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# EZ1<sup>®</sup> DSP DNA Blood Kit Instructions for Use (Handbook)



48

Version 4



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)



62124



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# Contents

Intended Use .....	4
Intended User .....	4
Description and Principle .....	5
Summary and explanation .....	5
Materials Provided .....	7
Kit contents .....	7
Components of the kit .....	8
Materials Required but Not Provided .....	9
Warnings and Precautions .....	11
Safety information .....	11
Precautions .....	13
Emergency information .....	13
Disposal .....	14
Reagent Storage and Handling .....	15
In-use stability .....	16
Specimen Storage and Handling .....	17
Elution volumes and DNA storage .....	19
Procedure .....	20
Working with EZ2 Connect MDx instruments .....	20
Working with EZ1 instruments .....	27
Protocol: Purification of Genomic DNA from Whole Blood Using the EZ2 Connect MDx ...	34
Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced XL ...	42

Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced (with V2.0 Card) .....	47
Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced (with V1.0 Card) .....	53
Protocol: Purification of Genomic DNA from Whole Blood Using the BioRobot EZ1 DSP ...	58
Quality Control.....	62
Limitations.....	62
Performance Characteristics .....	63
Troubleshooting Guide .....	64
Symbols.....	67
Contact Information .....	70
Appendix A: Display Messages on EZ1/EZ2 Instruments.....	71
Appendix B: Quantification and Determination of Purity of DNA .....	87
Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System .....	89
Ordering Information .....	91
Document Revision History .....	92

## Intended Use

The EZ1 DSP DNA Blood Kit utilizes magnetic particle technology for automated isolation and purification of human DNA from biological specimens.

The EZ1 DSP DNA Blood system 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 DNA purification with the convenient handling of magnetic particles (see the flowchart, page 6). DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in elution buffer.

## Summary and explanation

The EZ1 DSP DNA Blood Kit is for purification of genomic DNA from whole blood samples. Magnetic particle technology provides high-quality DNA that is suitable for direct use in downstream applications, 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.

Using the BioRobot EZ1 DSP or using the EZ1 Advanced with the protocol card V1.0, the sample input volume is 350  $\mu$ l and DNA elution takes place in 200  $\mu$ l of elution buffer. Using the EZ1 Advanced XL or using the EZ1 Advanced with the protocol card V2.0, or using the EZ2 Connect MDx, the sample input volume can be chosen from 200 or 350  $\mu$ l and the DNA elution volume can be chosen from 50, 100, or 200  $\mu$ l.

## EZ1 DSP DNA Blood Procedure

Whole blood



Lysis



Magnetic particles  
added to samples



DNA binds to  
magnetic particles



Magnetic separation



Wash



Magnetic separation



Elute



Pure, high-quality DNA

# Materials Provided

## Kit contents

<b>EZ1 DSP DNA Blood Kit</b>			<b>(48)</b>
<b>Catalog no.</b>			<b>62124</b>
<b>Number of preps</b>			<b>48</b>
RCB	Reagent Cartridge, Blood 350 µl*	<b>REAG</b> <b>CART</b> <b>BLOOD</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), skirted	<b>SAMP</b> <b>TUBE</b>	50
ET	Elution Tubes (1.5 ml)	<b>ELU</b> <b>TUBE</b>	50
	Q-Card†		1
	Instructions for Use		1

\* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See “Warnings and Precautions”, page 11, for safety information.

† 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>
RCB (Reagent Cartridge Blood)	Ethanol	≥50 to <70
	Guanidinium thiocyanate	≥50 to <70
	Guanidine hydrochloride	≥30 to <50
	Lithium chloride	≥1 to <10
	t-Octylphenoxyethoxyethanol	≥1 to <2.5

# 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 pipet tips
- Soft paper tissue
- Water
- 70% ethanol (for cleaning procedures)
- **Optional:** incubator\* (if reagent cartridges [RCB] contain precipitates at bottom of wells)
- **Optional:** microcentrifuge\* (if magnetic particles need to be removed from eluates)
- **Optional:** 80% ethanol<sup>†</sup> and 2 ml screw-capped tubes (if performing the optional 80% ethanol wash steps on the EZ1 Advanced using the V2.0 protocol card, on the EZ1 Advanced XL or on the EZ2 Connect MDx, see “Things to do before starting”, page 42)

 2 ml screw-capped tubes: Use Sarstedt® cat. no. 72.693 (non-skirted, with lid) tubes for preparation of optional 80% ethanol wash step.

## For BioRobot EZ1 users

- BioRobot EZ1 DSP instrument\* (discontinued)
- EZ1 DSP DNA Blood Card (cat. no. 9017713)

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

<sup>†</sup> Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

## For EZ1 Advanced users

- EZ1 Advanced instrument\* (discontinued)
- EZ1 Advanced DSP DNA Blood Card (cat. no. 9018305)

## For EZ1 Advanced XL users

- EZ1 Advanced XL instrument\* (cat. no. 9001492)
- EZ1 Advanced XL DSP DNA Blood Card (cat. no. 9018702)

## 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
    - Printer

## 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

# 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 (RCB) contain guanidine hydrochloride or guanidine isothiocyanate, which can form highly reactive compounds when combined with bleach.
- If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If liquid containing potentially infectious agents is spilled on EZ1/EZ2 instruments, disinfect the instrument using reagents described in the user manual supplied with your EZ1/EZ2 instrument.
- Broken or leaky reagent cartridges (RCB) must be handled and discarded according to local safety regulations. Do not use damaged reagent cartridges (RCB) 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 DNA Blood 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 DNA Blood Kit:

### Reagent Cartridge Blood (RCB)



Contains: ethanol, guanidine hydrochloride, guanidine thiocyanate, lithium chloride and t-octylphenoxypolyethoxyethanol. Danger! Highly flammable liquid and vapor. Harmful if swallowed, in contact with skin or if inhaled. Causes severe skin burns and eye damage. May cause respiratory irritation. 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. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician. Take off immediately all contaminated clothing and wash it before reuse. 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.

The product contains t-octylphenoxypolyethoxyethanol, an endocrine disrupting substance that can have adverse effects on the environment.

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 11.

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 (RCB) upright cooled at 2–8°C. The magnetic particles in the reagent cartridges (RCB) remain active when stored at this temperature. Do not freeze the reagent cartridges (RCB). When stored at 2–8°C, the reagent cartridges (RCB) are stable until the expiration date printed on the label and on the kit box. Upon removal from cooled storage, the reagent cartridges (RCB) may be stored once at 15–25°C, but must be used in a period of 4 weeks or until the expiration date printed on the label, the Q-Card, and on the kit box, whichever comes first.

- ⓘ The buffer in well 1 of the reagent cartridge (RCB) (the well that is nearest to the front of the EZ1/E2 instrument when the RCB is loaded) may form a precipitate upon storage. Before use, equilibrate the reagent cartridge (RCB) to room temperature. Thoroughly check well 1 for precipitates before loading by inverting 4 times. If necessary, redissolve by equilibrating up to 40°C and inverting 4 times without creating foam.

Make sure that no precipitates are visible before loading.

- ⓘ Do not use the EZ1 DSP DNA Blood Kit once it has expired. Avoid exposure of the RCB to UV light (e.g., used for decontamination) as this may cause accelerated aging of the buffers.
- ⓘ Do not use reagent cartridges (RCB) 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 (RCB) are for single-use only and do not provide in-use stability.

For the optional 80% ethanol wash step, always prepare the buffer fresh. Do not store residual buffer as this may lead to evaporation and wrong buffer concentration. See “Things to do before starting” for further preparation instructions.

# Specimen Storage and Handling

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

The purification procedure is optimized for use with 200 and 350  $\mu$ l sample volumes.

