July 2015

EZ1® DSP DNA Blood Kit Handbook

Σ 48

Version 3

IVD

For in vitro diagnostic use.

CE

REF 62124

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QIAGEN Sample and Assay Technologies

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Kit Contents

EZ1 DSP DNA Blood Kit			**(48)**
Catalog no.			**62124**
Number of preps			**48**
RCB	Reagent Cartridge, Blood 350 µl*	REAG CART BLOOD	48
DTH	Disposable Tip Holders	DISP TIP HOLD	50
DFT	Disposable Filter-Tips	DISP FILT TIP	50
ST	Sample Tubes (2 ml)	SAMP TUBE	50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
	Q-Card †		1
	Handbook	HB	1

* Contains sodium azide as a preservative. Contains a guanidine salt. Not compatible with disinfectants containing bleach. For more information, see page 6.

† The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ1 Advanced or EZ1 Advanced XL instrument.

Symbols

Σ 48	Contains reagents for 48 sample preparations
	To be used by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number
COMP	Components
NUM	Number
VOL	Volume

Temperature limitations

Legal manufacturer

Important note

USE — Only for use with

CONT — Contains

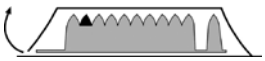
GITC — Guanidine isothiocyanate

GuHCl — Guanidine hydrochloride

EtOH — Ethanol

GTIN — Global Trade Item Number

Open on delivery; store reagent cartridges (RCB) at 2–8°C



This side down when opening

Storage

Store the reagent cartridges (RCB) 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 up 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.

Buffers in the reagent cartridge (RCB) (well 1) may form a precipitate upon storage. Equilibrate the reagent cartridge (RCB) to room temperature and check before use. Redissolve precipitates as described in “Precipitate in reagent cartridge (RCB)”, page 22.

Intended Use

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

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

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

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.

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.

Technical Assistance

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

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

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

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient

and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

Buffers in the reagent cartridges (RCB) contain guanidine hydrochloride/guanidine isothiocyanate, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If liquid containing potentially infectious agents is spilt on EZ1 instruments, disinfect the instrument using reagents described in the user manual supplied with your EZ1 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 kit components, since their use may lead to poor kit performance.

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 highly unlikely, but cannot be excluded completely. Therefore, residual liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

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

Reagent Cartridge Blood

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

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.

Introduction

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 or other enzymatic reactions. The EZ1 instrument performs all steps of the sample preparation procedure for up to 6 samples (using the EZ1 Advanced or the BioRobot® EZ1 DSP) or for up to 14 samples (using the EZ1 Advanced XL) in a single run.

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

Principle and procedure

Magnetic particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart, page 10). 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.

Performance characteristics of the EZ1 DSP DNA Blood system

System robustness

Various different primary tubes and anticoagulants can be used to collect blood samples for the EZ1 DSP DNA Blood procedure. Table 1 (page 11) provides an overview of the sample collection tubes that have been used for evaluation of the system. These tubes were selected in order to cover a range of different anticoagulants and manufacturers of blood collection tubes. Tubes from other manufacturers may also be used.

The average relative yields of DNA from blood samples using different primary tubes are shown in Figure 1 (page 12).

