

# EZ1<sup>®</sup> DSP Virus Kit

## Instructions for Use (Handbook)



48

Version 5



For In Vitro Diagnostic Use

For use with BioRobot<sup>®</sup> EZ1 DSP, EZ1 Advanced and EZ1 Advanced XL instruments

For use with EZ2<sup>®</sup> Connect MDx instrument (with software version 1.1 or higher)



62724



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## 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 EZ1 DSP Virus Kit is intended for in vitro diagnostic use.

## Intended User

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

# Description and Principle

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 the sections hereafter and the flowchart on page 7). Pretreatment of the sample is mandatory for stool. 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 3 washing steps, followed by rinse and air dry step..

## 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.

## Summary and explanation

The EZ1 DSP Virus Kit provides an automated procedure for simultaneous purification of viral nucleic acids and bacterial DNA from the following sample materials using EZ1 or EZ2 Connect MDx instruments:

- Serum and plasma
- Cerebrospinal fluid (CSF)
- Stool
- Nasopharyngeal swabs collected in UTM

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. The EZ1 (EZ1 Advanced, BioRobot EZ1 DSP, and EZ1 Advanced XL) and EZ2 Connect MDx instruments perform all steps of the sample preparation procedure for up to 6 samples (using the EZ1 Advanced or the BioRobot EZ1 DSP; both discontinued), for up to 14 samples (using the EZ1 Advanced XL), or for up to 24 samples (using the EZ2 Connect MDx) in a single run.

## EZ1 DSP Virus Procedure

Serum, plasma, CSF, stool, and nasopharyngeal swabs collected  
in UTM



Lysis with proteinase K and  
lysis buffer



Magnetic particles and binding  
buffer added to lysates



Nucleic acids bind to magnetic  
particles



Magnetic separation



Three washing steps  
followed by rinse and air  
dry



Magnetic separation




Elute with Elution Buffer (AVE)



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

# Materials Provided

## Kit contents

<b>EZ1 DSP Virus Kit</b>			<b>(48)</b>
<b>Catalog no.</b>			<b>62724</b>
<b>Number of preps</b>			<b>48</b>
RCV	Reagent Cartridge, Virus 350 µL*†	<b>REAG</b> <b>CART</b> <b>VIRUS</b>	48
DTH	Disposable Tip Holders	<b>DISP</b> <b>TIP</b> <b>HOLD</b>	50
DFT	Disposable Filter-Tips	<b>DISP</b> <b>FILT</b> <b>TIP</b>	50
ST	Sample Tubes (2 mL), non-skirted	<b>SAMP</b> <b>TUBE</b>	2 x 50
ET	Elution Tubes (1.5 mL)	<b>ELU</b> <b>TUBE</b>	2 x 50
CARRIER	Carrier RNA	<b>CAR</b> <b>RNA</b>	310 µg
AVE	Elution Buffer†	<b>ELU</b> <b>BUF</b>	3 x 2 mL
	Q-Card‡		1
	Instructions for Use		1

\* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 12 for **Safety information**.

† Contains sodium azide as a preservative.

‡ The information encoded in the barcode on the Q-Card is needed for reagent data tracking using the EZ1 Advanced, EZ1 Advanced XL and EZ2 Connect MDx instruments.



## Components of the kit

The principal components of the kit containing active ingredients are explained below.

**Table 1. Reagents supplied containing active ingredients**

<b>Reagent</b>	<b>Components</b>	<b>Concentration (w/w) [%]</b>
RCV (Reagent Cartridge Virus)	Ethanol	≥70 to <90
	Isopropanol	≥70 to <90
	Guanidinium thiocyanate	≥30 to <50
	Guanidine hydrochloride	≥30 to <50
	Proteinase K	≥1 to <10
	Lithium chloride	≥1 to <10

# Materials Required but Not Provided

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), available from the product supplier.

## All protocols

- Pipets\* and sterile, RNase-free pipet tips
- Reaction tubes (for specific sample types only)
- Soft paper tissue
- Water
- 70% ethanol (for cleaning procedures)
- **Optional:** Vortexer\* (if samples need to be mixed)
- **Optional:** microcentrifuge\* (if magnetic particles need to be removed from eluates)

## For pretreatment of stool

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

## For isolation of genomic DNA of Gram positive bacteria

- Lysozyme, Tris-HCl, EDTA, Triton X-100
- Thermo-shaker\* or 37°C water bath\*
- Centrifuge (able to run 5000 x *g*)

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

## For BioRobot EZ1 users

- BioRobot EZ1 DSP instrument\* (discontinued)
- EZ1 DSP Virus Card (cat. no. 9017707)

## For EZ1 Advanced users

- EZ1 Advanced instrument\* (discontinued)
- 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 (including monitor) with EZ1 Advanced Communicator Software (software supplied with EZ1 Advanced and EZ1 Advanced XL instruments)
  - Printer
  - For further details, see respective instrument handbook

## For EZ2 Connect MDx users

- EZ2 Connect MDx instrument\* (cat. no. 9003230)

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

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

# Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative and the regulatory authority in which the user and/or the patient is established.

For in vitro diagnostic use.

Read all instructions carefully before using the kit.

Please be aware of the following remaining risks:

- While using secondary tubes (sample tubes, "ST"), please ensure that the sample IDs are not mixed up during transfer of the sample ID from primary to secondary tube.
- Sample IDs can also be entered manually (for details refer to the EZ1 or EZ2 instrument user manuals). If wrong ID data are entered manually, wrong correlation between sample and patient can occur.

## 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 PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety), where you can find, view and print the SDS for each QIAGEN® kit and kit component.

### WARNING



### Risk of personal injury

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/EZ2 instruments, disinfect the instrument using reagents described in the user manual supplied with your EZ1/EZ2 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 damaged kit components because their use may lead to poor kit performance, user injury, or damage to the instrument.
- 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 unlikely but cannot be excluded completely. Therefore, residual liquid waste must be considered infectious and be handled and discarded according to local safety regulations.
- Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

## Precautions

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

### Reagent Cartridge, Virus Mini, v2.0 CE (RCV)



Contains: ethanol, guanidine hydrochloride, guanidine thiocyanate, isopropanol, lithium chloride, and proteinase K. Danger! Highly flammable liquid and vapor. Harmful if swallowed or if inhaled. May be harmful in contact with skin. Causes severe skin burns and eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause respiratory irritation. May cause drowsiness or dizziness. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician. Remove person to fresh air and keep comfortable for breathing. Wash contaminated clothing before reuse. Store in a well-ventilated place. Dispose of contents/container to an approved waste disposal plant.

## Emergency information

CHEMTREC

USA & Canada 1-800-424-9300

Outside USA & Canada +1 703-527-3887

## Disposal

The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed properly.

Dispose of as hazardous waste in compliance with local and national regulations. This also applies to unused products.

Do not dispose of liquid waste into sewer.

Follow recommendations in the Safety Data Sheet (SDS).

Refer to your local safety regulations for proper disposal procedures. See also “Warnings and Precautions”, starting on page 12.




For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

# 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, kit box, and barcode on the RCV.

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 buffer ASL during storage at room temperature. Incubate the bottle at 50–56°C for 15–20 minutes and shake bottle manually twice within this incubation period.