-  Do not use lower or higher sample volumes other than 200 or 350  $\mu$ l as this might lead to performance issues or might damage the instrument.

Whole blood samples treated with EDTA, ACD (citrate), or heparin\* can be used, and may be either fresh or frozen. Frozen samples should be thawed at room temperature (15–25°C) with mild agitation before beginning the procedure. Yield and quality of the purified DNA may depend on storage conditions of the blood. Fresh blood samples may yield better results. Do not re-freeze blood samples more than 2 times as this may result in decreasing DNA yield.

- For short-term storage (up to 7 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.
- For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store the tubes at –20°C for a maximum of 4 weeks. Longer storage may be possible depending on the downstream application but needs to be validated by the user.
- Do not use blood that shows signs of coagulation.

\* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Sample stability highly depends on various factors and relates to the specific downstream application. It has been established for the EZ1 DSP DNA Blood 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.

- ① 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. For further instructions for DNA extraction from venous whole blood also refer to ISO 20186-2:2019 (E).
  
- ① Please note that during development of the EZ1 DSP DNA Blood Kit no indications were observed that heparin has a negative impact on the performance. However, ISO 20186-2:2019(E) states that heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates could cause inhibitions in some downstream applications. Therefore it is the user's responsibility to validate if heparin has a negative influence on their workflow.

## Elution volumes and DNA storage

The final step of the purification procedure is elution of genomic DNA. The elution parameters which can be chosen are 50, 100, or 200  $\mu\text{l}$ .

We recommend storing the purified DNA at 2–8°C or at –20°C for up to 24 months. For extended storage time, we recommend storage at –20°C or –80°C for up to 36 months. The impact of DNA stability 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 Blood 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 DNA Blood 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 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

**i** 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 resource tab of the product page on [www.qiagen.com](http://www.qiagen.com)).

-  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 DNA Blood kit (Figure 1) by pressing the **Scan** button. Dedicated protocols are automatically displayed, when the Q-card is scanned.

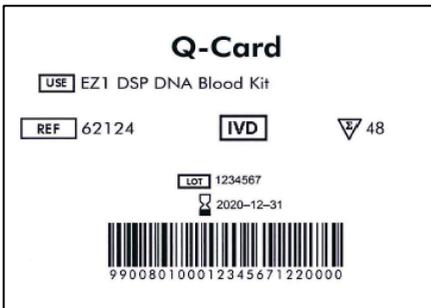
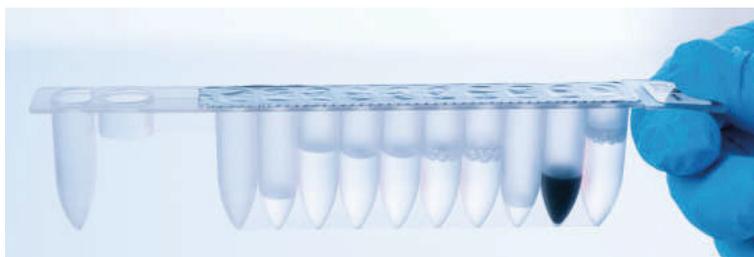


Figure 1. Q-card example.

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

## Reagent cartridges (RCB)

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



**Figure 2. Reagent cartridge (RCB).** Sealed, prefilled reagent cartridge (RCB) of the EZ1 DSP DNA Blood Kit.

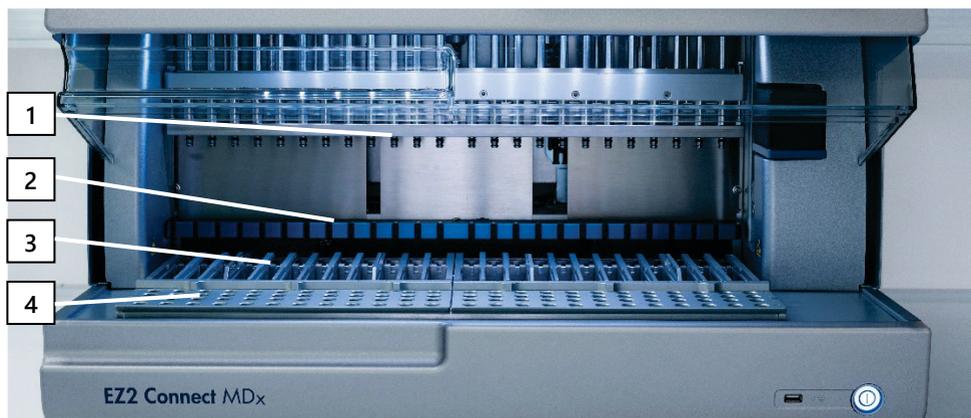


**Figure 3. Reagent cartridge rack.** The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges (RCB) must be loaded.

## Worktable

The worktable of EZ2 Connect MDx instruments is where the user loads samples and the components of the EZ1 DSP DNA Blood 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) Sample tubes (ST) (2 ml) loaded into row A. (2) Empty or optional: Tube (2 ml) containing 80% Ethanol for optional wash step, loaded into row B. (3) Disposable tip holders (DTH) containing disposable filter-tips (DFT) loaded into row C. (4) Elution tubes (ET) (1.5 ml) loaded into row D.

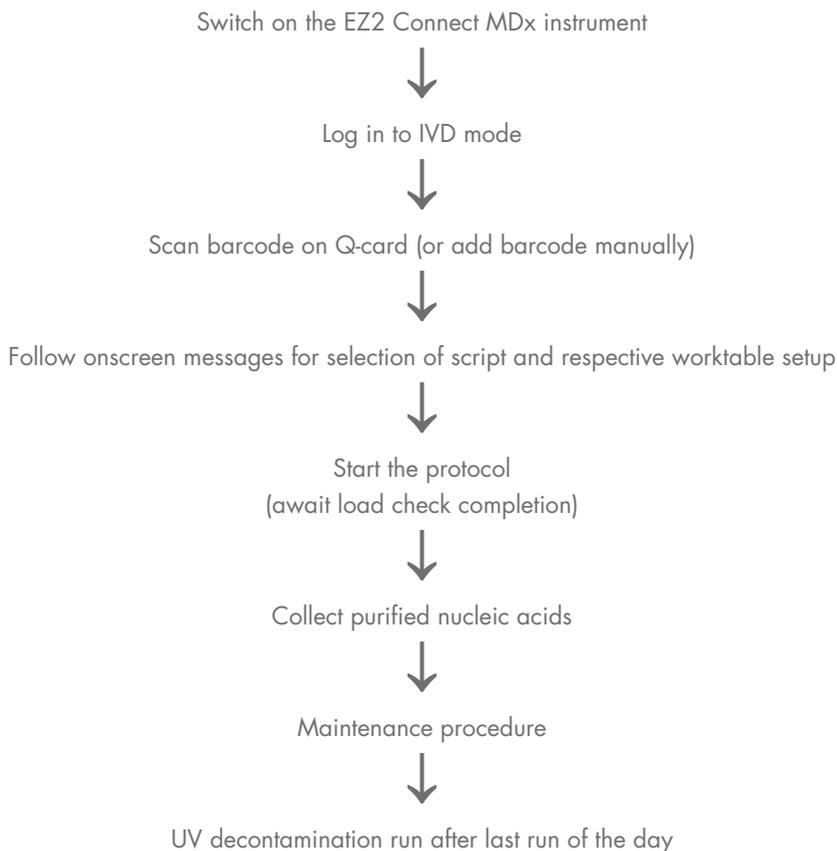
## Data tracking with the EZ2 Connect MDx

The EZ2 Connect MDx enables 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 DNA Blood 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 DNA Blood operation on EZ2 Connect MDx



## Working with EZ1 instruments

The main features of EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 (BioRobot EZ1 DSP and EZ1 Advanced) or 1–14 (EZ1 Advanced XL) samples per run
- Small footprint to save 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

**i** 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 DNA Blood protocol 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.