EZ1 DSP DNA Blood Procedure

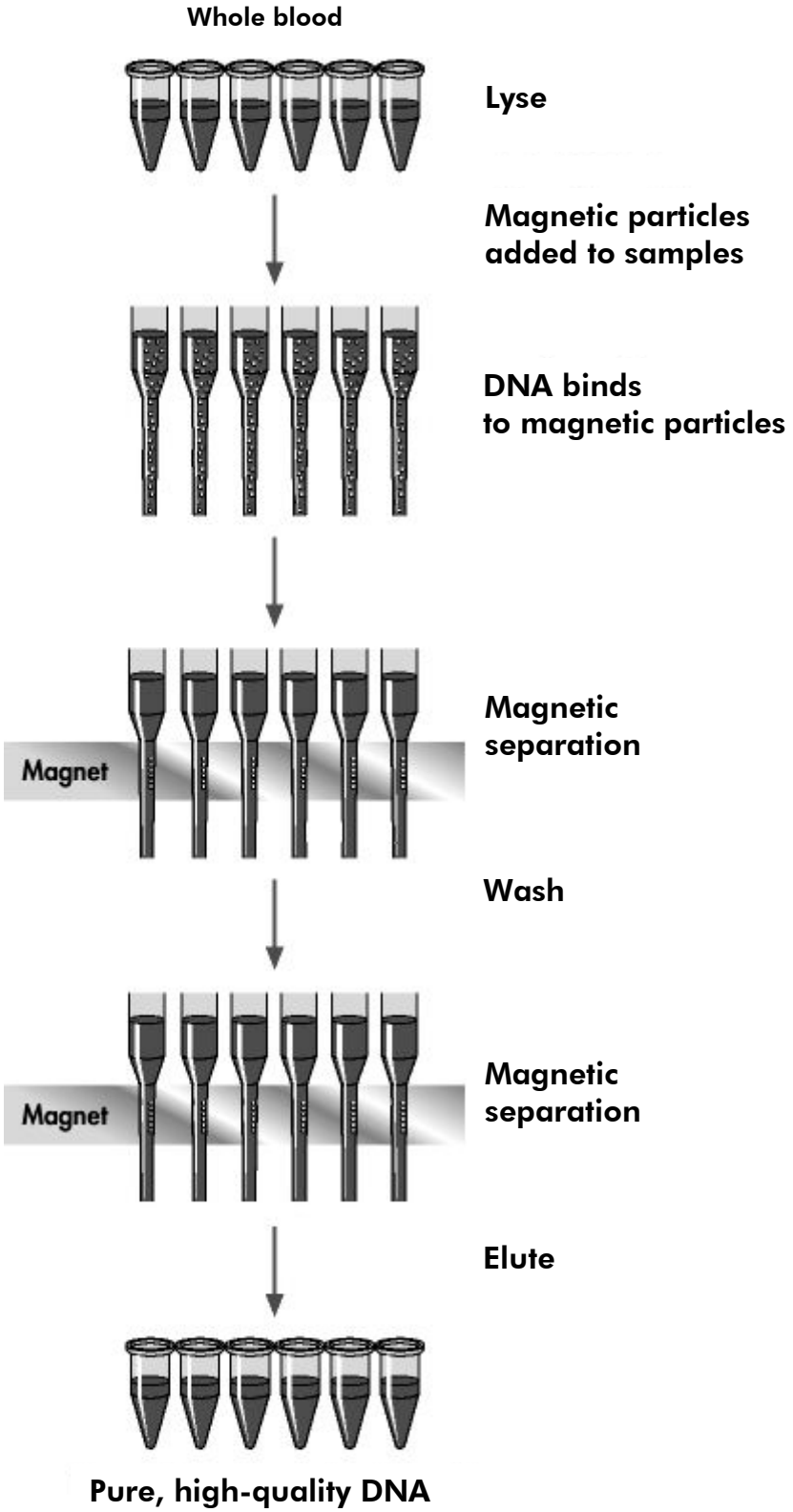


Table 1. Blood collection tubes tested with the EZ1 DSP DNA Blood system

Tube	Abbreviation	Manufacturer	Cat. no.*	Nominal draw volume (ml)
BD Vacutainer® 9NC	BD 9NC	Becton Dickinson	366007	9
BD Vacutainer K3E	BD K3E	Becton Dickinson	368457	10
BD Vacutainer K2E	BD K2E	Becton Dickinson	367864	6
Monovette® EDTA	EDTA	Sarstedt	21.066.001	9
Monovette LH	LH	Sarstedt	21.065.001	9
Monovette CDPA1	CPDA1	Sarstedt	11.610.001	8.5
Vacuette® K3E	K3E	Greiner Bio-One	455036	9
Vacuette 9NC	V 9NC	Greiner Bio-One	454382	9

* Catalog numbers are subject to change; please check with the manufacturer or supplier.