-  Do not use the EZ1 DSP Virus Kit or Buffer ASL once it has expired. Avoid exposure of the RCV or Buffer ASL to UV light (e.g., used for decontamination) as this may cause accelerated aging of the buffers.
-  Do not use reagent cartridges (RCV) if damaged or pre-opened.
-  Do not remove the foil from the reagent cartridges. It will be pierced automatically by the instrument.





## In-use stability

Reagent cartridges (RCV) are for single-use only and do not provide in-use stability.

The reconstituted Carrier RNA (CARRIER) stock solution has a concentration of 1 ng/ $\mu$ L and is stable for up to 4 weeks when stored at 2–8°C.


Pretreatment buffer ASL is stable for up to 6 months after first opening/use of the bottle when reclosed and stored at room temperature (15–25°C).

-  It is recommended to note the first opening/use date of buffer bottle ASL on the bottle to guarantee not exceeding the in-use stability.
-  If the remaining kit shelf life is shorter than 6 months, buffer ASL may not be used after the expiration date.


# Specimen Storage and Handling

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

The purification procedure is optimized for use with 100, 200, or 400  $\mu\text{L}$  sample volumes.

-  Do not use lower or higher samples volumes other than 100, 200, or 400  $\mu\text{L}$  as this might lead to performance issues or might damage the instrument.

Sample stability highly depends on various factors and relates to the specific downstream application. It has been established for the EZ1 DSP Virus Kit in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

-  For general collection, transport, and storage recommendations refer to the approved CLSI guideline MM13-A “Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods”. Furthermore, the manufacturer’s instructions for the used sample collection device/kit shall be followed during sample preparation, storage, transport, and general handling.

## Plasma and serum samples

For blood collection, follow the manufacturer’s instructions of the respective blood collection tubes (BCT) used. Especially the instructions for the correct positioning of the BCT during blood draw, required filling volume and the instructions for gentle mixing and inverting of BCT after blood collection shall be considered.

**Note:** Wrong and/or insufficient mixing of blood samples can be one of the most important pre-examination variables. Unless additives in the blood collection tubes are homogeneously mixed with the specimen, the viral NA quality can be compromised, which can impact the validity and reliability of the examination results.

Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation. Plasma and serum samples can be either fresh or frozen, provided that they have not been refrozen after thawing.

For examination of viral NA it is recommended to start the plasma preparation of the blood samples by centrifugation immediately after transport (maximum 2 hours at ambient temperature). In case of any delay, the EDTA and citrate blood collection tubes can be stored at 4°C for up to 6 hours until centrifugation and plasma preparation. Serum samples shall be stored at ambient condition for up to 2 hours until centrifugation. Storage conditions and duration shall be documented.

After plasma and serum preparation, for longer storage, it is recommended to store aliquots of samples at -20°C to -80°C. Thaw frozen sample aliquots at 25°C for 30–90 minutes. Invert the sample tubes at least 10 times, and process the samples immediately when they have equilibrated to room temperature. Do not refreeze the aliquots after thawing. Repeated freezing–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.

## Stool samples

After collection, store and transport stool samples at 2–8°C. A sample volume of 200 µL is recommended for extraction of viral or bacterial nucleic acids from stool. A pretreatment needs to be performed prior to extraction on the EZ1 or EZ2 instrument (see page 39 for “Protocol: Pretreatment of Stool”).

For general collection, transport, and storage recommendations refer to the approved CLSI guideline MM13-A “Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods”.

## Nasopharyngeal swabs collected in UTM

Nasopharyngeal swabs collected in UTM may be transported at room temperature.

For general collection, transport, and storage recommendations refer to the approved CLSI guideline MM13-A “Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods”.

## Cerebrospinal fluid (CSF) samples

For DNA studies, CSF samples should be transported at 2–8°C. For RNA studies, CSF samples should be transported frozen on dry ice.

For general collection, transport and storage recommendations refer to the approved CLSI guideline MM13-A “Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods”.

## Gram-positive bacteria samples

For the DNA extraction of difficult-to-lyse Gram positive bacteria, an additional pre-lysis step comprising lysozyme digestion may be performed prior to extraction on the EZ1 or EZ2 Connect MDx instrument (see page 41, "Protocol: Pretreatment for Isolation of genomic DNA of Gram Positive Bacteria").

## 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, 90, 120, or 150  $\mu\text{l}$ .

If the sample material is stool, it is recommended to choose an elution volume of 120–150  $\mu\text{l}$ .

If eluates obtained from stool are turbid, centrifuge at full speed (20,000  $\times g$ ) for 3 minutes 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, it is recommended to store the purified viral nucleic acids or bacterial DNA at 2–8°C. For long-term storage of over 24 hours, it is recommended to store at –80°C for up to 12 months or –20°C for up to 12 weeks. Stability of nucleic acids might be different for the specific downstream application being used and needs to be self-validated by the user.

Eluate stability highly depends on various factors and relates to the specific downstream application. It has been established for the EZ1 DSP DNA Virus Kit in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

# Procedure

The EZ1 DSP Virus Kit can be used on multiple types of instruments:

- The EZ2 Connect MDx
- The EZ1 Advanced XL and EZ1 Advanced (discontinued)
- The BioRobot EZ1 DSP (discontinued)

## Working with the EZ2 Connect MDx instruments

The main features of the EZ2 Connect MDx instruments include:

- Automated purification of high-quality nucleic acids from 1 to 24 samples per run
- Preinstalled ready-to-use protocols
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup
- An external barcode reader, which is used for reading sample IDs and kit IDs (Q-card)
- Graphical user interface (GUI)
- An internal camera, which is used for automated load checks and reagent cartridge barcode reading
- UV lamp to support decontamination of the worktable surfaces

Additional features of the EZ2 Connect MDx include:

- LIMS and QIASphere Connectivity (LAN or WiFi via USB ports)
- Extended User Management

- ① UV decontamination helps to reduce possible pathogen contamination of the EZ2 Connect MDx 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.

## Operating procedure EZ2 Connect MDx

Before proceeding, it is recommended that you familiarize yourself with the features of the instrument as described in the *EZ2 Connect MDx User Manual* (which can be found under the resources tab of the product page on [www.qiagen.com](http://www.qiagen.com)).

- ① The hood of the EZ2 Connect MDx must remain closed and will automatically lock during operation of the instrument. Only open the hood when instructed to do so by the instruction for use. The worktable of the EZ2 Connect MDx instrument moves during operation of the instrument. Never open the EZ2 Connect MDx hood while the instrument is operating.

To setup a protocol run, close the hood and switch on the instrument. For MDx applications, choose the IVD mode when logging in. Press the **Setup** tab on the Home screen and scan the 1D Barcode on the Q-card provided with the EZ1 DSP Virus kit (Figure 1) by pressing the **Scan** button. Dedicated protocols are automatically displayed when the Q-card is scanned.

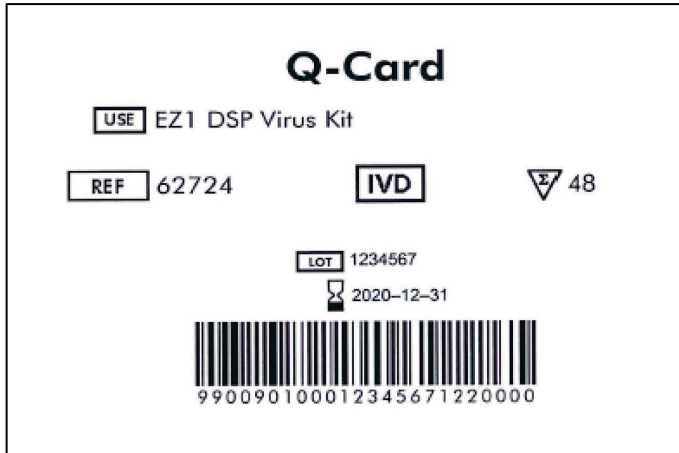


Figure 1. Q-card example.