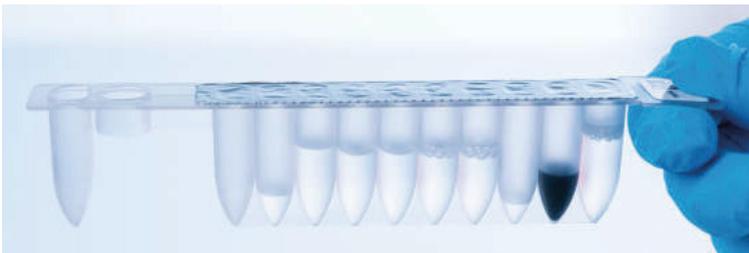
- ⓘ The instrument must only be switched on after an EZ1 Card is inserted and make sure that the EZ1 Card is completely inserted! Otherwise, essential instrument data will be lost, leading to a memory error. EZ1 Cards must not be exchanged while the instrument is switched on.



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

### Reagent cartridges (RCB)

Reagents for purification of nucleic acids from a single sample are contained in a single reagent cartridge (RCB) (Figure 8). Most wells of the cartridge (RCB) contain a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or 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.



**Figure 8. Reagent cartridge (RCB).** A sealed and prefilled RCB of the EZ1 DSP DNA Blood Kit.

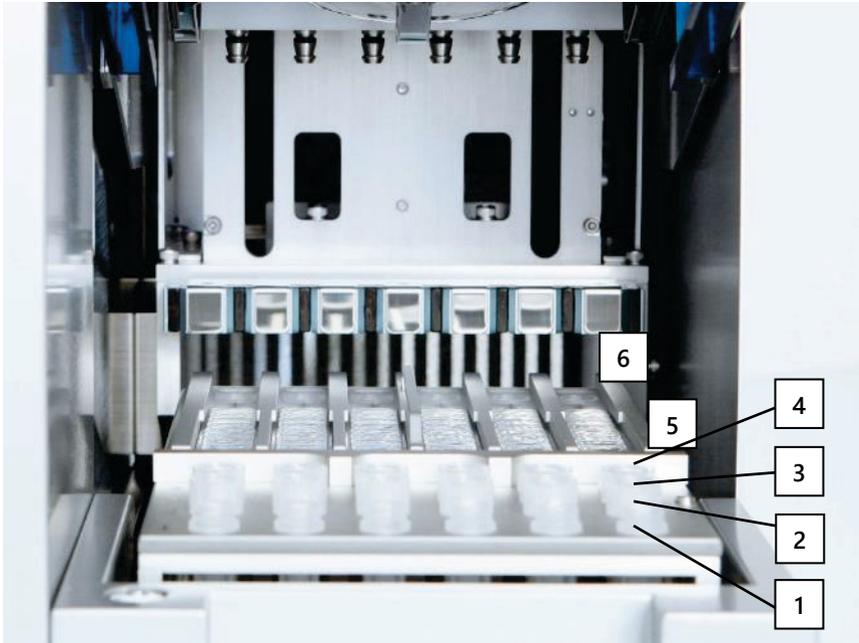


**Figure 9. Loading the reagent cartridge rack.** The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges (RCB) must be loaded.

## Worktable

The worktable of the EZ1 instrument is where the user loads samples and the components of the EZ1 DSP DNA Blood Kit (Figure 10).

Details on worktable setup are displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced or 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. 3: Row 3 is empty for the EZ1 DSP DNA Blood protocol. (**Optional:** If performing the optional 80% ethanol wash steps, the 2 ml tubes (non-skirted) containing 1800  $\mu$ l each of 80% ethanol are loaded into this row.). 4: Sample tubes (ST) (2 ml) loaded into row 4. 5: Reagent cartridges (RCB) loaded into the cartridge rack. 6: The Heating block is empty for the EZ1 DSP DNA Blood protocol.

## 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 DNA Blood operation on EZ1

Insert EZ1 DSP DNA Blood Card into the EZ1 Card slot



Switch on the EZ1 instrument



Follow onscreen message for data tracking\*



Follow onscreen messages for worktable setup



Start the protocol



Collect purified DNA



UV decontamination run\*

\* EZ1 Advanced and EZ1 Advanced XL only.

# Protocol: Purification of Genomic DNA from Whole Blood Using the EZ2 Connect MDx

## Important points before starting

- If using the EZ1 DSP DNA Blood Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with EZ2 Connect MDx instruments” starting on page 15.
- The reagent cartridges (RCB) contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 11 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- After receiving the kit, check the kit components for damage. If the reagent cartridges (RCB) or other kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Warnings and Precautions” (page 11). Do not use damaged reagent cartridges (RCB) 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 RCB.
- The yield of genomic DNA depends on the number of white blood cells in the sample. Blood samples with a white blood cell count from  $3 \times 10^6$  to  $1 \times 10^7$  WBC/ml are recommended to be used.

## Things to do before starting

- The lysis buffer in the reagent cartridge (RCB) may form a precipitate upon storage. Before use, equilibrate reagent cartridge (RCB) to room temperature. Check the RCB for precipitates by inverting the cartridge 4 times. If necessary, redissolve by equilibrating up to 40°C, inverting 4 times without creating foam, and then placing at room temperature.

- The protocol includes an option to perform washes with 80% ethanol instead of washing with one buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, per sample a 2 ml tube (Sarstedt, cat. no. 72.693, non-skirted) containing 1800 µl of 80% ethanol should be placed in row B of the worktable (Figure 5). For preparation of 80% ethanol sufficient for 24 samples, add 10 ml nuclease-free water to 40 ml 96–100% ethanol. \* Follow the instructions given in the onscreen messages.

## Procedure

1. Equilibrate up to 24 whole blood samples at room temperature. Transfer either 200 or 350 µl sample into the 2 ml sample tubes (ST) (skirted) provided with the kit.
  - ① Only use the 2 ml tubes (ST) (skirted) provided with the kit.
  - ① Make sure that samples that have been frozen are thawed completely and equilibrated to room temperature for a sufficient period of time to equilibrate. If samples have been stored at 2–8°C, they must also be equilibrated to room temperature. The temperature of all samples should be 15–25°C before starting the procedure to ensure optimal yield and DNA purity.
  - ① Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.
2. Switch on the EZ2 Connect MDx instrument.

The power switch is located at the right front of the instrument.
3. Log in to the instrument choosing the **IVD mode** of the software. Enter user ID and password.

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

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*.

4. 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 RCB as this will lead to impaired performance; the samples will be flagged as invalid.

5. Tap **Next** to continue.

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

6. Choose the different protocol parameters by tapping the box next to each parameter option.

7. Tap **Next** to continue.

8. 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.

9. Tap **Next** to continue.

10. Enter the sample ID's, 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.
11. Tap **Next** to continue.
  12. 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.
  13. Invert reagent cartridges (RCB) 4 times to mix the magnetic particles. See “Things to do before starting” before using the RCB.
  14. Place the RCB into the cartridge rack, press down the cartridge until it clicks into place.
  15. Once all RCB are prepared, place both cartridge racks onto the worktable.
    -  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.
  16. Tap **Next** to continue.
  17. **Optional:** If “Pure ethanol wash” has been chosen, load the 2 ml tubes (non-skirted, Sarstedt cat. no. 72.693), containing 1800 µl of 80 % ethanol, into row B of the tip rack (“labware holder”).