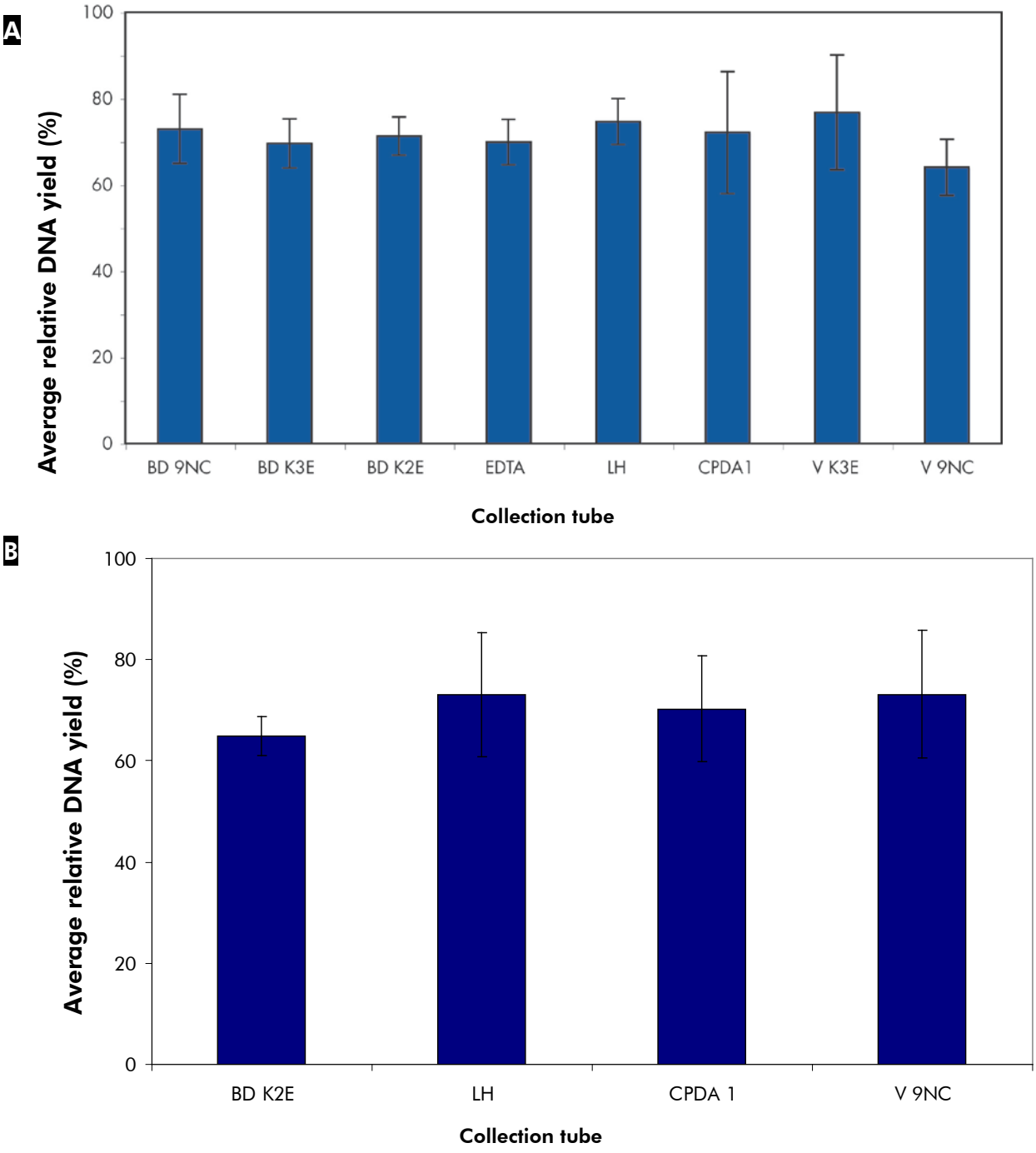


Figure 1. System robustness using different collection tubes and anticoagulants. Whole blood was collected from healthy donors in different types of tubes with replicates of 3 per donor and tube. The tubes used are listed in Table 1 (page 11). **A** Blood was collected from 6 donors in 8 different types of tubes. Genomic DNA was purified from 350 µl samples, with elution in 200 µl. **B** Blood was collected from 4 donors in 4 different types of tubes. Genomic DNA was purified from 200 µl samples using the EZ1 DSP DNA Blood system on the EZ1 Advanced XL, with elution in 200 µl. Theoretical DNA yields from each donor and tube were determined by white blood cell counts. The bars show the mean relative DNA yield (in comparison with the theoretical yield) with standard deviation.