The EZ2 Connect MDx software will guide you through the protocol run setup process.

## Reagent cartridges (RCV)

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (RCV) (Figure 2). Most wells of the cartridge (RCV) contain a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or RNase-free elution buffer (AVE). Because 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).





**Figure 2. Reagent cartridge (RCV).** Sealed, prefilled reagent cartridge (RCV) of the EZ1 DSP Virus Kit.



**Figure 3. Reagent 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 EZ2 Connect MDx instruments is where the user loads samples and the components of the EZ1 DSP Virus Kit (Figure 4 and Figure 5).

Details on worktable setup are displayed on the touchscreen of the GUI.



**Figure 4. Overview of an EZ2 Connect MDx instrument.** (1) Pipettor head, (2) magnet module, (3) cartridge rack, and (4) tip rack (labware holder).



**Figure 5. Worktable of an EZ2 Connect MDx instrument.** (1) Heating block with 2 mL tubes (ST) loaded in the reagent cartridges (RCV) for lysis. (2) Sample tubes (ST) (2 mL) loaded into row A. (3) Tube (ET) (1.5 mL) containing carrier RNA (CARRIER) and internal control (IC) (if used) in elution buffer (AVE), loaded into row B. (4) Disposable tip holders (DTH) containing disposable filter-tips (DFT) loaded into row C. (5) Elution tubes (ET) (1.5 mL) loaded into row D.

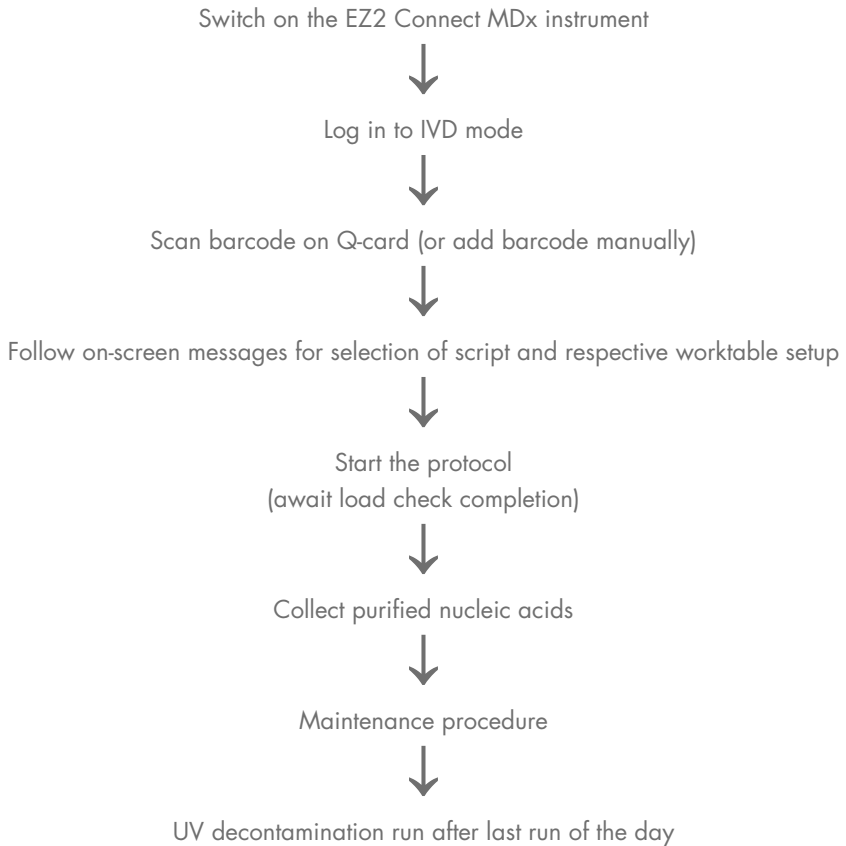
## Data tracking with the EZ2 Connect MDx

The EZ2 Connect MDx enables complete tracking of a variety of data for increased process control and reliability. The user ID is tracked via log in to the software. The EZ1 DSP Virus Kit lot number and expiration date are entered at the start of the protocol using the Q-Card barcode or entered manually using the touchscreen. Sample information and run settings are entered during the setup of the protocol. At the end of the protocol run, a report file can be generated. In the “Data” section of the GUI, run reports can be downloaded to a USB stick (always in both file formats “.pdf” and “.xml”).

If WiFi/LAN connectivity has been established for the EZ2 Connect MDx instrument, run and sample information can directly be processed via LIMS (if configured).

For further details about EZ2 Connect MDx instrument setup, see the *EZ2 Connect MDx User Manual* (which can be found under the resource tab of the product page on [www.qiagen.com](http://www.qiagen.com)).

## Workflow of EZ1 DSP Virus operation on EZ2 Connect MDx




## Working with EZ1 instruments

The main features of EZ1 instruments include:

- Purification of high-quality nucleic acids from 1 to 6 (BioRobot EZ1 DSP and EZ1 Advanced) or 1 to 14 (EZ Advanced XL) 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:

- Barcode reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV lamp to support decontamination of the worktable surfaces

 UV decontamination helps 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 EZ1 DSP Virus protocol for purification of viral nucleic acids and bacterial DNA is stored on the preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Advanced XL DSP Card into the EZ1 Advanced XL, an EZ1 Advanced DSP Card into the EZ1 Advanced, or an EZ1 DSP Card\* into the BioRobot EZ1 DSP instrument, and the instrument is then ready to run a protocol (Figure 6 and Figure 7).



**Figure 6. Ease of protocol setup using EZ1 DSP Cards.** Inserting an EZ1 Card, preprogrammed with the protocol, into the EZ1 instrument.

- ⓘ The instrument must only be switched on after an EZ1 Card is inserted and make sure that the EZ1 Card is completely inserted! Otherwise, essential instrument data will be lost, leading to a memory error. EZ1 Cards must not be exchanged while the instrument is switched on.



**Figure 7. Card completely inserted into EZ1 Card slot.**

## Reagent cartridges (RCV)

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (RCV) (Figure 8 and Figure 9). Most wells of the cartridge (RCV) contain a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or RNase-free elution buffer (AVE). Because 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).