18. Place the tips into the tip holder and load them into row C of the rack.
  -  When preparing the tips and tip holder, only touch the upper part of the tips with gloves.
19. Load the 1.5 ml elution tubes (ET) into row D of the rack.
  -  Ensure that the elution tubes are loaded without a lid.
20. Load the 2 ml sample tubes (ST) (skirted) containing either 200 or 350  $\mu$ l sample (according to the selected protocol parameter) into row A of the rack.
  -  Ensure that the sample tubes are loaded into the correct positions as selected in step 10. **Optional:** Use the template from “Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System” to track sample ID and orientation.
  -  Ensure that the sample tubes are loaded without a lid.
  -  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.
  -  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.
  -  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.
21. Once all tubes and tips are loaded, place each tip rack (left and right rack) onto the worktable and close the hood.
  -  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.
22. Tap **Next** to continue.

23. Check the on-screen information of the run setup overview for correct protocol, sample and elution volume, and number of samples.

24. If all information is correct, tap **Start** to proceed with the protocol run.

 To make any modifications, tap Return to go back to the run setup.

25. The load check will now be performed. The protocol will start automatically after the load check is successfully completed.

 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 28 after successful load check.

26. 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*.

27. 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 ethanol (if applicable) from the worktable, close the tubes, and store them at appropriate conditions. Recalibrate the camera and contact QIAGEN Technical Support for additional support.

28. 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.

29. When the protocol has successfully finished, the **Protocol run completed** screen appears.

30. Open the hood, carefully remove the tip racks and place them onto the bench. First, remove the elution tubes 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.

31. Discard the sample preparation waste from row A. \* Discard the tip holders and tips as well as the ethanol tubes (if used).

 Follow the local safety regulations for waste disposal.

32. Remove the cartridge racks and discard the RCB.

 Follow the local safety regulations for waste disposal (see also “Warnings and Precautions”, page 11).

33. 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*.

\* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 11 for Safety information.

34. 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.
35. After the last run of each day, perform the daily maintenance procedure followed by UV decontamination.
36. Perform the weekly maintenance procedure, if required, after the daily maintenance.

# Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced XL

## Important points before starting

If using the EZ1 DSP DNA Blood Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with EZ1 instruments” starting on page 15.

- The reagent cartridges (RCB) contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 11 for 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 (RCB) or other kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Warnings and Precautions ” (page 11). Do not use damaged reagent cartridges (RCB) 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 RCB.
- The yield of genomic DNA depends on the number of white blood cells in the sample. Blood samples with a white blood cell count from  $3 \times 10^6$  to  $1 \times 10^7$  WBC/ml are recommended to be used.

## Things to do before starting

- The lysis buffer in the reagent cartridge (RCB) may form a precipitate upon storage. Before use, equilibrate reagent cartridge (RCB) to room temperature. Check the RCB for precipitates by inverting the cartridge 4 times. If necessary, redissolve by equilibrating up to 40°C, inverting 4 times without creating foam, and then placing at room temperature.

- The protocol includes an option to perform washes with 80% ethanol instead of washing with one buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, per sample a 2 ml tube (Sarstedt cat. no. 72.693, non-skirted) containing 1800 µl of 80% ethanol should be placed in row 3 of the worktable (see Figure 10, page 31). For preparation of 80% ethanol sufficient for 14 samples, add 6 ml nuclease-free water to 24 ml 96–100% ethanol. \* Follow the instructions given in the onscreen messages.

## Procedure

1. Equilibrate up to 14 whole blood samples at room temperature. Transfer either 200 or 350 µl sample into 2 ml sample tubes (ST) (skirted) provided with the kit.
  - ⓘ Make sure that samples that have been frozen are thawed completely and equilibrated to room temperature for a sufficient period of time to equilibrate. If samples have been stored at 2–8°C, they must also be equilibrated to room temperature. The temperature of all samples should be 15–25°C before starting the procedure to ensure optimal yield and DNA purity.
  - ⓘ Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.
2. Insert the EZ1 Advanced XL DSP DNA Blood Card completely into the EZ1 Card slot of the EZ1 Advanced XL.
3. Switch on the EZ1 instrument.

The power switch is located at the rear of the instrument.
4. Press **START** to start protocol and worktable setup of the EZ1 DSP DNA Blood protocol.
5. Follow the onscreen instructions for worktable setup, protocol variable selection, and data tracking.

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

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

6. Press **1** to start worktable setup for the 200 µl DSP Protocol or **2** to start the 350 µl DSP Protocol.
7. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.
8. Choose **yes** if you wish to perform the optional 80% ethanol washes.

The text summarizes the following steps, which describe the loading of the worktable.

9. Open the instrument door.
10. Invert reagent cartridges (RCB) 4 times to mix the magnetic particles.
11. Load the reagent cartridges into the cartridge rack.

**i** After sliding a reagent cartridge (RCB) into the cartridge rack, press down on the cartridge until it clicks into place.

**i** For data tracking, always start loading samples in 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.

12. Follow the onscreen instructions for further worktable setup.

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

**i** Ensure that the elution tubes (ET, 1.5 ml tubes) are loaded without a lid.

**i** Ensure that the sample tubes are loaded into the correct positions as selected in step 5. **Optional:** Use the template from "Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System" to track sample ID and orientation.

-  Ensure that the sample tubes are loaded without a lid.
-  Ensure that the sample tubes contain the correct volume of sample material.
-  Avoid formation of foam or bubbles on top of the sample or at the rim of the sample tubes.
-  Immediately start the protocol after placing the samples onto the worktable as prolonged storage time onboard the instrument may lead to evaporation.

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

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

14. Close the instrument door.

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

16. When the protocol ends, the display shows "Protocol finished". Press **ENT** to generate the report file.

The 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.

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

18. Remove the elution tubes (ET) containing the purified DNA from row 1. Avoid touching other tubes while removing the single elution tubes. Close the ET with the lids provided with the kit.

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

19. Discard the sample-preparation waste\*. Discard the tip holders and tips as well as the ethanol tubes (if used).

\* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 11 for Warnings and Precautions.

20. Remove the cartridge rack and discard the RCB.

-  Follow the local safety regulations for waste disposal “Warnings and Precautions”, page 11.

21. Recommended: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

22. Carry out the regular maintenance procedure as described in the user manual supplied with your EZ1 instrument.

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

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

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

23. To run another protocol, press **START**, carry out step 1 of the protocol, and then follow the protocol from step 4. Otherwise press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

Steps 2 and 3 are not necessary when running another protocol. Skip these steps.

# Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced (with V2.0 Card)

This protocol is for use with the EZ1 Advanced DSP DNA Blood Card V2.0, an updated version of the original V1.0 card. When using the V1.0 card, follow “Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced (with V1.0 Card)”.

The protocol on the V2.0 card includes additional protocol options enabling use of different sample input and elution volumes as well as optional 80% ethanol wash steps. The protocol on the V2.0 card is equivalent to the original V1.0 card when the original input and elution volumes and wash buffers are used.

## Important points before starting

If using the EZ1 DSP DNA Blood Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with EZ1 instruments” starting on page 15.

- The reagent cartridges (RCB) contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 11 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- After receiving the kit, check the kit components for damage. If the reagent cartridges (RCB) or other kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Warnings and Precautions” (page 11). Do not use damaged reagent cartridges (RCB) 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 RCB

- The yield of genomic DNA depends on the number of white blood cells in the sample. Blood samples with a white blood cell count from  $3 \times 10^6$  to  $1 \times 10^7$  WBC/ml are recommended to be used.

