaw the aliquots more than 2 times.

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

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

Note: The purification procedure is optimized so that 3 µg carrier RNA (CARRIER) is added per sample. If a different amount of carrier RNA (CARRIER) has been shown to be better for a specific amplification system, change the volume of carrier RNA (CARRIER) stock solution mixed with elution buffer (AVE) or use a different concentration of stock solution. The total volume of carrier RNA–elution buffer (CARRIER–AVE) solution per sample should be 60 µl, of which 50 µl is transferred to the lysis mix. Use of different amounts of carrier RNA (CARRIER) must be validated for each particular sample type and downstream assay.

Using an internal control (IC)

Using the EZ1 DSP Virus Kit in combination with commercially available amplification systems may require introducing an internal control (IC) into the purification procedure to monitor the efficiency of sample preparation.

Internal control DNA or RNA should be combined with carrier RNA (CARRIER) stock solution (3.6 µl) in one mixture. For each sample, the carrier RNA–internal control (CARRIER–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 (CARRIER) stock solution plus 47 µl elution buffer (AVE) and/or internal control solution.

Note: If the internal control (IC) 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 sample preparation.

Refer to the manufacturer's instructions to determine the optimal amount of internal control (IC) for specific downstream applications. Using an amount other than that recommended may reduce amplification efficiency. To determine the amount of internal control (IC) needed for the EZ1 DSP Virus protocol, the volume of the eluate needs to be taken into account. See "Appendix B: Calculating the Amount of Internal Control", page 58, for detailed instructions on how to calculate the correct volume of internal control (IC).

Internal controls (IC) are not provided in the EZ1 DSP Virus Kit.

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 ± 30 seconds to clear the eluates. This treatment will improve performance of turbid eluates in downstream applications.

Storing viral nucleic acids/bacterial DNA

For short-term storage of up to 24 hours, we recommend storing the purified viral nucleic acids or bacterial DNA at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –80°C to –20°C.

Performance Characteristics

For any additional information that may be available in your country, please visit the QIAGEN website:
http://www.qiagen.com/literature/handbooks/literature.aspx?id=1001022

Protocol: Pretreatment of Urine

This protocol is intended for pretreatment of urine prior to nucleic acid purification (page 29).

Procedure

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

Table 9. Urine and ATL volumes

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

ATL should be ordered separately, see ordering information, page 64.

2. **Mix the solution by carefully pipetting up and down, or by inverting the closed tube 3 times.**
3. **Proceed to the purification protocol (page 29)**

Protocol: Pretreatment of Whole blood

This protocol is intended for pretreatment of whole blood samples prior to nucleic acid purification (page 29).

Procedure

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

Table 10. Whole blood and ATL volumes

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

ATL should be ordered separately, see ordering information, page 64.

2. **Mix the solution by carefully pipetting up and down, or by inverting the closed tube 3 times.**
3. **Proceed to the purification protocol (page 29).**

Protocol: Pretreatment of Stool

This protocol is intended for pretreatment of solid as well as liquid stool samples prior to nucleic acid purification (page 29).

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 pretreatment for extraction with the EZ1 instrument.

2. **Vortex the sample vigorously for 1–2 minutes 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 pipet tip. Some particles will remain insoluble and will be removed during the next step.

3. **Incubate the sample for 10 minutes ± 1 minute at room temperature on the bench to allow for 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 stool 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 minutes ± 1 minute at 70°C ± 3°C in a water bath* or thermoshaker*.**

6. **Proceed to the purification protocol (page 29).**

   Note: For stool samples, it is recommended to use 200 µl sample volume for extraction 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 minutes ± 30 seconds 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.

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

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 29).

Procedure

1. **Add 600 µl of 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 extraction.

2. **Incubate the swab for 15 minutes ± 1 minute at 56°C ± 3°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 29).**

Protocol: Pretreatment of Viscous 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 29).