Freeze–thawing of samples

Fresh or frozen human whole blood samples can be used (see "Storage of blood samples", page 22). The effects of freezing and thawing blood samples on DNA purification using the EZ1 DSP DNA Blood system has been determined (Figure 2).

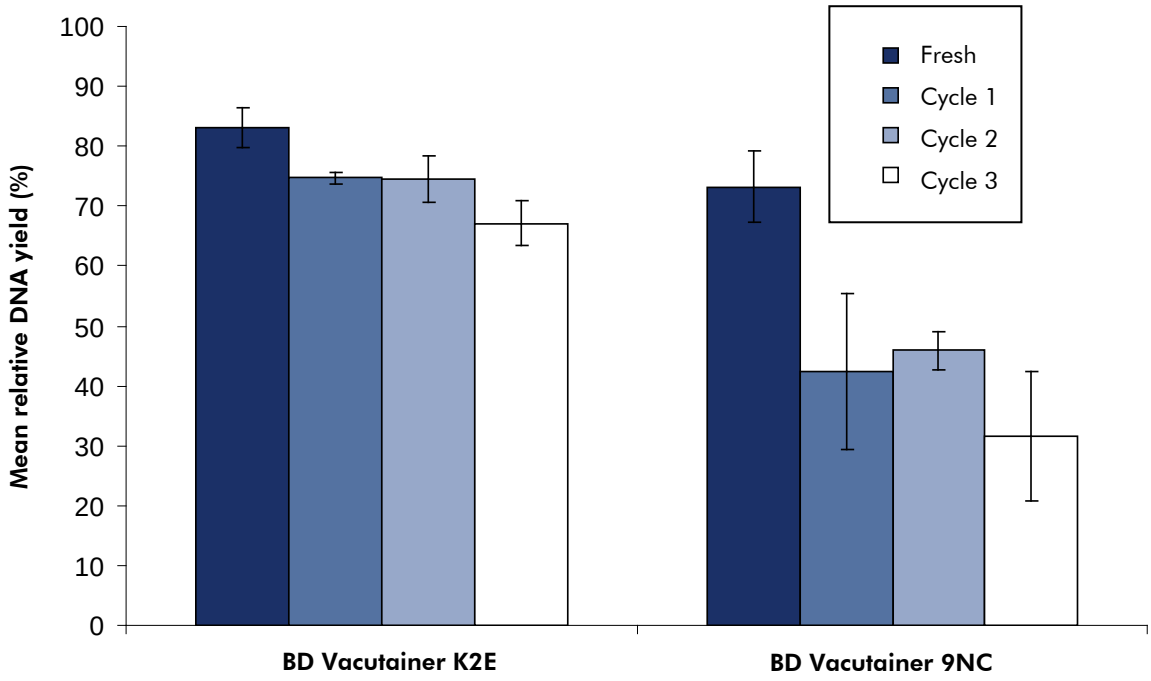


Figure 2. Influence of freeze–thaw cycles on DNA yields. Whole blood was collected from 3 healthy donors in the indicated tubes with 6 replicates each. The tubes used are listed in Table 1. Genomic DNA was purified from 350 µl of each sample using the EZ1 DSP DNA Blood system, and mean values of relative DNA yield (**Fresh**) was calculated for each donor and tube. The tubes containing the blood were frozen and thawed 3 times. Genomic DNA was purified after each freeze–thaw cycle (**Cycle 1 – Cycle 3**) using the EZ1 DSP DNA Blood system, and the relative DNA yield was determined. For freeze–thawing, tubes with EDTA as an anticoagulant are recommended.