## Things to do before starting

- The lysis buffer in the reagent cartridge (RCB) may form a precipitate upon storage. Before use, equilibrate the reagent cartridge (RCB) to room temperature. Check the RCB for precipitates by inverting the cartridge 4 times. If necessary, redissolve by equilibrating up to  $40^\circ\text{C}$ , inverting 4 times without creating foam, and then placing at room temperature.
- The protocol includes an option to perform washes with 80% ethanol instead of washing with one buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, per sample a 2 ml tube (Sarstedt cat. no. 72.693, non-skirted) containing 1800  $\mu\text{l}$  of 80% ethanol should be placed in row 3 of the worktable (Figure 10). For preparation of 80% ethanol sufficient for 6 samples, add 3 ml nuclease-free water to 12 ml 96–100% ethanol. \* Follow the instructions given in the onscreen messages.

## Procedure

24. Equilibrate up to 6 whole blood samples at room temperature. Transfer either 200 or 350  $\mu\text{l}$  sample into 2 ml sample tubes (ST) (skirted) provided with the kit.



Make sure that samples that have been frozen are thawed completely and equilibrated to room temperature for a sufficient period of time to equilibrate. If samples have been stored at  $2\text{--}8^\circ\text{C}$ , they must also be equilibrated to room temperature. The temperature of all samples should be  $15\text{--}25^\circ\text{C}$  before starting the procedure to ensure optimal yield and DNA purity.

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

-  Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.

25. Insert the EZ1 Advanced DSP DNA Blood Card (V2.0) completely into the EZ1 Card slot of the EZ1 Advanced.

26. Switch on the EZ1 instrument.

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

28. Press **START** to start protocol and worktable setup of the EZ1 DSP DNA Blood protocol.

29. Follow the onscreen 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.

30. Press **1** to start worktable setup for the 200 µl DSP Protocol or **2** to start the 350 µl DSP Protocol.

31. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl or **3** to elute in 200 µl.

32. Choose **Yes** if you wish to perform the optional 80% ethanol washes.

33. The text summarizes the following steps, which describe the loading of the worktable.

34. Open the instrument door.

35. Invert the reagent cartridges (RCB) 4 times to mix the magnetic particles. Then tap the cartridges (RCB) to deposit the reagents to the bottom of their wells.

36. Load the reagent cartridges into the cartridge rack.

-  After sliding a reagent cartridge (RCB) into the cartridge rack, press down on the cartridge until it clicks into place.

- ① For data tracking, always start loading samples in position A on the EZ1 Advanced. 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.

37. Follow the onscreen instructions for further worktable setup.

- ① When preparing the tips and tip holder, only touch the upper part of the tips with gloves.
- ① Ensure that the elution tubes (ET, 1.5 ml tubes) are loaded without a lid.
- ① Ensure that the sample tubes are loaded into the correct positions as selected in step 5. **Optional:** Use the template from “Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System” to track 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.
- 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.

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

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

39. Close the instrument door.

40. Press **START** to start the protocol.
41. When the protocol ends, the display shows "Protocol finished". Press **ENT** to generate the report file.

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

42. Open the instrument door, carefully remove the tip rack and place it onto the bench.
43. Remove the elution tubes (ET) containing the purified DNA from row 1. Avoid touching other tubes while removing the single elution tubes. Close the ET with the lids provided with the kit.

 Immediately remove and store eluates after the run has finished.

44. Discard the sample-preparation waste. \* Discard the tip holders and tips as well as the ethanol tubes (if used).

45. Remove the cartridge rack and discard the RCB.

 Follow the local safety regulations for waste disposal "Warnings and Precautions", page 11.

46. **Optional:** Follow the onscreen instructions to perform UV decontamination procedure of the worktable surfaces.

 After the last run of the day and subsequent regular maintenance, UV decontamination procedure is recommended.

47. Carry out the regular maintenance procedure as described in the user manual supplied with your EZ1 instrument.

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

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

\* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 11 for Warnings and Precautions.



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

48. To run another protocol, press **START**, carry out step 24 of the protocol, and then follow the protocol from step 28. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

Steps 25 and 26 are not necessary when running another protocol. Skip these steps.

# Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced (with V1.0 Card)

This protocol is for use with the original EZ1 Advanced DSP DNA Blood Card V1.0. When using the V2.0 card, follow “Protocol: Purification of Genomic DNA from Whole Blood Using the EZ1 Advanced (with V2.0 Card)”, page 47. This protocol is for use with 350 µl sample volume.

The protocol on the V2.0 card includes additional protocol options enabling use of different sample input and elution volumes as well as optional 80% ethanol wash steps. The protocol on the V2.0 card is equivalent to the original V1.0 card when the original input and elution volumes and wash buffers are used.

## Important points before starting

If using the EZ1 DSP DNA Blood Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with EZ1 instruments” starting on page 15.

- The reagent cartridges (RCB) contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 11 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- After receiving the kit, check the kit components for damage. If the reagent cartridges (RCB) or other kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Warnings and Precautions” (page 11). Do not use damaged reagent cartridges (RCB) 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 RCB.

- The yield of genomic DNA depends on the number of white blood cells in the sample. Blood samples with a white blood cell count from  $3 \times 10^6$  to  $1 \times 10^7$  WBC/ml are recommended to be used.

## Things to do before starting

- The lysis buffer in the reagent cartridge (RCB) may form a precipitate upon storage. Before use, equilibrate the reagent cartridge (RCB) to room temperature. Check the RCB for precipitates by inverting the cartridge 4 times. If necessary, redissolve by equilibrating up to 40°C, inverting 4 times without creating foam, and then placing at room temperature.

## Procedure

1. Equilibrate up to 6 whole blood samples at room temperature. Transfer 350  $\mu$ l sample into 2 ml sample tubes (ST) provided with the kit.
  - ❗ Make sure that samples that have been frozen are thawed completely and equilibrated to room temperature for a sufficient period of time to equilibrate. If samples have been stored at 2–8°C, they must also be equilibrated to room temperature. The temperature of all samples should be 15–25°C before starting the procedure to ensure optimal yield and DNA purity.
  - ❗ Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.
2. Insert the EZ1 Advanced DSP DNA Blood Card (V1.0) completely into the EZ1 Card slot of the EZ1 Advanced.
3. Switch on the EZ1 instrument.

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

4. Press **START** to start worktable setup of the EZ1 DSP DNA Blood protocol.
5. Open the instrument door.
6. Invert 1–6 reagent cartridges (RCB) 4 times to mix the magnetic particles. Then tap the cartridges (RCB) to deposit the reagents to the bottom of their wells.
7. Follow the onscreen instructions for worktable setup, protocol variable selection, and data tracking.

 After sliding a reagent cartridge (RCB) into the cartridge rack, press down on the cartridge until it clicks into place.

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

 For data tracking, always start loading samples in position A on the EZ1 Advanced. 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.

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

 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 5. Optional: Use the template from “Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System” to track sample ID and orientation.

 Ensure that the sample tubes are loaded without a lid.

-  Ensure that the sample tubes contain the correct volume of sample material.
  -  Avoid formation of foam or bubbles on top of the sample or at the rim of the sample tubes.
8. Load the prepared cartridge rack and tip rack into the instrument.
-  Do not interchange the cartridge racks and tip racks between different instruments.
9. Close the instrument door.
10. Press **START** to start the protocol.
11. When the protocol ends, the display shows “Protocol finished”. Press “ENT” to generate the report file.
- The EZ1 Advanced can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
12. Open the instrument door, carefully remove the tip rack and place it onto the bench.
13. Remove the elution tubes (ET) containing the purified DNA from row 1. Avoid touching other tubes while removing the single elution tubes. Close the ET with the lids provided with the kit.
-  Immediately remove and store eluates after the run has finished.
14. Remove the cartridge rack and discard the RCB.
-  Follow the local safety regulations for waste disposal (see also “Warnings and Precautions”, page 11).
15. Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
-  After the last run of the day and subsequent regular maintenance, UV decontamination procedure is recommended.