Procedure

1. **Add 1 volume of Sputasol solution to 1 volume of sample and shake well.**
2. **Place in a water bath* or thermoshaker* and incubate at 37°C ± 3°C with periodic shaking until the sample is completely liquefied.**
3. **Proceed to the purification protocol (page 29).**

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

Protocol: Pretreatment for Isolation of genomic DNA of Gram Positive Bacteria

DNA extraction 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 minutes ± 1 minute at 5000 x *g* (7500 rpm in a microcentrifuge).**
2. **Suspend bacterial pellet in 180 µl of the enzyme solution (20 mg/ml lysozyme; 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 minutes at 37°C ± 3°C.**
4. **Briefly centrifuge the tube to remove drops from the inside of the lid.**
5. **Proceed to the purification protocol (page 29).**

Protocol: Purification of Viral Nucleic Acids and Bacterial DNA

Important points before starting

- If using the EZ1 DSP Virus Kit for the first time, read "Procedure" (page 14).
- The reagent cartridges (RCV) contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 11 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- After receiving the kit, check the kit components for damage. If the reagent cartridges (RCV) or other kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to "Warnings and Precautions" (page 11). Do not use damaged reagent cartridges (RCV) or other kit components, since their use may lead to poor kit performance.
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● if using the BioRobot EZ1 DSP*.

Things to do before starting

- The lysis buffer in the reagent cartridge (RCV) may form precipitates upon storage. If necessary, redissolve by warming at 30–40°C and then place at room temperature.
- Prepare serum, plasma, CSF, or transport media samples as described in "Specimen Handling and Storage", page 13. If cryoprecipitates are visible in the thawed samples, centrifuge at 6800 x *g* for 3 minutes ± 30 seconds, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.
- Prepare urine samples as described in "Protocol: Pretreatment of Urine", page 23.
- Prepare whole blood samples as described in "Protocol: Pretreatment of Whole blood", page 24.
- Prepare stool samples as described in "Protocol: Pretreatment of Stool", page 25.

* Not available in the US or Canada.

- Prepare dried swab samples as described in "Protocol: Pretreatment of Dried Swabs", page 26.
- Prepare viscous respiratory samples as described in "Protocol: Pretreatment of Viscous Respiratory Samples", page 27. Nonviscous respiratory samples do not require pretreatment.
- Prepare a carrier RNA (CARRIER) stock solution (with optional internal control [IC]) before using it for the first time. Dissolve the lyophilized carrier RNA (CARRIER) in 310 µl elution buffer (AVE) (provided in the kit), and mix it with the internal control (IC) (optional) as described in "Preparing carrier RNA (CARRIER)" and "Using an internal control (IC)", pages 20–21.

Procedure

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

   The 1.5 ml tube (ET) is loaded into the third row, as specified in the onscreen instructions.

   Note: Make sure that the carrier RNA (CARRIER) solution is at the bottom of the 1.5 ml tube (ET) so 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 (ST), and equilibrate to room temperature (15–25°C) 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 2 ml tubes (ST) provided with the kit.

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

   We recommend using 100 µl, 200 µl, or 400 µl sample volume. A sample volume of 200 µl is recommended for extraction of viral/bacterial nucleic acids from stool. For pretreatment of samples, refer to the appropriate pretreatment protocol. If you want to use less sample, bring the volume up to 100 µl, 200 µl, or 400 µl with the appropriate amount of elution buffer (AVE) (extra elution buffer [AVE] not supplied; available separately).

   Note: Do not use sample volumes greater than 100 µl, 200 µl, or 400 µl. After lysis and binding of viral nucleic acids or bacterial DNA to the magnetic particles, a portion of the lysate is transferred to the sample tube (ST) to inactivate residual viruses. Any sample left in the sample tube (ST) after sample transfer will therefore be lost.

3. **Insert ▲ the EZ1 Advanced DSP Virus Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DSP Virus Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL, or ● the EZ1 DSP Virus Card* completely into the EZ1 Card slot of the BioRobot EZ1 DSP*.**
4. **Switch on the EZ1 instrument.**
   The power switch is located at the left rear of the instrument.
5. **Press "START" to start worktable setup of the EZ1 DSP Virus protocol.**
6. **Open the instrument door.**
7. **Invert reagent cartridges (RCV) 3 times to mix the magnetic particles. Then tap the cartridges (RCV) 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 (RCV) into the cartridge rack, press down on the cartridge until it clicks into place.

   Note: If there are fewer than 6 (BioRobot EZ1 DSP*, EZ1 Advanced) or 14 (EZ1 Advanced XL) reagent cartridges (RCV), 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: Make sure that the sample volumes correspond to the sample volume in the protocol chosen.