Yield of purified DNA

Genomic DNA was purified from 350 µl blood samples from healthy donors. The amount of DNA purified using the EZ1 DSP DNA Blood procedure depends on the white blood cell content of each blood sample, and yields can vary from donor to donor (Figure 3).

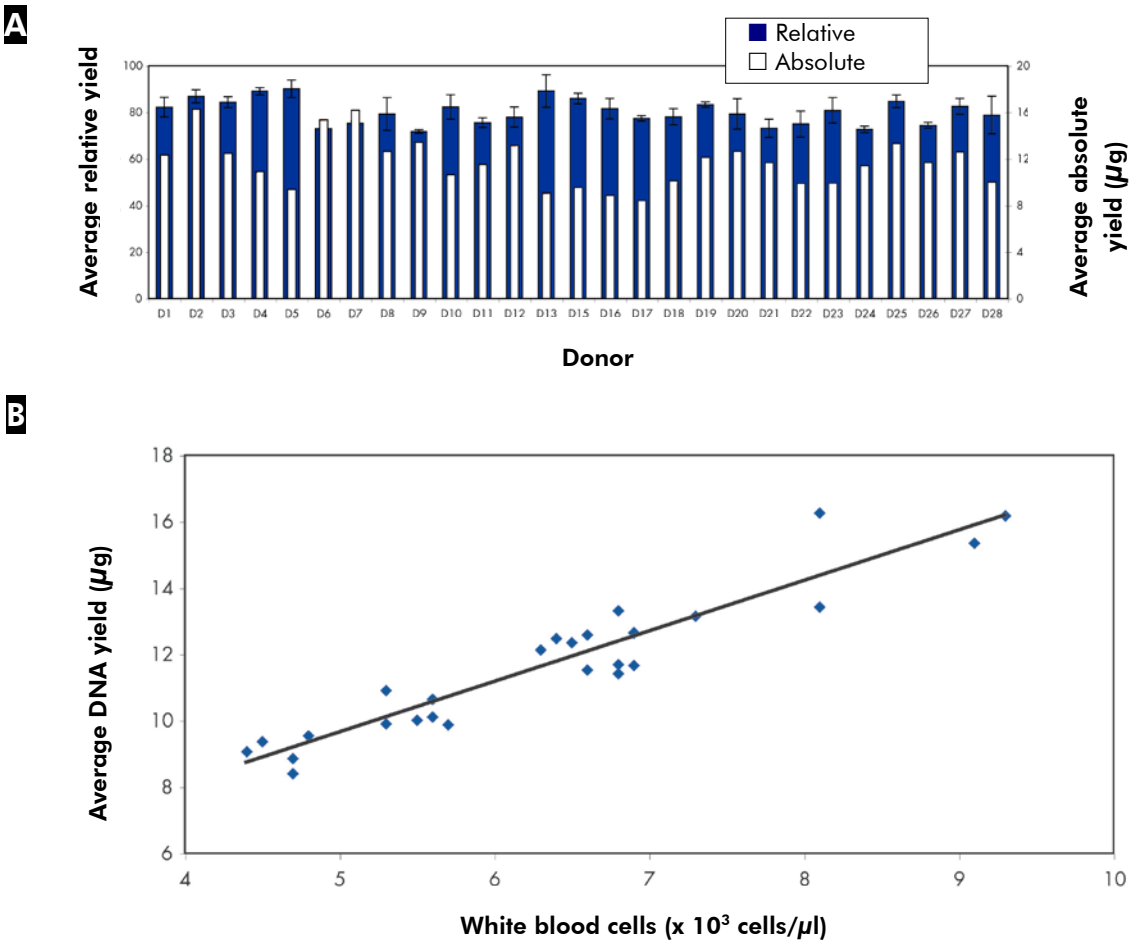


Figure 3. Average absolute and relative DNA yields from different donors. Whole blood was collected from 27 donors in triplicate. Genomic DNA was purified from 350 µl of each sample using the EZ1 DSP DNA Blood system. **A** Theoretical DNA yield was determined by white blood cell counts. Mean absolute (**Absolute**) and relative (**Relative**) (in comparison with calculated theoretical yield) DNA yields are shown for each donor. **B** Mean absolute yields are shown for each donor in relation to white blood cell counts.