**Figure 8. Reagent cartridge (RCV).** A sealed, prefilled RCV of the EZ1 DSP Virus Kit.



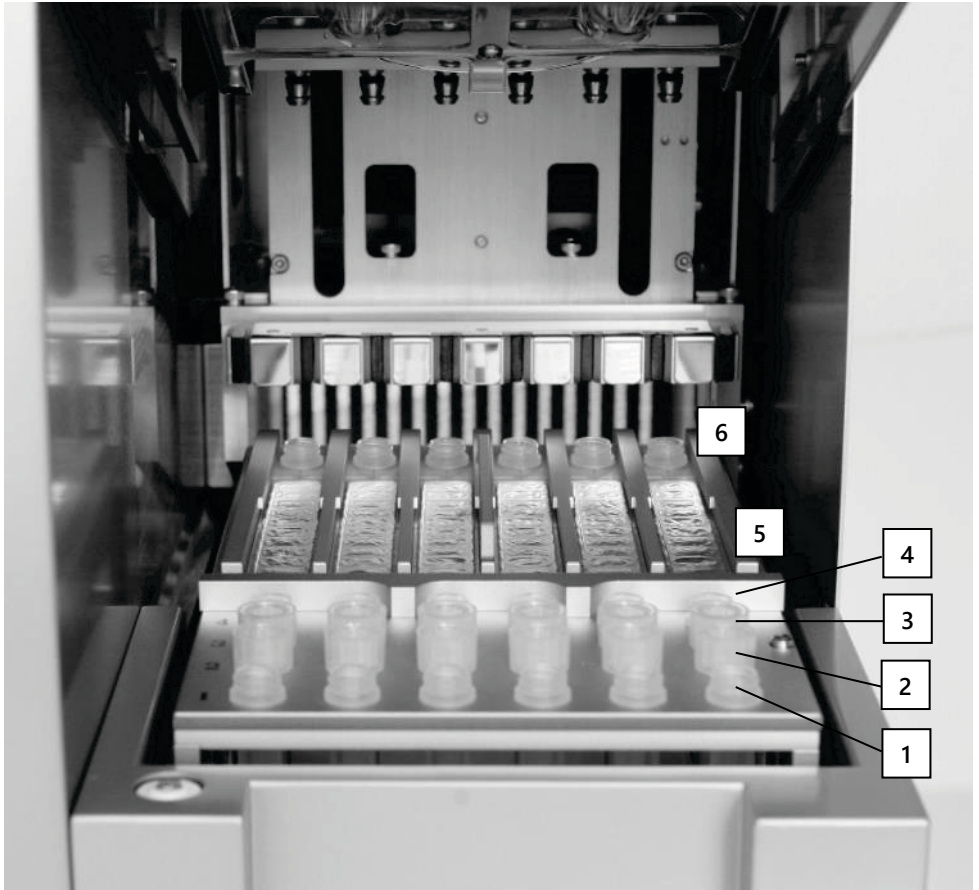
**Figure 9. Loading the reagent cartridge rack.** The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges (RCV) must be loaded.

## Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1 DSP Virus Kit (Figure 10).

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.






**Figure 10. Worktable of an EZ1 instrument.** (1) Elution tubes (ET) (1.5 mL) loaded into row 1. (2) Disposable tip holders (DTH) containing disposable filter-tips (DFT) loaded into row 2. (4) Tube (ET) (1.5 mL) containing carrier RNA (CARRIER) and internal control (IC) (if used) in elution buffer (AVE), loaded into row 3. (4) Sample tubes (ST) (2 mL) loaded into row 4. (5) Reagent cartridges (RCV) loaded into the cartridge rack. (6) Heating block with 2 mL tubes (ST) in the reagent cartridges for lysis.

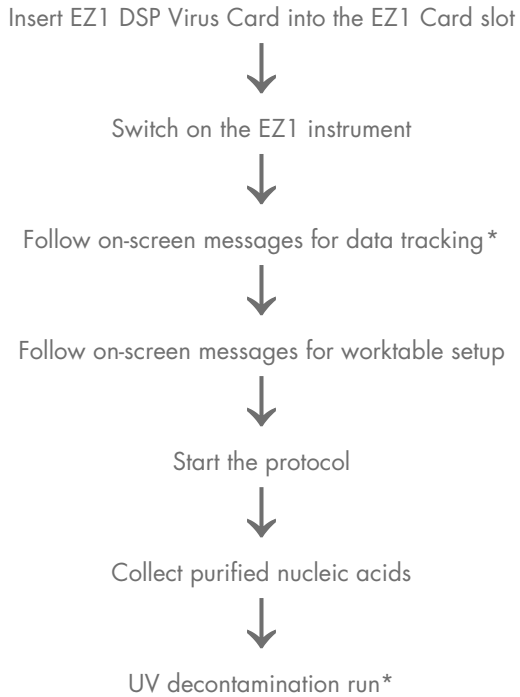
## Data tracking with the EZ1 Advanced and EZ1 Advanced XL

The EZ1 Advanced and EZ1 Advanced XL enable tracking of a variety of data for increased process control and reliability. The EZ1 kit lot number and expiration dates are entered at the start of the protocol using the Q-Card barcode. A user ID and the Q-Card barcode can be entered manually via the keypad or by scanning barcodes using the handheld barcode reader. Sample and assay information as well as notes can also be optionally entered at the start of the protocol. At the end of each protocol run, a report file is automatically generated. The EZ1 Advanced and EZ1 Advanced XL can store up to 10 result files, and the data can be transferred to a PC or directly printed on a printer.

-  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 further details about data tracking, see the respective user manual, which can be found under the resource tab of the product page on [www.qiagen.com](http://www.qiagen.com).

## Workflow of EZ1 DSP Virus operation on EZ1



\* 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  $\mu\text{L}$  elution buffer (AVE), divide it into conveniently sized aliquots, and store at 2–8°C. The reconstituted CARRIER stock solution has a concentration of 1 ng/ $\mu\text{L}$  and is stable for up to 4 weeks.

For each sample processed, dilute 3.6  $\mu\text{L}$  of carrier RNA (CARRIER) stock solution in a total volume of 60  $\mu\text{L}$  using elution buffer (AVE) (and/or an internal control solution). A 50  $\mu\text{L}$  volume of this carrier RNA–elution buffer (CARRIER–AVE) solution is transferred by the EZ1/EZ2 instrument to the lysis mix, corresponding to 3  $\mu\text{g}$  carrier RNA (CARRIER).


If you want to use an internal control (IC), see “Using an internal control (IC)” hereafter.

**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.

-  Do not add the internal control (IC) directly to the sample. Only use IC in combination with the CARRIER solution in one mixture.

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 82, 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.


# Protocol: Pretreatment of Stool

This protocol is intended for pretreatment of solid as well as liquid stool samples prior to nucleic acid purification (page 42 for EZ2 Connect MDx instruments and page 50 for EZ1 instruments).


## Procedure

1. Resuspend 100 mg of solid or liquid stool in 900  $\mu\text{L}$  Buffer ASL.

Buffer ASL should be ordered separately, see Ordering Information, page 88.


-  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  $\mu\text{L}$  sample volume after pretreatment for extraction with the EZ1/EZ2 instrument.

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

-  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 may 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 for sedimentation of large stool particles.

4. Transfer at least 400  $\mu\text{L}$  supernatant from the top of the suspension to a fresh 1.5 mL screw cap tube without carryover of large stool particles.

-  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/EZ2 instrument.

5. Incubate the sample for 10 min at 70°C in a water bath\* or thermo-shaker.\*
6. Proceed to the purification protocol (page 42 or 50).
  - ① 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.
  - ① If eluates obtained from stool are turbid, we recommend centrifugation at full speed (20,000 × *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.

\* 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/EZ2 Connect MDx instrument. This protocol is not intended for use with stool samples.

## Procedure:

1. Pellet bacteria by centrifugation for 10 min at 5000 x *g*.
2. Suspend bacterial pellet in 180  $\mu$ L 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. Place in a water bath\* or thermo-shaker\* and 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 42 or 50).