16. Carry out the regular maintenance procedure as described in the user manual supplied with your EZ1 instrument.

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



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

17. To run another protocol, press **START**, carry out step 1 of the protocol, and then follow the protocol from step 4. Otherwise press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

Steps 2 and 3 are not necessary when running another protocol. Skip these steps.

# Protocol: Purification of Genomic DNA from Whole Blood Using the BioRobot EZ1 DSP

## Important points before starting

If using the EZ1 DSP DNA Blood Kit for the first time, read “Reagent Storage and Handling”, “Specimen Storage and Handling”, and “Working with EZ1 instruments” starting on page 15.

- The reagent cartridges (RCB) contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 11 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- After receiving the kit, check the kit components for damage. If the reagent cartridges (RCB) or other kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Warnings and Precautions” (page 11). Do not use damaged reagent cartridges (RCB) 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 RCB.
- The yield of genomic DNA depends on the number of white blood cells in the sample. Blood samples with a white blood cell count from  $3 \times 10^6$  to  $1 \times 10^7$  WBC/ml are recommended to be used.

## Things to do before starting

- The lysis buffer in the reagent cartridge (RCB) may form a precipitate upon storage. Before use, equilibrate the reagent cartridge (RCB) to room temperature. Check the RCB for precipitates by inverting the cartridge 4 times. If necessary, redissolve by

equilibrating up to 40°C, inverting 4 times without creating foam, and then placing at room temperature.

## Procedure

1. Equilibrate up to 6 whole blood samples at room temperature. Transfer 350 µl sample into 2 ml sample tubes (ST) provided with the kit.
  -  Make sure that samples that have been frozen are thawed completely and equilibrated to room temperature for a sufficient period of time to equilibrate. If samples have been stored at 2–8°C, they must also be equilibrated to room temperature. The temperature of all samples should be 15–25°C before starting the procedure to ensure optimal yield and DNA purity.
  -  Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.
2. Insert the EZ1 DSP DNA Blood Card completely into the EZ1 Card slot of the BioRobot EZ1 DSP.
3. Switch on the EZ1 instrument.

The power switch is located at the rear of the instrument.
4. Press **START** to start worktable setup of the EZ1 DSP DNA Blood protocol.
5. Open the instrument door.
6. Invert the reagent cartridges (RCB) 4 times to mix the magnetic particles. Then tap the cartridges (RCB) to deposit the reagents to the bottom of their wells.
7. Follow the onscreen instructions for worktable setup and protocol variable selection.
  -  After sliding a reagent cartridge (RCB) into the cartridge rack, press down on the cartridge until it clicks into place.
  -  Do not interchange the cartridge racks and tip racks between different instruments.

- ① If there are fewer than 6 reagent cartridges (RCB), they can be loaded in any order on the rack. However, when loading the other labware, ensure that they also follow the same order.
  - ① Immediately start the protocol after placing the samples onto the worktable as prolonged storage time onboard the instrument may lead to evaporation.
  - ① When preparing the tips and tip holder, only touch the upper part of the tips with gloves.
  - ① Ensure that the elution tubes (ET, 1.5 ml tubes) are loaded without a lid.
  - ① Ensure that the sample tubes are loaded into the correct positions as selected in step 5. **Optional:** Use the template from “Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System” to track 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.
  - ① Avoid formation of foam or bubbles on top of the sample or at the rim of the sample tubes.
8. Load the prepared cartridge rack and tip rack into the instrument.
    - ① Do not interchange the cartridge racks and tip racks between different instruments.
  9. Close the instrument door.
  10. Press **START** to start the protocol.
  11. When the protocol ends, the display shows “Protocol finished”.
  12. Open the instrument door, carefully remove the tip rack and place it onto the bench.

13. Remove the elution tubes (ET) containing the purified DNA from row 1. Avoid touching other tubes while removing the single elution tubes. Close the ET with the lids provided with the kit.

 Immediately remove and store eluates after the run has finished.

14. Discard the sample-preparation waste. \* Discard the tip holders and tips.

15. Remove the cartridge rack and discard the RCB.

 Follow the local safety regulations for waste disposal (see also “Warnings and Precautions”, page 11).

16. Carry out the regular maintenance procedure as described in the user manual supplied with your EZ1 instrument.

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

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

17. To run another protocol, press **START**, carry out step 1 of the protocol, and then follow the protocol from step 4. Otherwise press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

Steps 2 and 3 are not necessary when running another protocol. Skip these steps.

\* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 11 for Warnings and Precautions.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1 DSP DNA Blood 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 human whole blood for isolation of genomic 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 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/EZ2 Connect MDx 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 DNA yield**

- |    |   |  |
|----|---|--|
| a) | Magnetic particles not completely resuspended   | Ensure that you resuspend the magnetic particles thoroughly before loading the reagent cartridges (RCB) into the holder.   |
| b) | Precipitates visible at the bottom of the wells of the reagent cartridges (RCB)             | Before use, equilibrate the reagent cartridges (RCB) to room temperature. Thoroughly check well 1 for precipitates before loading by inverting 4 times. If necessary, redissolve by equilibrating the RCB up to 40 °C and inverting 4 times without creating foam.<br><br>Do not use the reagent cartridges (RCB) if the precipitates do not redissolve. |
| c) | Wrong sample volume in sample tube  | Ensure to pipette the exact sample volume into the sample tube.  |
| 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) | Frozen blood samples not homogenized properly after thawing                                 | Thaw frozen blood samples in an incubator* or water bath* at 30–40°C with mild agitation to ensure thorough mixing.  |
| f) | Clogged blood samples in sample tubes   | Avoid transfer of clogged sample material into the sample tubes. This may lead to abortion of the procedure and a potential instrument crash.  |
| g) | Reagents loaded onto worktable in wrong order   | Ensure that all tubes (ET, ST, optional EtOH) and the tip holders (DTH) with the tips (DFT) are loaded onto the worktable in the correct order. Follow the onscreen instructions. Repeat the purification procedure with new samples.  |

**DNA does not perform well in downstream applications**

- |    |   |  |
|----|---|--|
| a) | Insufficient DNA used in downstream application | Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of DNA”, page 87).  |
| b) | Excess DNA used in downstream application       | Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of DNA”, page 87). |

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

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### Comments and suggestions

- |   |  |
|---|--|
| c) Inhibition of downstream application | Some downstream applications may show superior performance if 80% ethanol washes are performed instead of washes using buffers in the reagent cartridges. This option is available when using the EZ1 Advanced DSP DNA Blood Card V2.0 (see page 47) or the EZ1 Advanced XL DSP DNA Blood Card (see page 42) as well as the EZ2 Connect MDx (see page 34).   |
| d) Carryover of magnetic particles      | Carryover of magnetic particles in the eluates will not affect most downstream applications. If the risk of magnetic particle carryover needs to be minimized, first place the tubes containing eluate in a suitable magnetic separator for 1 minute, and then transfer the eluates to clean tubes. If a suitable magnet is not available, centrifuge the tubes containing eluates in a microcentrifuge at full speed for 1 minute to pellet any remaining magnetic particles, and transfer the supernatants to clean tubes. |

#### Low $A_{260}/A_{280}$ ratio for purified nucleic acids

Absorbance reading at 320 nm not subtracted from the absorbance readings obtained at 260 and 280 nm

To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 and 280 nm.