   Note: Make sure that the elution volumes correspond to the elution volume in the protocol chosen.

   ▲ **Note**: For data tracking, always start loading samples in position A on the EZ1 Advanced and position 1 on the EZ1 Advanced XL. Place the remaining samples consecutively into the next open positions on the worktable.

   ▲ **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.

* Not available in the US or Canada.

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

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 22 for safety information.

Quality Control

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

Limitations

It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance evaluation studies.

The system performance has been established in performance evaluation studies using plasma, serum, CSF, urine, whole blood, stool, transport media, dried swabs and respiratory samples for isolation of viral nucleic acids and bacterial DNA. The performance evaluation was carried out only with the combinations of pathogen and sample material listed within the performance data of the handbook.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonisation of Technical Requirements (ICH) in *ICH Q2(R1) Validation Of Analytical Procedures: Text And Methodology* are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Symbols

Kit contains reagents for 48 sample preparations


To be used by

IVD — In vitro diagnostic medical device

REF — Catalog number

LOT — Lot number

MAT — Material number

COMP — Components

NUM — Number

VOL — Volume

GTIN — Global Trade Item Number


Temperature limitations


Legal manufacturer

USE — Only for use with

CONT — Contains

GITC — Guanidine isothiocyanate

GuHCl — Guanidine hydrochloride

EtOH — Ethanol

IPA — Isopropanol

PROTK — Proteinase K


This side down when opening

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Contact Information

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding the EZ1 DSP Virus Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
General handling	
a) Error message in instrument display	Refer to the user manual supplied with your 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 nucleic acids or bacterial DNA	
a) Magnetic particles not completely resuspended	Ensure that you resuspend the magnetic particles thoroughly before loading the reagent cartridges (RCV) into the holder.
b) Insufficient reagent aspirated	After inverting the reagent cartridges (RCV) to resuspend the magnetic particles, ensure that you tap the cartridges (RCV) to deposit the reagents at the bottom of the wells.

	Comments and suggestions
c) Reagents loaded onto worktable in wrong order	Ensure that all tubes (ET, ST) and the tip holders (DTH) with the tips (DFT) are loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.
d) Carrier RNA (CARRIER) not added	Reconstitute the lyophilized carrier RNA (CARRIER) in 310 µl elution buffer (AVE). For each sample, use 3.6 µl of this carrier RNA (CARRIER) stock solution, mixed with internal control (IC) (optional) and additional elution buffer (AVE) to a final volume of 60 µl, as described in "Preparing carrier RNA (CARRIER)" and "Using an internal control (IC)", pages 20–21. Repeat the purification procedure with new samples.
e) Carrier RNA (CARRIER) and elution buffer (AVE) not sufficiently mixed	Mix carrier RNA (CARRIER), internal control (IC) (optional), and elution buffer (AVE) by pipetting at least 10 times.
f) RNA degraded	RNA may have been degraded by RNases in the original 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 (RCV) into a shaker–incubator, and incubate at 30–40°C with mild agitation for up to 2 hours. Do not use the reagent cartridges (RCV) if the precipitates do not redissolve.
RNA or DNA does not perform well in downstream applications	
a) Little or no nucleic acid in the eluate	See "Low yield of viral nucleic acids 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 seconds.

	Comments and suggestions
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 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 (RCV) were stored at elevated temperatures for too long, leading to inactivation of proteinase K. Repeat the purification procedure using new samples and reagent cartridges (RCV).
e) Salt carryover during elution	For best results, ensure that the reagent cartridges (RCV) are at 20–30°C.
f) Too much or too little carrier RNA (CARRIER) in the eluate	Determine the maximum amount of carrier RNA (CARRIER) suitable for your amplification reaction. Adjust the concentration of carrier RNA (CARRIER) 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 (RCV) may have separated due to long-term storage. Always shake the cartridges (RCV) thoroughly and tap them before starting a purification procedure.
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 minutes ± 30 seconds to clear eluates. This will not have a negative impact on clear eluates, but will improve performance of turbid eluates in downstream applications.

	Comments and suggestions
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 (CARRIER) added to elution 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 minute, 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 minute to pellet any remaining magnetic particles, and transfer the supernatants to clean tubes.