Concentration of purified DNA using different elution volumes

Genomic DNA was purified from 250 µl and 350 µl blood samples from healthy donors using the EZ1 DSP DNA Blood procedure on the EZ1 Advanced XL with three different elution volumes (Figure 4).

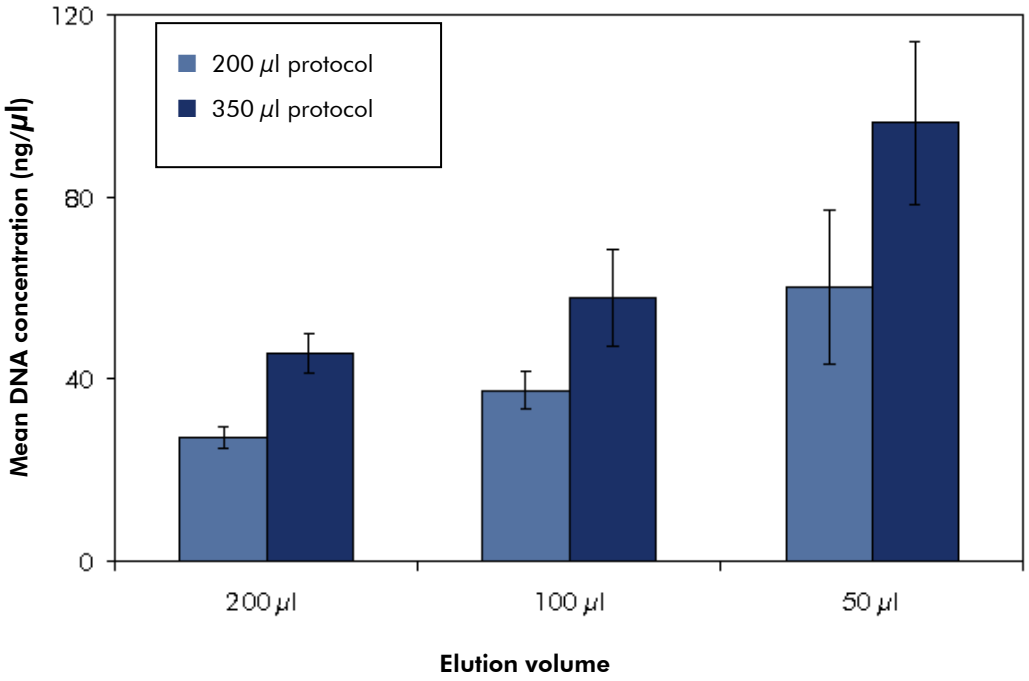


Figure 4. Mean DNA concentration obtained with different elution volumes. Whole blood was collected from 3 donors. Genomic DNA was purified from 200 µl and 350 µl of each sample and eluted in 200 µl, 100 µl, and 50 µl, each in triplicate, using the EZ1 DSP DNA Blood system on the EZ1 Advanced XL. Mean DNA concentration is shown for each protocol and elution volume.

ⓘ Due to the low elution buffer volume and heating of the elution buffer during the process, elution with 50 µl may lead to final eluate volumes less than 50 µl.

Inhibition test

The effects of increasing amounts of eluate used in PCR on PCR performance have been determined (Figure 5).