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

# Protocol: Purification of Viral Nucleic Acids and Bacterial DNA Using the EZ2 Connect MDx

## Important points before starting

- If using the EZ1 DSP Virus Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with the EZ2 Connect MDx instruments” starting on page 16.
- 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 12 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 12). Do not use damaged reagent cartridges (RCV) or other kit components because their use may lead to poor kit performance, user injury, or damage to the instrument. Do not remove the foil from the RCV.

## Things to do before starting


- Prepare serum, plasma, CSF, or nasopharyngeal swabs in UTM as described in “Specimen Storage and Handling”, page 18. 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.
- Prepare stool samples as described in “Specimen Storage and Handling”, page 18, and “Protocol: Pretreatment of Stool”, page 39.

- For isolation of DNA from Gram-positive bacterial, prepare samples as described in “Protocol: Pretreatment for Isolation of genomic DNA of Gram Positive Bacteria” (page 41).
- 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  $\mu$ L elution buffer (AVE) (provided in the kit), and mix it with the internal control (IC) (optional) as described in “Preparing carrier RNA (CARRIER)” (page 36) and “Using an internal control (IC)” (page 37).

## Procedure



1. For each sample, prepare a 60  $\mu$ L carrier RNA solution containing 3.6  $\mu$ L 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 row B, as specified in the on-screen instructions.

-  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 EZ2 Connect MDx instrument.

2. Equilibrate up to 24 samples to room temperature (15–25°C) and transfer 100, 200, or 400  $\mu$ L sample into 2 mL sample tubes (ST) (non-skirted; provided with the kit), before loading on the worktable. If using frozen samples, thaw and equilibrate at room temperature, and mix well by vortexing.

A sample volume of 200  $\mu$ L is recommended for extraction of viral/bacterial nucleic acids from stool. For pretreatment of samples, refer to the appropriate pretreatment protocol.

-  Only use the 2 mL tubes (ST) (non-skirted) provided with the kit.
-  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 or bacterial DNA.


-  Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.
-  Do not use sample volumes greater than 100, 200, 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) Do not reuse any sample material left in the sample tube (ST).

3. Switch on the EZ2 Connect MDx instrument.

The power switch is located at the right front of the instrument.




4. Log in to the instrument choosing the IVD mode of the software. Enter user ID and password.

The EZ2 Connect MDx software will guide you through the protocol run setup process. The process is started tapping either the **SCAN** or the **LIMS** button on the setup tab.

-  To set-up a run using the LIMS function/button, please refer to the *EZ2 Connect MDx User Manual*.








5. Press **Scan** and tap into the field showing up in the next screen. Scan the 1D Barcode on the Q-Card provided with the kit.

By scanning the 1D Barcode on the Q-Card, the protocol type is automatically selected.

-  If scanning of the Q-card fails, you can also type the kit number via the user interface.
-  Scanning the Q-card is only possible if all required maintenance procedures have been finalized. Otherwise, start the maintenance procedure first before scanning the Q-card.
-  Do not use expired RCV as this will lead to impaired performance; the samples will be flagged as invalid.

6. Tap **Next** to continue.

**Note:** To return to the Setup screen, tap **Back** or **Cancel**.

7. Choose the different protocol parameters by tapping the box next to each parameter option.
8. Tap **Next** to continue.
9. To select the positions of your samples, tap the relevant rows on the worktable diagram or tap the corresponding row numbers underneath the diagram. The selected positions are highlighted. To select or deselect all positions, tap the **Select all** toggle.
  -  After at least one sample position is selected, the **Next** button is enabled.
10. Tap **Next** to continue.
11. Enter the sample IDs, either manually or by using the handheld barcode scanner.
  -  When using the barcode scanner, ensure that the barcode used is of appropriate type and quality to be read by the scanner.
  -  Sample IDs can be changed manually by tapping on the ID and using the on-screen keyboard.
  -  Sample IDs must be unique. The **Next** button is not active until unique sample IDs have been entered for all samples.
  -  Check sample ID for correctness before proceeding with the setup.
12. Tap **Next** to continue.
13. Open the instrument door and remove both the cartridge racks and tip racks (also referred to as labware holder) from the instrument. Place them safely onto the bench. To remove a tip rack, grab both sides of the rack and gently pull up.
  -  Depending on which positions were chosen for the samples, remove racks from the left and/or right side of the worktable.
  -  Do not interchange the cartridge racks and tip racks between different instruments.

14. Invert reagent cartridges (RCV) 4 times to mix the magnetic particles. See “Things to do before starting” before using the RCV.
15. Place the RCV into the cartridge rack, press down the cartridge until it clicks into place.
16. Place an empty sample tube (ST) (non-skirted; provided with the kit) into well 11 of each RCV loaded.

**i** Ensure that the empty sample tube (ST) is loaded without a lid.  
The empty tube is required for the lysis step of the protocol. The EZ2 Connect MDx instrument does not detect the presence of the tube.

17. Once all RCV are prepared, place both cartridge racks onto the worktable.

**i** Ensure that the racks are placed in the correct position and position numbers are engraved onto the rack. Numbering reads 1 to 24 from left to right.

18. Tap **Next** to continue.

19. Load the CARRIER (IC) tubes (1.5 mL elution tubes, ET; provided with the kit) into row B of the tip rack (“labware holder”).

See “Preparing carrier RNA (CARRIER)” (page 36) and “Appendix B: Calculating the Amount of Internal Control (IC)” (page 82) for details on preparing the CARRIER (IC) mix.

**i** Ensure that the 1.5 mL elution tubes (ET) containing sufficient volume of CARRIER (IC) are loaded without a lid.

20. Place the tips into the tip holder and load them into row C of the rack.

**i** When preparing the tips and tip holder, only touch the upper part of the tips with gloves.

21. Load the 1.5 mL elution tubes (ET) into row D of the rack.




**i** Ensure that the elution tubes are loaded without a lid.


22. Load the 2 mL sample tubes (ST) (non-skirted) containing either 100, 200, or 400  $\mu$ L sample (according to the selected protocol parameter) into row A of the rack.

- ① Ensure that the sample tubes are loaded into the correct positions as selected in step 11. **Optional:** Use the template from “Appendix C: Sample Sheet for Use with the EZ1 DSP Virus System” to track the sample ID and orientation.
  - ① Ensure that the sample tubes are loaded without a lid.
  - ① Ensure that the sample tubes contain the correct volume of sample material. The load check does not detect if the correct sample volume is loaded.
  - ① Avoid formation of foam or bubbles on top of the sample or at the rim of the sample tubes as this may lead to load check errors.
  - ① Immediately start the protocol after placing the samples onto the worktable as prolonged storage time onboard the instrument may lead to evaporation or could affect onboard stability.
23. Once all tubes and tips are loaded, place each tip rack (left and right rack) onto the worktable and close the hood.
- ① Ensure that the racks are placed in the correct position, position numbers are engraved onto the rack. Numbering reads 1 to 24 from left to right. Always place both tip racks onto the worktable independent of used sample positions.
24. Tap **Next** to continue.
25. Check the on-screen information of the run setup overview for correct protocol, sample and elution volume, and number of samples.
26. If all information is correct, tap **Start** to proceed with the protocol run.
- ① To make any modifications, tap **Return** to go back to the run setup.
27. The load check will now be performed. The protocol will start automatically after the load check is successfully completed.
- ① Wait until the load check has completed successfully before leaving the instrument unattended. Upon a failure of the load check (e.g., due to errors

during worktable setup), the run will not start and the operator action will be required. If the instrument is unattended for an extended time period, stability of samples and reagents may be impaired.