Appendix A: Display Messages

The messages displayed by the software protocol during worktable setup, during the protocol run, and after the protocol run are listed in Tables 11–13. The numbers of the messages listed in the tables correspond to the numbers of the messages displayed by the software.

For general error messages on the EZ1 instrument display, see the user manual supplied with your EZ1 instrument.

Table 11. Messages in the EZ1 Advanced XL DSP Virus Procedure

Message number	Message type	EZ1 Advanced XL message text
None	Guidance	Date/time START: Run 1: UV 2: Man 3: Test 4: Setup
1	Guidance	EZ1 Advanced XL DSP Virus Version 1.0
2	Data tracking	Enter user ID ENT: Next
3	Data tracking	Enter Q-Card bar code ENT: Next
4	Guidance	Wrong kit! Please load EZ1 DSP Virus Kit ENT: Back
5	Guidance	Kit expired MMYY ENT: Use new kit ESC: Stop protocol
6	Data tracking	Use Q-Card data with sample 1 to xx Enter 1 to 14 ENT: Next

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
7	Guidance	Do you want to process more samples with another kit lot ENT: Yes, ESC: no
8	Data tracking	Do you want to add sample ID? ENT: Yes ESC: No
9	Data tracking	Enter sample ID for sample no. [x] ENT: Next
10	Data tracking	Do you want to check sample IDs? ENT: Yes ESC: No
11	Data tracking	ID 1: ID 2: ID 3: DOWN: Next
12	Data tracking	ID 4: ID 5: ID 6: DOWN: Next, UP: Back
13	Data tracking	ID 7: ID 8: ID 9: DOWN: Next, UP: Back
14	Data tracking	ID 10: ID 11: ID 12: DOWN: Next, UP: Back

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
15	Data tracking	ID 13: ID 14: ESC: Rescan DOWN: Next, UP: Back
16	Data tracking	Do you want to add assay information? ENT: Yes, ESC: No
17	Data tracking	Enter assay ID for sample no. [x] ENT: Next
18	Data tracking	Do you want to check assay IDs? ENT: Yes ESC: No
19	Data tracking	Do you want to add notes? ENT: Yes ESC: No
20	Data tracking	Enter notes for sample no. [x] ENT: Next
21	Data tracking	Do you want to check notes? ENT: Yes ESC: No
22	Selection	Select sample volume: 1: 100 ul 2: 200 ul 3: 400 ul

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
23	Selection	Select elution volume: 1: 60 ul 2: 90 ul 3: 120 ul 4: 150 ul
24	Guidance	You have chosen: Sample volume: xxxul Elution volume:yyyul ENT: Next, ESC: Back
25	Guidance	Load cartridges at same positions as samples ENT: Next, ESC: Back
26	Guidance	Load empty 2 ml tubes into heating block ENT: Next, ESC: Back
27	Guidance	Load elution tubes (1.5 ml) into first row ENT: Next, ESC: Back
28	Guidance	Load tip holders and tips into second row ENT: Next, ESC: Back
29	Guidance	Load 1.5ml tubes containing cRNA and IC into third row ENT: Next, ESC: Back
30	Guidance	Load 2 ml tubes with sample into fourth row ENT: Next, ESC: Back

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
31	Guidance	Loading finished Close door and press START ESC: Back
32	Guidance	Please close door! ENT: Next
33	Guidance	Checking temperature Set: Cur:
34	Status	Protocol started
35	Status	Piercing foil [x] of 43 min left
36	Status	Collecting elution buffer AVE [x] of 43 min left
37	Status	Collecting cRNA + IC [x] of 43 min left
38	Status	Collecting Lysis Buffer [x] of 43 min left
39	Status	Collecting Sample [x] of 43 min left
40	Status	Collecting Proteinase K [x] of 43 min left

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
41	Status	Mixing lysate [x] of 43 min left
42	Status	15 min Incubation [x] of 43 min left
43	Status	Tip touch [x] of 43 min left
44	Status	Collecting Binding Buffer [x] of 43 min left
45	Status	Collecting Lysis Buffer [x] of 43 min left
46	Status	Collecting Beads [x] of 43 min left
47	Status	Resuspending Beads in Binding Buffer [x] of 43 min left
48	Status	Transfering Lysate [x] of 43 min left
49	Status	Binding Magnetic Separation [x] of 43 min left
50	Status	Wash 1 Magnetic Separation [x] of 43 min left