# Symbols

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

Symbol	Symbol definition
	Contains reagents 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
	Contains
	Number
	Volume

## Symbol

## Symbol definition

	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
	Instructions for use
	Warning/caution
	Only for use with
	RCB: Reagent Cartridge Blood
	DFT: Disposable Filter Tips
	DTH: Disposable Tip Holder
	ST: Sample Tube
	ET: Elution Tube

Symbol	Symbol definition
<b>GITC</b>	Guanidine isothiocyanate
<b>GuHCl</b>	Guanidine hydrochloride
<b>EtOH</b>	Ethanol
<b>LiCl</b>	Lithium chloride
	Open on delivery; store reagent cartridges (RCB) at 2–8°C
	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 to Table 5. 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 DNA Blood protocol**

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 DNA Blood Version 1.0
2	Data tracking	Enter user ID ENT: Next
3	Data tracking	Enter Q-Card barcode ENT: Next
4	Guidance	Wrong kit! Please load DSP DNA Blood kit ENT: Back
5	Guidance	Kit expired! MMYY: ENT: Use new kit ESC: Stop protocol

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

Message number	Message type	EZ1 Advanced XL message text
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	Guidance	Select protocol 1: 200 µl DSP Blood 2: 350 µl DSP Blood Choose 1 or 2
23	Guidance	Select elution volume: 1: 50 µl 2: 100 µl 3: 200 µl
24	Guidance	Pure ethanol wash? 1: No 2: Yes Choose 1 or 2
25	Guidance	You have chosen: [xxx] µl blood, EtOH [xxx] µl elution ENT: Next, ESC: Back
26	Guidance	Load cartridges at same positions as samples ENT: Next, ESC: Back
27	Guidance	Load elution tubes (ET) (1.5 ml) into first row ENT: Next, ESC: Back
28	Guidance	Load tip holders (DTH) and tips (DFT) into second row ENT: Next, ESC: Back
29	Guidance	Load 2 ml tubes with 1800 µl 80% EtOH into third row ENT: Next, ESC: Back
30	Guidance	Load 2 ml tubes (ST) with sample into fourth row ENT: Next, ESC: Back
31	Guidance	Loading finished Close door and press START ESC: Back

Message number	Message type	EZ1 Advanced XL message text
32	Guidance	Please close door! ENT: Next
33	Status	Protocol started
34	Status	Piercing foil [x] of [x] min left
35	Status	Collecting Elution Buffer [x] of [x] min left
36	Status	Deliver at heat block [x] of [x] min left
37	Status	Collecting Beads [x] of [x] min left
38	Status	Resuspension of Beads [x] of [x] min left
39	Status	Collecting Lysis Buffer [x] of [x] min left
40	Status	Mixing Lysate [x] of [x] min left
41	Status	Collecting Beads [x] of [x] min left
42	Status	DNA binding to Beads Magnetic separation [x] of [x] min left
43	Status	Wash 1 Magnetic separation [x] of [x] min left
44	Status	Wash 2 Magnetic separation [x] of [x] min left
45	Status	Wash 3 Magnetic separation [x] of [x] min left
46	Status	Wash 4 Magnetic separation [x] of [x] min left
47	Status	Rinse [x] of [x] min left

Message number	Message type	EZ1 Advanced XL message text
48	Status	Check Temp. Set: Cur: [x] of [x] min left
49	Status	Elution [x] of [x] min left
50	Guidance	Protocol finished! ENT: Next
51	Status	Transferring report file Attempt no.
52	None	
None	Guidance	SEND REPORT Print out o.k.? 1: o.k. 2: not o.k. ESC:Back
53	Status	Report file sent ENT: Next
54	Status	Report file could not be sent ENT: Resend
55	Guidance	Perform UV run? ENT: Yes ESC: No
56	Guidance	Remove eluates and consumables from the worktable ENT: Next
57	Guidance	UV lamps expire soon UV runs left: ENT: Next
58	Guidance	UV lamps are expired ENT: Next ESC: Abort
59	Guidance	UV decontamination. Enter 20 to 60 ENT: Next
60	Guidance	UV decontamination time must be between 20-60 min ESC: Back

Message number	Message type	EZ1 Advanced XL message text
61	Guidance	UV lamp did not ignite! ESC: Back
62	Guidance	UV decontamination Total time: min Time left: min
63	Status	Decontamination UV lamps cooling Please stand by
64	Guidance	Perform regular maintenance after each run ESC: Main menu

**Table 3. Messages in the EZ1 Advanced DSP DNA Blood protocol (V2.0)**

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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 DNA Blood Version 2.0
2	Data tracking	Enter user ID ENT: Next
3	Data tracking	Enter Q-Card barcode ENT: Next
4	Guidance	Wrong kit! Please load DSP DNA Blood kit ENT: Back
5	Guidance	Kit expired! MMYY: ENT: Use new kit ESC: Stop protocol
6	Data tracking	Use Q-Card data with sample 1 to [X] Enter 1 to 6 ENT: Next
7	Data tracking	Do you want to process more samples with another kit lot ENT: Yes, ESC: No
8	Data tracking	Do you want to add sample IDs? ENT: Yes ESC: No

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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: ENT: Next; Esc: Rescan
13	None	
14	None	
15	None	
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	Guidance	Select protocol 1: 200 µl DSP Blood 2: 350 µl DSP Blood Choose 1 or 2
23	Guidance	Select elution volume: 1: 50 µl 2: 100 µl 3: 200 µl

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
24	Guidance	Pure ethanol wash? 1: No 2: Yes Choose 1 or 2
25	Guidance	You have chosen: [xxx] µl blood, EtOH [xxx] µl elution ENT: Next, ESC: Back
26	Guidance	Load cartridges at same positions as samples ENT: Next, ESC: Back
27	Guidance	Load elution tubes (ET) (1.5 ml) into first row ENT: Next, ESC: Back
28	Guidance	Load tip holders (DTH) and tips (DFT) into second row ENT: Next, ESC: Back
29	Guidance	Load 2 ml tubes with 1800 µl 80% EtOH into third row ENT: Next, ESC: Back
30	Guidance	Load 2 ml tubes (ST) 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
33	Status	Protocol started
34	Status	Piercing foil [x] of [x] min left
35	Status	Collecting Elution Buffer [x] of [x] min left
36	Status	Deliver at heat block [x] of [x] min left
37	Status	Collecting Beads [x] of [x] min left
38	Status	Resuspension of Beads [x] of [x] min left
39	Status	Collecting Lysis Buffer [x] of [x] min left

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
40	Status	Mixing Lysate [x] of [x] min left
41	Status	Collecting Beads [x] of [x] min left
42	Status	DNA binding to Beads Magnetic separation [x] of [x] min left
43	Status	Wash 1 Magnetic separation [x] of [x] min left
44	Status	Wash 2 Magnetic separation [x] of [x] min left
45	Status	Wash 3 Magnetic separation [x] of [x] min left
46	Status	Wash 4 Magnetic separation [x] of [x] min left
47	Status	Rinse [x] of [x] min left
48	Status	Check Temp. Set: Cur: [x] of [x] min left
49	Status	Elution [x] of [x] min left
50	Guidance	Protocol finished! ENT: Next
51	Status	Transferring report file Attempt no.
52	None	
None	Guidance	SEND REPORT Print out o.k.? 1 = o.k. 2 = not o.k. Key: 1, 2, ESC

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
53	Status	Report file sent ENT: Next
54	Status	Report file could not be sent ENT: Resend
55	Guidance	Perform UV run? ENT: Yes ESC: No
56	Guidance	Remove eluates and consumables from the worktable ENT: Next
57	Guidance	UV lamps expire soon UV runs left: ENT: Next
58	Guidance	UV lamps are expired ENT: Next ESC: Abort
59	Guidance	UV decontamination. Enter 20 to 60 ENT: Next
60	Guidance	UV decontamination time must be between 20-60 min ESC: Back
61	Guidance	UV lamp did not ignite! ESC: Back
62	Guidance	UV decontamination Total time: min Time left: min
63	Status	Decontamination UV lamps cooling Please stand by
64	Guidance	Perform regular maintenance after each run ESC: Main menu