Proceed to step 30 after successful load check.

28. If the load check fails, the “Load check failed” screen is shown. Incorrect labware placements are marked in red. Tap on the respective columns for details on the load check error.
  -  Visually check the loading of the highlighted positions on the worktable. Do not repeatedly rerun a failed load check without first completing the visual inspection.
  -  For detailed information about load check limitations and failure, refer to the *EZ2 Connect MDx User Manual*.
29. Once correct loading of the worktable has been confirmed, tap **Next** in the “Load the tip rack” screen. The “Run setup selection overview” screen is shown, where a **Skip load check** button is now available. Either tap **Skip load check** or **Start** to proceed with the protocol run.
  -  When choosing the **Skip load check** option, it is the operator’s responsibility to visually check to confirm correct placement of ALL consumables in ALL worktable positions.

**Important:** The skipped load check will be recorded in the run report and all samples will be flagged as invalid.
  -  **Important:** If the load check fails the second time, remove the samples and CARRIER (IC) from the worktable, close the tubes, and store them at appropriate conditions. Recalibrate the camera and contact QIAGEN Technical Support for additional support.
30. After successful completion of the load check, progress of the run and the elapsed run time are displayed on the “Protocol run in progress” screen.
31. When the protocol has successfully finished, the “Protocol run completed” screen appears.



32. Open the hood, carefully remove the tip racks and place them onto the bench. First, remove the purified DNA/RNA from row D. Avoid touching other tubes while removing the single elution tubes (ET). Close the elution tubes with the lids provided with the kit.
- ❗ Immediately remove and store eluates after the run has finished.
33. Discard the sample preparation waste from row A\*. Discard the tip holders and tips as well as the CARRIER (IC) tubes.
- ❗ Follow the local safety regulations for waste disposal.
34. Remove the cartridge racks and discard the RCV and the tube from well 11.
- ❗ Remove and discard the tube from well 11 of each cartridge first before removing the RCV. Otherwise, the RCV cannot be removed from the cartridge rack.
  - ❗ Follow the local safety regulations for waste disposal (see also “Warnings and Precautions”, page 12).
35. Follow the “After run maintenance” instructions and tap the check box afterwards.
- ❗ The piercing unit is sharp! Use of double gloves is recommended.
  - ❗ For further maintenance procedures, refer to the *EZ2 Connect MDx User Manual*.
36. Press the **Finish** button to create the run report and to go back to the Home screen. The time of finishing the run and the maintenance status are not transferred to the run report until the **Finish** button has been pressed.
37. After the last run of each day, perform the daily maintenance procedure followed by UV decontamination.
38. Perform the weekly maintenance procedure, if required, after the daily maintenance.

\* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 12 for **Safety information**.

# Protocol: Purification of Viral Nucleic Acids and Bacterial DNA using the EZ1 instruments

## Important points before starting

- If using the EZ1 DSP Virus Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with EZ1 instruments” starting on page 16.
- 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 12 for Warnings and Precautions.
- 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 12). Do not use damaged reagent cartridges (RCV) or other kit components because their use may lead to poor kit performance, user injury, or damage to the instrument. Do not remove the foil from the RCV.
- 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

- Prepare serum, plasma, CSF, or nasopharyngeal swabs in UTM as described in “Specimen Storage and Handling”, page 18. 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.

- Prepare stool samples as described in “Specimen Storage and Handling”, page 18, and “Protocol: Pretreatment of Stool”, page 39.
- For isolation of DNA from Gram-positive bacterial, prepare samples as described in “Protocol: Pretreatment for Isolation of genomic DNA of Gram Positive Bacteria” (page 41)
- 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  $\mu\text{L}$  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 36–37.

## Procedure

1. For each sample, prepare a 60  $\mu\text{L}$  solution containing 3.6  $\mu\text{L}$  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 row 3, as specified in the on-screen instructions.

- ⓘ 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. Equilibrate samples to room temperature (15–25°C) and transfer 100, 200, or 400  $\mu\text{L}$  sample into 2 mL sample tubes (ST) (non-skirted; provided with the kit) before loading on the worktable. If using frozen samples, thaw and equilibrate at room temperature, and mix well by vortexing.

A sample volume of 200  $\mu\text{L}$  is recommended for extraction of viral/bacterial nucleic acids from stool. For pretreatment of samples, refer to the appropriate pretreatment protocol.

- ⓘ Only use the 2 mL tubes (ST) (non-skirted) provided with the kit.

-  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 or bacterial DNA.
-  Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.
-  Do not use sample volumes greater than 100, 200, 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). Do not reuse any sample material left in the sample tube (ST).


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. Follow the on-screen instructions for worktable setup, protocol variable selection, and ▲ data tracking.

-  Immediately start the protocol after placing the samples onto the worktable as prolonged storage time onboard the instrument may lead to evaporation.


7. Open the instrument door.

8. Invert the reagent cartridges (RCV) 4 times to mix the magnetic particles.

9. Load the reagent cartridges into the cartridge rack, and press down the cartridge until it clicks into place.





-  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.
-  ▲: 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.
-  ▲: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mix up.

10. Place an empty 2 mL tube (ST) (non-skirted; provided with the kit) into well 11 of each RCV.

-  Ensure that the empty sample tube (ST) is loaded without a lid.  
The empty tube is required for the lysis step of the protocol.

11. Follow the on-screen instructions for further worktable setup.

Preparation of elution tubes, tips and tip holder, CARRIER (IC) tubes as well as sample tubes are required.

-  When preparing the tips and tip holder, only touch the upper part of the tips with gloves.
-  Ensure that the elution tubes (ET, 1.5 mL tubes) are loaded without a lid.
-  Ensure that the sample tubes are loaded into the correct positions as selected in step 9.  
**Optional:** Use the template from “Appendix C: Sample Sheet for Use with the EZ1 DSP Virus System” to track sample ID and orientation.
-  Ensure that the sample tubes are loaded without a lid.

- ① Ensure that the sample tubes contain the correct volume of sample material.
- ① Avoid formation of foam or bubbles on top of the sample or at the rim of the sample tubes.
- ① Immediately start the protocol after placing the samples onto the worktable as prolonged storage time onboard the instrument may lead to evaporation.

12. Load the prepared cartridge rack and tip rack into the instrument.

- ① Do not interchange the cartridge racks and tip racks between different instruments.

13. Close the instrument door.

14. Press **START** to start the protocol.

15. 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.

16. Open the instrument door, carefully remove the tip rack and place it onto the bench.

17. Remove the elution tubes (ET) containing the purified viral nucleic acids and/or bacterial DNA from row 1. Avoid touching other tubes while removing the single elution tubes. Close the ET with the lids provided with the kit.

- ① Immediately remove and store eluates from the worktable after the run has finished.

18. Discard the sample-preparation waste. \* Discard the tip holders and tips as well as the CARRIER (IC) tubes.

19. Remove the cartridge rack and discard the RCV including the tube from well 11.

\* Sample waste contains guanidine salts and it therefore not compatible with bleach. See page 12 for **Warnings and Precautions**.

- ① Follow the local safety regulations for waste disposal (see also “Warnings and Precautions”, page 12).

20. ▲ Recommended: Follow the on-screen instructions to perform UV decontamination of the worktable surfaces.

21. Carry out the regular maintenance procedure, for example, UV run, 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.