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
51	Status	Wash 2 Magnetic Separation [x] of 43 min left
52	Status	Wash 3 Magnetic Separation [x] of 43 min left
53	Status	Drying Beads [x] of 43 min left
54	Status	Rinse [x] of 43 min left
55	Status	Elution [x] of 43 min left
56	Guidance	Check transfer of cRNA + IC (row 3) ENT: Next
57	Guidance	Check transfer of sample (row 4) ENT: Next
58	Guidance	Protocol finished ENT: Next
59	Data tracking	Transferring report file Attempt no.
60	None	

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
None	Guidance	Report file sent Print out o.k.? 1: o.k. 2: not o.k.
61	Guidance	Report file sent ENT: Next
62	Guidance	Report file could not be sent ENT: Resend
63	Guidance	Perform UV run? ENT: Yes ESC: No
64	Guidance	Remove eluates and consumables from the worktable ENT: Next
65	Guidance	UV decontamina- tion: Enter 20-60 min ENT: Next
66	Guidance	UV decontamina- tion time must be between 20-60 min ESC: Back
67	Guidance	UV decontamination Total time: min Time left: min
68	Guidance	Perform regular maintenance after each run ESC: Main menu

Table continued on next page.

Table 11. Continued

Message number	Message type	EZ1 Advanced XL message text
69	Guidance	UV lamps expire soon UV runs left: ENT: Next
70	Guidance	UV lamps are expired ENT: Next ESC: Abort
71	Guidance	Decontamination UV lamps cooling Please stand by
72	Guidance	Perform regular maintenance after each run ESC: Main menu

Table 12. Messages in the EZ1 Advanced DSP Virus Procedure

Message number	Message type	EZ1 Advanced message text
None	Guidance	Date/Time START:Run 1: UV 2: Man 3: Test 4: Setup Key: START, 1, 2, 3, 4
1	Guidance	EZ1 Advanced DSP Virus Version 1.0
2	Data tracking	Scan/enter user ID
3	Data tracking	Scan/enter Q-Card bar code
4	Guidance	Wrong kit! Please load EZ1 DSP Virus Kit ENT=back
5	Guidance	Kit expired ENT: Use new kit ESC: Stop protocol
6	Data tracking	Use Q-Card data with sample no. 1 to Enter 1 to 6
7	Guidance	Do you want to process more samples with another kit lot ENT: Yes, ESC: no
8	Data tracking	Do you want to add sample ID? ENT: Yes ESC: No
9	Data tracking	Scan/enter sample ID sample no. [x]

Table continued on next page.

Table 12. Continued

Message number	Message type	EZ1 Advanced message text
10	Data tracking	ID1: ID2: ID3: Next=ENT
11	Data tracking	ID4: ID5: ID6: Next=ENT, ID1-3=Up
12	Data tracking	Do you want to add assay information? ENT: Yes, ESC: No
13	Data tracking	Scan/enter assay ID ID sample no. [x]
14	Data tracking	Do you want to add notes? ENT: Yes ESC: No
15	Data tracking	Scan/enter notes sample no. [x]
16	Guidance	Select sample volume: 1: 100 ul 2: 200 ul 3: 400 ul
17	Guidance	Select elution volume: 1: 60 ul 2: 90 ul 3: 120 ul 4: 150 ul
18	Guidance	You have chosen: Sample volume: [xxx] ul Elution volume: [yyy] ul Next=Any, Prev=Esc
19	Guidance	Load cartridges at same positions as sample Next=Any, Prev=Esc

Table continued on next page.

Table 12. Continued

Message number	Message type	EZ1 Advanced message text
20	Guidance	Load empty 2.0 ml tubes at heating block Next=Any, Prev=Esc
21	Guidance	Load elution tubes (1.5 ml) into first row Next=Any, Prev=Esc
22	Guidance	Load tip holders and tips into second row Next=Any, Prev=Esc
23	Guidance	Load 1.5 ml tubes containing cRNA and IC in third row Next=Any, Prev=Esc
24	Guidance	Load 2.0 ml tubes with sample in fourth row Next=Any, Prev=Esc
25	Guidance	Loading finished. Close door and press START Prev=Esc
26	Guidance	Please close door!
27	Guidance	Checking temperature Set: Cur:
28	Status	Protocol started
29	Status	Piercing foil
30	Status	Collecting Elution Buffer AVE
31	Status	Collecting cRNA + IC
32	Status	Collecting Lysis Buffer
33	Status	Collecting Sample
34	Status	Collecting Proteinase K
35	Status	Mixing Lysate
36	Status	15 min Incubation [x] of 43 min left

Table continued on next page.