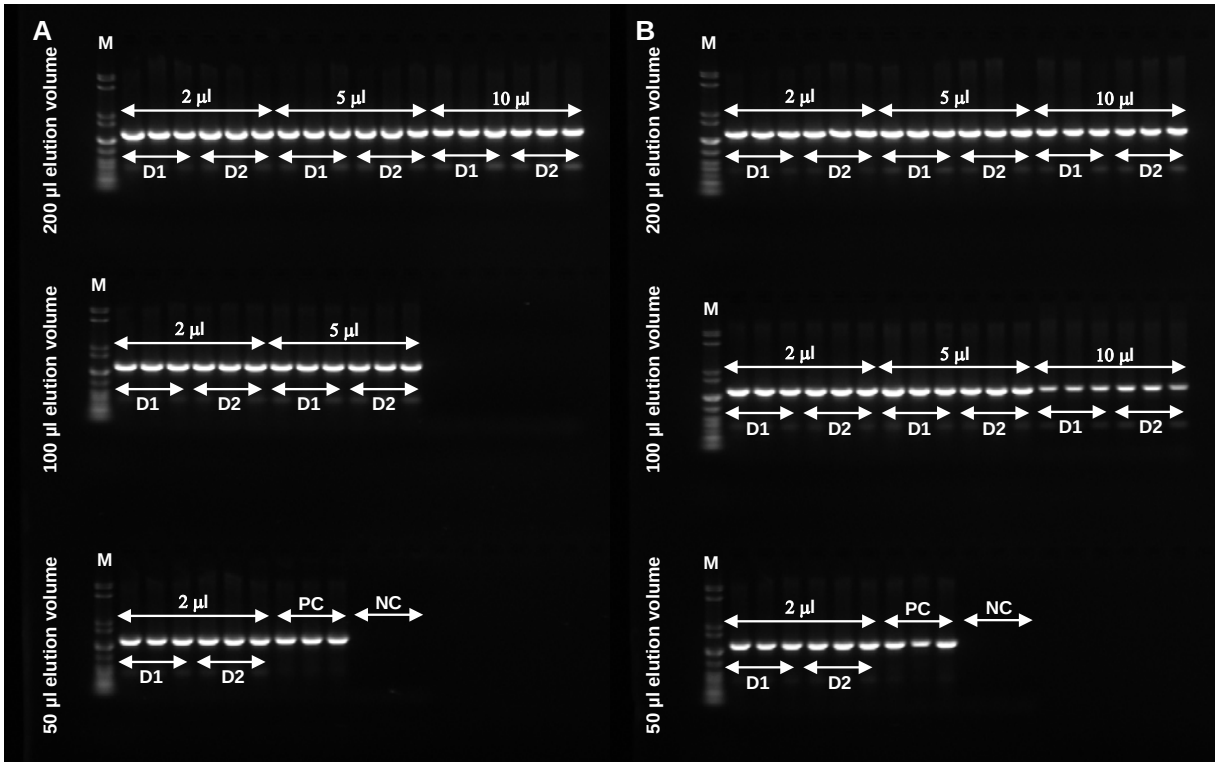


Figure 5. Effects of eluate volume used in PCR on PCR performance. Blood was collected from two healthy donors (**D1**, **D2**) in BD K2E tubes. Genomic DNA was purified from 350 µl (**A**) and 200 µl (**B**) aliquots in triplicate using the EZ1 DSP DNA Blood system. DNA was eluted in 200 µl, 100 µl, or 50 µl (**elution volume**). The indicated amount of eluate was used in a 50 µl PCR with primers for a 1100 bp single-copy human gene fragment. **PC**: Positive control. **NC**: Negative control. **M**: Low DNA mass ladder. (Note that using large amounts of high concentrations of DNA can cause overloading of the PCR, as shown, for example, by the weaker bands when using 10 µl of a 100 µl elution in the PCR.)

Precision analysis

DNA yields from 350 µl human whole blood were compared for different runs using the EZ1 DSP DNA Blood system on the EZ1 Advanced and the EZ1 Advanced XL. The inter-run precision data are shown as standard deviations of the DNA yields (Figure 6).