- ① The piercing unit is sharp! Use of double gloves is recommended.

- ① For further maintenance procedures, refer to the *EZ1 Advanced XL User Manual*.

22. 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 and 4 are not necessary when running another protocol. Skip these steps.

# 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, stool, and nasopharyngeal swabs in UTM, for isolation of viral nucleic acids and bacterial DNA and exemplary downstream applications. As overall performance highly depends on the downstream application, it is the user's responsibility to validate the performance of the entire diagnostic workflow, including the sample preparation and the specific downstream application.

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.

## Performance Characteristics

The applicable performance characteristics can be found under the resources tab of the product page on [www.qiagen.com](http://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](http://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/or protocols in this handbook or sample and assay technologies (for contact information, visit [www.qiagen.com](http://www.qiagen.com)).

## Comments and suggestions

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### General handling

- |                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Error message in instrument display           | Refer to the user manual supplied with your EZ1 or EZ2 instrument.                                                                                                                                                                                                                                                                                                                                                                                                |
| b) Report file not printed (for EZ1)             | Check whether the printer is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port.<br>Check whether the serial port is set for use with a printer.                                                                                                                                                                                                                                                                                   |
| c) Report file not sent to the PC (for EZ1)      | Check whether the PC is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port.<br>Check whether the serial port is set for use with a PC.                                                                                                                                                                                                                                                                                             |
| d) Wrong Q-Card ID entered (for EZ1)             | 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 <b>STOP</b> twice to go to the main menu.                                                                                                                                                                                                                               |
| e) Wrong Q-Card ID entered (for EZ2 Connect MDx) | If the wrong ID was entered instead of the Q-card ID, the EZ2 Connect MDx will not display the correct protocol to be used. Enter the correct Q-card ID for the required protocol to be displayed.<br><br>The EZ2 Connect MDx checks during the load check, if the chosen protocol and the loaded reagent cartridges fit. If the wrong protocol has been chosen due to wrong Q-Card ID, abort the run and start setting up the instrument run from the beginning. |

### 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 the reagents of the RCV are settled at the bottom of the wells. |
| c) Wrong sample volume in sample tube            | Ensure to pipette the exact sample volume into the sample tube.                                                                                               |

### Comments and suggestions

d)	Wrong amount of sample transferred (less volume transferred from sample tube than expected)	Check the sample tubes to be almost empty after the run. Check that selected and provided sample volume were consistent. Check that remaining sample material in tubes contains no clots or precipitates. Check greasing status of the pipettor O-rings (weekly maintenance).
e)	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.
f)	Carrier RNA (CARRIER) not added	Reconstitute the lyophilized carrier RNA (CARRIER) in 310 $\mu\text{L}$ elution buffer (AVE). For each sample, use 3.6 $\mu\text{L}$ of this carrier RNA (CARRIER) stock solution, mixed with internal control (IC) (optional) and additional elution buffer (AVE) to a final volume of 60 $\mu\text{L}$ , as described in "Preparing carrier RNA (CARRIER)" and "Using an internal control (IC)", pages 36-37. Repeat the purification procedure with new samples.
g)	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.
h)	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.

### 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 59, 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 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.











### Comments and suggestions

- |                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|---------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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 invert the cartridges (RCV) thoroughly and ensure that the reagents of the RCV are settled at the bottom of the wells.                                                                                                                                                                                                                                                                                                                   |
| 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.<br><br>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. Transfer the eluate after centrifugation into a new tube without disturbing the pellet.                                             |
| j) New combination of reverse transcriptase and <i>Taq</i> 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 magnetic separator 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. |

# Symbols

The following symbols appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
 $\Sigma$ <N>	Contains reagents sufficient for <N> reactions
	Use by
	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Unique device identifier
	Components

Symbol	Symbol definition
	Contains
	Number
	Volume
	Global Trade Item Number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Address/ Legal manufacturer
	Important note
	Consult instructions for use
	Keep away from sunlight
	Warning/caution

## Symbol

## Symbol definition

**USE**

Only for use with

**REAG** **CART** **VIRUS**

RCV: Reagent Cartridge Virus

**CAR** **RNA**

CARRIER: Carrier RNA

**ELU** **BUF**

AVE: Elution Buffer AVE

**DISP** **FILT** **TIP**

DFT: Disposable Filter Tips

**DISP** **TIP** **HOLD**

DTH: Disposable Tip Holder

**SAMP** **TUBE**

ST: Sample Tube

**ELU** **TUBE**

ET: Elution Tube

**GITC**

Guanidine isothiocyanate

**GuHCl**

Guanidine hydrochloride

**EtOH**

Ethanol

**IPA**

Isopropanol

**LiCl**

Lithium chloride



## Symbol

## Symbol definition



Proteinase K



This side down when opening

# Contact Information

For technical assistance and more information, please see our Technical Support Center at [www.qiagen.com/Support](http://www.qiagen.com/Support), call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

# Appendix A: Display Messages on EZ1 /EZ2 Instruments

The messages displayed by the software protocol on the EZ1 instruments during worktable setup, during the protocol run, and after the protocol run are listed in Table 2–Table 4. 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.

For general error messages shown on the EZ2 Connect MDx instrument, see the respective User Manual. Contact QIAGEN Technical Service for troubleshooting support.

**Table 2. 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

Table continued on next page.

**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
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
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

Table continued on next page.

**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
14	Data tracking	ID 10: ID 11: ID 12: DOWN: Next, UP: Back
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 µl 2: 200 µl 3: 400 µl
23	Selection	Select elution volume: 1: 60 µl 2: 90 µl 3: 120 µl 4: 150 µl

Table continued on next page.

**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
24	Guidance	You have chosen: Sample volume: xxx µl Elution volume:yyy µl 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 1.5 ml 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
31	Guidance	Loading finished Close door and press START ESC: Back
32	Guidance	Please close door! ENT: Next

Table continued on next page.

**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
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
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

Table continued on next page.

**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
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	Transferring 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
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

Table continued on next page.



**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
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	
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 decontamination: Enter 20-60 min ENT: Next
66	Guidance	UV decontamination time must be between 20-60 min ESC: Back

Table continued on next page.

**Table 2. Messages in the EZ1 Advanced XL DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced XL message text
67	Guidance	UV decontamination Total time: min Time left: min
68	Guidance	Perform regular maintenance after each run ESC: Main menu
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 3. 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

Table continued on next page.

**Table 3. Messages in the EZ1 Advanced DSP Virus procedure (continued)**

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 barcode
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]
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

Table continued on next page.

**Table 3. Messages in the EZ1 Advanced DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced message text
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 µl 2: 200 µl 3: 400 µl
17	Guidance	Select elution volume: 1: 60 µl 2: 90 µl 3: 120 µl 4: 150 µl
18	Guidance	You have chosen: Sample volume: [xxx] µl Elution volume: [yyy] µl Next=Any, Prev=Esc
19	Guidance	Load cartridges at same positions as sample Next=Any, Prev=Esc
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!

Table continued on next page.

**Table 3. Messages in the EZ1 Advanced DSP Virus procedure (continued)**

<b>Message number</b>	<b>Message type</b>	<b>EZ1 Advanced message text</b>
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
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

Table continued on next page.

**Table 3. Messages in the EZ1 Advanced DSP Virus procedure (continued)**

Message number	Message type	EZ1 Advanced message text
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
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

Table continued on next page.