Table 12. Continued

Message number	Message type	EZ1 Advanced message text
37	Status	Kick [x] of 43 min left
38	Status	Collecting Binding Buffer [x] of 43 min left
39	Status	Collecting Lysis Buffer [x] of 43 min left
40	Status	Collecting Beads [x] of 43 min left
41	Status	Resuspension of Beads in Binding Buffer [x] of 43 min left
42	Status	Transferring Lysate [x] of 43 min left
43	Status	Binding Magnetic Separation [x] of 43 min left
44	Status	Wash 1 Magnetic Separation [x] of 43 min left
45	Status	Wash 2 Magnetic Separation [x] of 43 min left
46	Status	Wash 3 Magnetic Separation [x] of 43 min left
47	Status	Dry Beads [x] of 43 min left
48	Status	Rinse [x] of 43 min left
49	Status	Elution [x] of 43 min left

Table continued on next page.

Table 12. Continued

Message number	Message type	EZ1 Advanced message text
50	Guidance	Check transfer of cRNA + IC (row 3) Next=Any
51	Guidance	Check transfer of sample (row 4) Next=Any
52	Guidance	Protocol finished
53	Data tracking	Transfer Report file, attempt no.
54	Guidance	Report file sent Next=ENT
55	Guidance	Report file could not be sent Resend=ENT
56	Guidance	Perform UV run? ENT: Yes ESC: No
57	Guidance	UV decontamination Set time min Key:0-9, ENT
58	Guidance	UV decontamination. Time must be between 20-60 min Key:ESC
59	Guidance	UV decontamination Time left: min
60	Guidance	Perform regular maintenance after each run ESC=Main menu
61	Guidance	UV lamp expires soon UV runs left: ENT=continue

Table continued on next page.

Table 12. Continued

Message number	Message type	EZ1 Advanced message text
62	Guidance	UV lamp is expired ENT=continue ESC=abort
63	Guidance	Decontamination UV lamp cooling Please stand by

Table 13. Messages in the BioRobot EZ1 DSP* Virus Procedure

Message number	Message type	BioRobot EZ1 DSP message text
None	Guidance	Choose button: START: Protocols 1: Tools 2: Tests
1	Guidance	BioRobot EZ1 DSP Virus Version
2	Guidance	Select sample volume: 1: 100ul 2: 200ul 3: 400ul
3	Guidance	Select elution volume: 1: 60ul 2: 90ul 3: 120ul 4: 150ul
4	Guidance	You have chosen: Sample Volume:[sample volume]ul Elution Volume:[elution volume]ul Next=Any, Prev=ESC
5	Guidance	Load cartridges (RCV) at same positions as samples Next=Any, Prev=ESC
6	Guidance	Load empty 2.0ml tubes (ST) at heating block Next=Any, Prev=ESC
7	Guidance	Load elution tubes (ET) (1.5ml) into first row Next=Any, Prev=ESC
8	Guidance	Load tip holders (DTH) and tips (DFT) into second row Next=Any, Prev=ESC
9	Guidance	Load 1.5ml tubes (ET) with (CARRIER) + IC in third row Next=Any, Prev=ESC

Table continued on next page.

* Not available in the US or Canada.

Table 13. Continued

Message number	Message type	BioRobot EZ1 DSP message text
10	Guidance	Load 2.0ml tubes (ST) with sample in fourth row Next=Any, Prev=ESC
11	Guidance	Start protocol Press START Prev=ESC
12	Status	Checking Temperature Set: 63.0 [deg] Cur: [deg]
13	Status	Protocol started
14	Status	Piercing Foil
15	Status	Collecting Elution Buffer (AVE)
16	Status	Collecting cRNA (CARRIER) + IC
17	Status	Collecting Lysis Buffer
18	Status	Collecting Sample
19	Status	Collecting
20	Status	Mixing Lysate
21	Status	Checking Temperature Set: 56.0 [deg] Cur: [deg]
22	Status	15 min Incubation
23	Status	Kick
24	Status	Collecting Binding Buffer
25	Status	Collecting Lysis Buffer
26	Status	Collecting Beads
27	Status	Resuspension of Beads in Binding Buffer
28	Status	Transferring Lysate
29	Status	Binding Magnetic Separation

Table continued on next page.