**Table 4. Messages in the EZ1 Advanced DSP DNA Blood protocol (V1.0)**

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
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 DNA Blood 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 DNA Blood 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	ID1: ID2: ID3: Next = ENT, ID1-3 = Up
12	Data tracking	Do you want to add assay information? ENT: Yes, ESC: No
13	Data tracking	Scan/enter assay ID sample no. [x]

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
14	Data tracking	Do you want to add notes? ENT: Yes ESC: No
15	Data tracking	Scan/enter notes sample no. [x]
16	Guidance	The protocol use Sample Volume: 350 µl Elution Volume: 200 µl Next = Any
17	Guidance	Load cartridges at same positions as samples Next = Any, Prev = Esc
18	Guidance	Load elution tubes (ET) (1.5 ml) into first row Next = Any, Prev = Esc
19	Guidance	Load tip holders (DTH) and tips (DFT) into second row Next = Any, Prev = Esc
20	Guidance	Leave third row empty Next = Any, Prev = Esc
21	Guidance	Load 2.0 ml tubes (ST) with sample in fourth row Next = Any, Prev = Esc
22	Guidance	Loading finished. Close door and press START Prev = Esc
23	Guidance	Please close door!
24	Status	Protocol started
25	Status	Piercing Foil [x] of 23 min left
26	Status	Collecting Elution Buffer [x] of 23 min left
27	Status	Deliver at Heat Block [x] of 23 min left
28	Status	Collecting Magnetic Beads [x] of 23 min left
29	Status	Resuspension of Magnetic Beads [x] of 23 min left
30	Status	Adding Lysis Buffer [x] of 23 min left

<b>Message number</b>	<b>Message type</b>	<b>EZ1 Advanced message text (V1.0 protocol)</b>
31	Status	Mixing Lysate [x] of 23 min left
32	Status	Adding Magnetic Beads [x] of 23 min left
33	Status	DNA binding to Magnetic Beads Magnetic separation [x] of 23 min left
34	Status	Wash 1 Magnetic separation [x] of 23 min left
35	Status	Wash 2 Magnetic separation [x] of 23 min left
36	Status	Wash 3 Magnetic separation [x] of 23 min left
37	Status	Wash 4 Magnetic separation [x] of 23 min left
38	Status	Rinse [x] of 23 min left
39	Status	Checking Temperature Set: Cur:
40	Status	Elution [x] of 23 min left
41	Guidance	Protocol finished
42	Data tracking	Transfer Report file, attempt no.
43	Guidance	Report file sent Next = ENT
44	Guidance	Report file could not be sent Resend = ENT
45	Guidance	Perform UV run? ENT: Yes ESC: No

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
46	Guidance	UV DECONTAMINATION Set time min Key:0-9, ENT
47	Guidance	UV lamp expires soon UV runs left ENT= continue
48	Guidance	UV lamp is expired ENT=continue ESC=abort
49	Guidance	UV DECONTAMINATION Time must be between 20–60 min Key:ESC
50	Guidance	UV DECONTAMINATION Total Time: min Time left: min
51	Guidance	Decontamination UV lamp cooling Please stand by
52	Guidance	Perform regular maintenance before next run! ESC = Main menu

**Table 5. Messages in the BioRobot EZ1 DSP DNA Blood protocol**

Message number	Message type	BioRobot EZ1 DSP message text
None	Guidance	Choose button: START: Protocols 1 : Tools 2 : Tests
1	Guidance	EZ1 DSP DNA Blood Version 1.0.0
2	Guidance	The protocol uses Sample Volume: [SampleVolume] $\mu$ l Elution Volume: [ElutionVolume] $\mu$ l Next = Any
3	Guidance	Load sufficient cartridges (RCB) for samples Next = Any, Prev=ESC
4	Guidance	Load elution tubes (ET) (1.5ml) into first row Next = Any, Prev = ESC
5	Guidance	Load tip holders (DTH) and tips (DFT) into second row Next = Any, Prev = ESC
6	Guidance	Leave third row Empty Next = Any, Prev = ESC
7	Guidance	Load 2.0ml tubes (ST) with sample in fourth row Next = Any, Prev = ESC
8	Guidance	Start protocol Press START Prev = ESC
9	Status	Protocol started
10	Status	Piercing Foil
11	Status	Collecting Elution Buffer
12	Status	Deliver at Heat Block
13	Status	Collecting Magnetic Beads
14	Status	Resuspension of Magnetic Beads
15	Status	Adding Lysis Buffer
16	Status	Mixing Lysate
17	Status	Adding Magnetic Beads
18	Status	DNA binding to Magnetic Beads Magnetic Separation

<b>Message number</b>	<b>Message type</b>	<b>BioRobot EZ1 DSP message text</b>
19	Status	Wash 1 Magnetic Separation
20	Status	Wash 2 Magnetic Separation
21	Status	Wash 3 Magnetic Separation
22	Status	Wash 4 Magnetic Separation
23	Status	Rinse
24	Status	Checking Temperature Set: 65 [deg] Cur: [deg]
25	Status	Elution
26	Guidance	Protocol finished! Press ESC to return to menu

# Appendix B: Quantification and Determination of Purity of DNA

## Quantification of DNA

The concentration of DNA can be estimated by measuring the absorbance at 260 nm ( $A_{260}$ ) in a spectrophotometer. Use a buffer of neutral pH (e.g. 10 mM Tris-Cl, \* pH 7.0) to dilute the samples and to calibrate the spectrophotometer. Carryover of magnetic particles in the eluate may affect the  $A_{260}$  reading, but should not affect the performance of the DNA in downstream applications. If the purified DNA is to be analyzed by fluorescent capillary sequencing, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

To quantify DNA isolated using the EZ1 DSP DNA Blood system:

- If beads are visible in the eluate, it is recommended to apply the tube containing the DNA to a suitable magnetic separator for 1 minute. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, perform quantification as described above.
- Measure the absorbance at 320 and 260 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

\* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

## Purity of DNA

- ① The preservative contained in the elution buffer may interfere with the measurement. If you require spectrophotometric determination of DNA purity, please contact QIAGEN Technical Services.

# Appendix C: Sample Sheet for Use with the EZ1 DSP DNA Blood System

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

## EZ1 DSP DNA Blood system

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

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

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

Position on worktable	Sample ID	Sample material	RCB loaded?	ST loaded?	ET loaded?	DTH with DFT loaded?	80% EtOH loaded (optional)?
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	RCB loaded?	ST loaded?	ET loaded?	DTH with DFT loaded?	80% EtOH loaded (optional)?
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 DNA Blood Kit (48)	For 48 DNA preps: Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes	62124
EZ1 Advanced XL DSP DNA Blood Card	Preprogrammed card for EZ1 DSP DNA Blood protocol; for use with the EZ1 Advanced XL instrument	9018702
EZ1 Advanced DSP DNA Blood Card	Preprogrammed card for EZ1 DSP DNA Blood protocol; for use with the EZ1 Advanced instrument	9018305
EZ1 DSP DNA Blood Card	Preprogrammed card for EZ1 DSP DNA Blood protocol; for use with the BioRobot EZ1 DSP instrument	9017713
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
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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit Instructions for Use. QIAGEN kit Instructions for Use are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

# Document Revision History

Revision	Description
R1, June 2022	<ul style="list-style-type: none"><li>● New Kit Version V4 according to the new EU regulation 2017/746 (IVDR)</li><li>● Addition of usage of the EZ2 Connect MDx instrument</li><li>● Update of Materials Provided (add active ingredients)</li><li>● Update of Warnings and Precautions</li><li>● Update of Reagent Storage and Handling</li><li>● Addition of section Disposal</li><li>● Update of Troubleshooting guide</li><li>● Update of Appendix B: recommendations for spectrophotometric measurements</li></ul>

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