**Table 3. Messages in the EZ1 Advanced DSP Virus procedure (continued)**

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

**Table 4. Messages in the BioRobot EZ1 DSP Virus procedure**

Message number	Message type	BioRobot EZ1 DSP 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	Guidance	Select elution volume: 1: 60 µl 2: 90 µl 3: 120 µl 4: 150 µl
4	Guidance	You have chosen: Sample Volume:[sample volume] µl Elution Volume:[elution volume] µl Next=Any, Prev=ESC
5	Guidance	Load cartridges (RCV) at same positions as samples Next=Any, Prev=ESC

Table continued on next page.

**Table 4. Messages in the BioRobot EZ1 DSP Virus procedure (continued)**

Message number	Message type	BioRobot EZ1 DSP message text
6	Guidance	Load empty 2.0 ml tubes (ST) at heating block Next=Any, Prev=ESC
7	Guidance	Load elution tubes (ET) (1.5 ml) 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 1.5 ml tubes (ET) with (CARRIER) + IC in third row Next=Any, Prev=ESC
10	Guidance	Load 2.0 ml 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]

Table continued on next page.



**Table 4. Messages in the BioRobot EZ1 DSP Virus procedure (continued)**

Message number	Message type	BioRobot EZ1 DSP message text
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
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  $\mu\text{L}$  of internal control solution (ICLB) to 8.4 mL of lysis buffer (LB) and 140  $\mu\text{L}$  of carrier RNA (CARRIER). Using the manual reference procedure for the assay system, 625  $\mu\text{L}$  of lysis buffer (LB) is added per sample ( $\text{LB}_{\text{SAM}}$ ), and an elution volume of 75  $\mu\text{L}$  ( $\text{EL}_{\text{SAM}}$ ) is used. User 1 uses 50  $\mu\text{L}$  of eluate per downstream reaction ( $\text{EL}_{\text{RXN}}$ ). The volume of internal control solution in each downstream reaction ( $\text{IC}_{\text{RXN}}$ ) is:

$$\text{IC}_{\text{RXN}} = \frac{39 \mu\text{L} \times 625 \mu\text{L} \times 50 \mu\text{L}}{(8540 \mu\text{L} + 39 \mu\text{L}) \times 75 \mu\text{L}} = 1.89 \mu\text{L}$$

The final downstream reactions for the given assay system contain 1.89  $\mu\text{L}$  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 ( $\text{IC}_{\text{RXN}}$ ), then you need to determine the amount of internal control (IC) to be diluted with elution buffer (AVE) and carrier RNA (CARRIER) ( $\text{IC}_{\text{AVE}}$ ) before starting the purification. To calculate this value, use the formula:

$$\text{IC}_{\text{AVE}} = \frac{\text{IC}_{\text{RXN}} \times \text{IC}_{\text{TOT}} \times \text{EL}_{\text{SAM}}}{\text{IC}_{\text{SAM}} \times \text{EL}_{\text{RXN}}}$$

where:

$\text{IC}_{\text{AVE}}$  = Volume of internal control (IC) diluted in elution buffer–carrier RNA (AVE–CARRIER)

$\text{IC}_{\text{RXN}}$  = Volume of internal control (IC) per downstream reaction

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

$IC_{SAM}$  = Volume of diluted internal control (IC) added per sample (50  $\mu$ L)

$EL_{SAM}$  = 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  $\mu$ L of internal control solution per reaction ( $IC_{RXN}$ ) and 20  $\mu$ L of eluate per reaction ( $EL_{RXN}$ ). User 2 follows the EZ1 DSP Virus protocol, and a 60  $\mu$ L elution volume ( $EL_{SAM}$ ) has been selected. For each processed sample, a volume of 60  $\mu$ L of diluted internal control (IC) has to be manually pipetted into the 1.5 mL tube (ET) in position 3 of the EZ1 worktable or row B of the EZ2 worktable, but during the sample preparation process of the EZ1 DSP Virus protocol, the EZ1/EZ2 instrument will only transfer 50  $\mu$ L of diluted internal control ( $IC_{SAM}$ ) from well 3/row B 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  $\mu$ L

$$= 6 \times 60 \mu\text{L} = 360 \mu\text{L}$$

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

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

For each sample, 3.6  $\mu\text{L}$  carrier RNA (CARRIER) stock solution with 1  $\mu\text{g}/\mu\text{L}$  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  $\mu\text{L}$  carrier RNA stock = 21.6  $\mu\text{L}$

For a final total volume of 360  $\mu\text{L}$  of diluted internal control (IC), the user has to add elution buffer (AVE):

$$\begin{aligned}\text{Volume of elution buffer (AVE)} &= \text{IC}_{\text{TOT}} - \text{IC}_{\text{AVE}} - \text{Volume carrier RNA (CARRIER)} \\ &= 360 \mu\text{L} - 21.6 \mu\text{L} - 21.6 \mu\text{L} = 316.8 \mu\text{L}\end{aligned}$$

User 2 needs to add 21.6  $\mu\text{L}$  of internal control solution to 316.8  $\mu\text{L}$  elution buffer (AVE) and 21.6  $\mu\text{L}$  of carrier RNA (CARRIER) stock to obtain 360  $\mu\text{L}$  of diluted internal control (IC). From this diluted internal control (IC), 60  $\mu\text{L}$  has to be manually transferred into 1.5 mL tubes (ET) in position 3 of the EZ1 worktable or row B of the EZ2 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 or printed and labeled with descriptions of the samples and details of the run.

## EZ1 DSP Virus system

Date/time: \_\_\_\_\_ Kit lot number: \_\_\_\_\_

Operator: \_\_\_\_\_ Run ID: \_\_\_\_\_

EZ1 serial number: \_\_\_\_\_

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

Date/time: \_\_\_\_\_ Kit lot number: \_\_\_\_\_

Operator: \_\_\_\_\_ Run ID: \_\_\_\_\_

**EZ2** serial number: \_\_\_\_\_

Position on worktable	Sample ID	Sample material	RCV and empty tube loaded?	ST loaded?	ET loaded?	DTH with DFT loaded?	ET with CARRIER and IC loaded?
1 (left)							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24 (right)							

# 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 XL DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol; for use with the EZ1 Advanced XL instrument	9018703
EZ1 Advanced DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol; for use with the EZ1 Advanced instrument	9018306
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

\* 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.



Product	Contents	Cat. no.
EZ2 Connect MDx	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled EZ1 Kit cartridges; includes 1-year warranty on parts and labor WiFi Connectivity for LIMS and QIASphere ease-of-use	9003230
Buffer ASL (4 x 140 mL)	4 x 140 mL Buffer ASL	19082

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# Document Revision History

Revision	Description
R1, June 2022	<ul style="list-style-type: none"><li>• New Kit Version V5 according to the new EU regulation 2017/746 (IVDR)</li><li>• Addition of usage of the new EZ2 Connect MDx instrument</li><li>• Update of Materials Provided (add active ingredients)</li><li>• Update of limitations section: removal of sample material whole blood, urine, dried swabs, sputum from intended use</li><li>• Update of Warnings and Precautions</li><li>• Update of Reagent Storage and Handling</li><li>• Update of Carrier-RNA in-use stability</li><li>• Addition of section Disposal</li><li>• Update of Troubleshooting guide</li></ul>
R2, November 2022	Corrected the catalog number and reagent name under Kit content table.

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