Table 13. Continued

Message number	Message type	BioRobot EZ1 DSP message text
30	Status	Wash 1 Magnetic Separation
31	Status	Wash 2 Magnetic Separation
32	Status	Wash 3 Magnetic Separation
33	Status	Dry Beads
34	Status	Kick
35	Status	Dry Beads
36	Status	Kick
37	Status	Rinse
38	Status	Checking Temperature Set: 65.0 [deg] Cur: [deg]
39	Status	Elution
40	Guidance	Check transfer of cRNA (CARRIER)+ IC (tube [ET], row 3) Next=Any
41	Guidance	Check transfer of sample (tube [ST], row 4) Next=Any
42	Guidance	Protocol finished! Press ESC to return to Menu

Appendix B: Calculating the Amount of Internal Control (IC)

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

Determining how much internal control (IC) will be in downstream reactions

To determine the volume of internal control (IC) that will be present in a given downstream assay, use the formula:

$$IC_{RXN} = \frac{IC_{LB} \times LB_{SAM} \times EL_{RXN}}{(LB_{TOT} + IC_{LB}) \times EL_{SAM}}$$

where:

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

IC_{LB} = Volume of internal control (IC) added to lysis buffer (LB)

LB_{SAM} = Volume of lysis buffer (LB) per sample

EL_{RXN} = Volume of eluate per downstream reaction

LB_{TOT} = Total volume of lysis buffer (LB) plus carrier RNA (CARRIER) used in the protocol

EL_{SAM} = Volume of eluate per sample

As an example, using a previously established assay system, User 1 adds 39 µl of internal control solution (ICLB) to 8.4 ml of lysis buffer (LB) and 140 µl of carrier RNA (CARRIER). Using the manual reference procedure for the assay system, 625 µl of lysis buffer (LB) is added per sample (LB_{SAM}), and an elution volume of 75 µl (EL_{SAM}) is used. 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:

$$IC_{RXN} = \frac{39\ \mu l \times 625\ \mu l \times 50\ \mu l}{(8540\ \mu l + 39\ \mu l) \times 75\ \mu l} = 1.89\ \mu l$$

The final downstream reactions for the given assay system contain 1.89 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 (IC) that you want to have present in the downstream assay (IC_{RXN}), then you need to determine the amount of internal control (IC) to be diluted with elution buffer (AVE) and carrier RNA (CARRIER) (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_{AVE} = Volume of internal control (IC) diluted in elution buffer–carrier RNA (AVE–CARRIER)

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

IC_{TOT} = Total volume of diluted internal control (IC) in elution buffer–carrier (AVE–CARRIER) RNA per run

IC_{SAM} = Volume of diluted internal control (IC) added per sample (50 µl)

EL_{SAM} = Volume of eluate per sample

EL_{RXN} = Volume of eluate per downstream reaction

As an example, User 2 is working with an assay that is optimized for use with 1.0 µl of internal control solution per reaction (IC_{RXN}) and 20 µl of eluate per reaction (EL_{RXN}). User 2 follows the EZ1 DSP Virus 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 (IC) has to be manually pipetted into the 1.5 ml tube (ET) in position 3 of the EZ1 worktable, but during the sample preparation process of the EZ1 DSP Virus protocol the EZ1 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:

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

For each sample, 3.6 µl carrier RNA (CARRIER) 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 (IC), the user has to add elution buffer (AVE):

Volume of elution buffer (AVE) = IC_{TOT} – IC_{AVE} – Volume carrier RNA (CARRIER)

= 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 elution buffer (AVE) and 21.6 µl of carrier RNA (CARRIER) stock in order to obtain 360 µl of diluted internal control (IC). From this diluted internal control (IC),60 µl has to be manually transferred into 1.5 ml tubes (ET) in position 3 of the EZ1 worktable before starting the EZ1 DSP Virus protocol.

Appendix C: Sample Sheet for Use with the EZ1 DSP Virus System

This sample sheet template may be useful for recordkeeping when using the EZ1 DSP Virus procedure. This sheet can be photocopied and labeled with descriptions of the samples and details of the run.

EZ1 DSP Virus system

Date/time: _____ **Kit lot number:** _____

Operator: _____ **Run ID:** _____

Instrument serial number: _____

Position on worktable	Sample ID	Sample material	RCV available?	ST available?	ET available?	DTH with DFT available?	ET with CARRIER and IC available?
1 (left)							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14 (right)							

Appendix D: 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: ..... "123456789"
User ID: ..... "964"
Firmware version: ..... "V 1.0.0"
Installation date of instrument: ..... " , "
Weekly maintenance done on: ..... "Feb 26, 2008"
Yearly maintenance done on: ..... "Nov 06, 2007"
Date of last UV-run: ..... "Mar 03, 2008"
Start of last UV-run: ..... "14:48"
End of last UV-run: ..... "14:52"
Status of last UV-run: ..... "UV run aborted"

Protocol name: ..... "Virus DSP"
..... "Version 1.0"

Date of run: ..... "Mar 03, 2008"
Start of run: ..... "14:54"
End of run: ..... "15:40"
Status run: ..... "o.k"
Error Code: ..... "---"
Sample input Volume [ul]: ..... " 400"
Elution volume [ul]: ..... " 60"

Channel A:
Sample ID: ..... "717"
Reagent Kit number: ..... "9801401"
Reagent Lot number: ..... "1181234567"
Reagent Expiry date: ..... "1210"
Assay Kit ID: ..... "717"
Note: ..... "717"

Channel B:
Sample ID: ..... "393"
Reagent Kit number: ..... "9801401"
Reagent Lot number: ..... "1181234567"
Reagent Expiry date: ..... "1210"
Assay Kit ID: ..... "393"
Note: ..... "393"

Channel C:
Sample ID: ......"163"
Reagent Kit number: ....."9801401"
Reagent Lot number: ..... "1181234567"
Reagent Expiry date: ....."1210"
Assay Kit ID: ....."163"
Note: ....."163"

Channel D:
Sample ID: ....."149"
Reagent Kit number: ....."9801401"
Reagent Lot number: ..... "1181234567"
Reagent Expiry date: ....."1210"
Assay Kit ID: ....."149"
Note: ....."149"

Channel E:
Sample ID: ....."719"
Reagent Kit number: ....."9801401"
Reagent Lot number: ..... "1181234567"
Reagent Expiry date: ....."1210"
Assay Kit ID: ....."719"
Note: ....."719"

Channel F:
Sample ID: ....."407"
Reagent Kit number: ....."9801401"
Reagent Lot number: ..... "1181234567"
Reagent Expiry date: ....."1210"
Assay Kit ID: ....."407"
Note: ....."407"

[Checksum E95974AC]

Ordering Information

Product	Contents	Cat. no.
EZ1 DSP Virus Kit (48)	For 48 preps of viral nucleic acids and/or bacterial DNA: Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, Carrier RNA	62724
EZ1 Advanced DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol; for use with the EZ1 Advanced instrument	9018306
EZ1 Advanced XL DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol; for use with the EZ1 Advanced XL instrument	9018703
EZ1 DSP Virus Card*	Preprogrammed card for EZ1 DSP Virus protocol; for use with the BioRobot EZ1 DSP instrument*	9017707
EZ1 Advanced XL	Robotic instrument for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty on parts and labor*†	9001492
EZ1 Advanced	Robotic instrument for automated purification of nucleic acids using EZ1 Kits, 1-year warranty on parts and labor†	9001411
ATL (4x 50 ml)	4x 50 ml ATL	939016
Buffer ASL (4x 140 ml)	4x 140 ml Buffer ASL	19082

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* Not available in the US or Canada.

† Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and repair parts.

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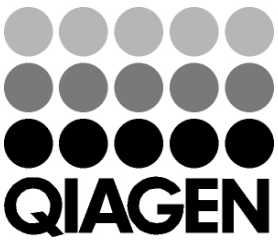

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