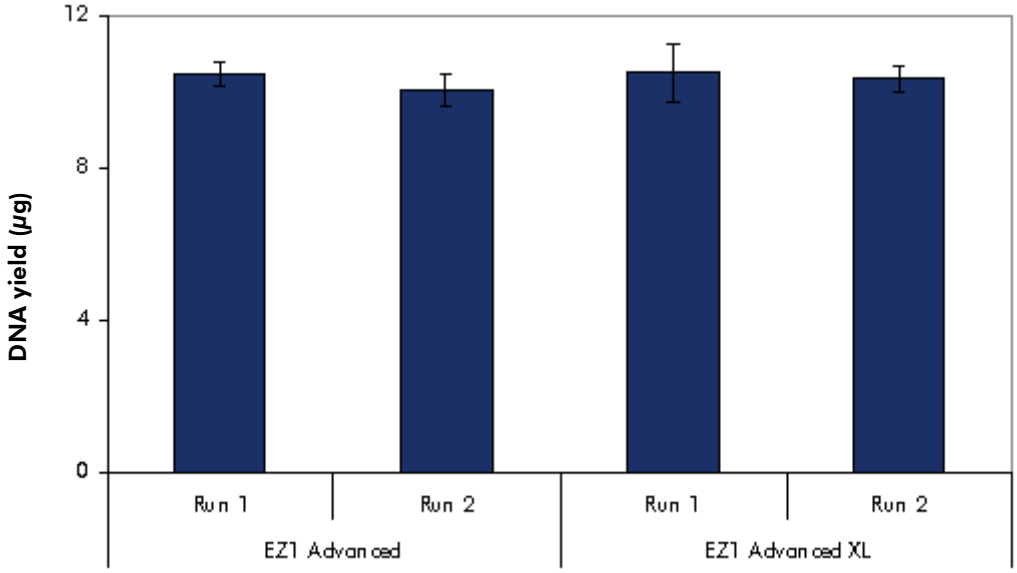


Figure 6. Intra- and inter-run precision using the EZ1 DSP DNA Blood system. Blood was collected from a healthy donor in BD K2E tubes and pooled before use. Genomic DNA was purified from twelve 350 µl aliquots in 2 runs (**Run 1**, **Run 2**) of 6 replicates each on the EZ1 Advanced, and from twenty-eight 350 µl aliquots in 2 runs (**Run 1**, **Run 2**) of 14 replicates each on the EZ1 Advanced XL using the EZ1 DSP DNA Blood system. Mean total DNA yield and standard deviation are shown for each run. Intra-run precision values were 2.90% (Run 1, EZ1 Advanced), 3.80% (Run 2, EZ1 Advanced), 7.17% (Run 1, EZ1 Advanced XL), and 3.45% (Run 2 EZ1 Advanced XL), and total precision was 5.17%.

Eluate stability

Stability of genomic DNA in EZ1 eluates has been demonstrated for 24 months when stored at 5°C and for 36 months when stored at –20°C or –80°C.

Exclusion of sample carryover

Twelve runs using the EZ1 Advanced (with the V2.0 protocol card; 350 µl input, 200 µl elution) and nine runs using the EZ1 Advanced XL (200 µl input, 200 µl elution) were performed with the EZ1 DSP DNA Blood system to evaluate the risk of cross-contamination events during and between EZ1 DSP DNA Blood procedures. To detect sample-to-sample carryover, the runs were performed with male (positive) and female (negative) blood samples in alternating positions, as shown in Table 2 and Table 3. Every third run was performed using only female blood samples. All eluates were tested for amplification of a 78 bp fragment of the Y-chromosome specific single-copy gene SRY using the QIAGEN QuantiTect® Probe PCR Kit.

Table 2. EZ1 Advanced cross-contamination test setup and C_T values for positive (male) samples

	Position					
Run	**1**	**2**	**3**	**4**	**5**	**6**
1	23.37	F	23.14	F	23.22	F
2	F	23.41	F	23.15	F	23.44
3	F	F	F	F	F	F
4	23.53	F	23.27	F	23.39	F
5	F	23.28	F	23.39	F	23.46
6	F	F	F	F	F	F
7	23.14	F	23.50	F	23.17	F
8	F	23.21	F	23.46	F	23.44
9	F	F	F	F	F	F
10	23.29	F	23.45	F	23.47	F
11	F	23.53	F	23.39	F	23.42
12	F	F	F	F	F	F

F: Female (negative) samples.

Numbers: C_T values for male (positive) samples.

Table 3. EZ1 Advanced XL cross-contamination test setup and C_T values for positive (male) samples

	Position						
Run	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	24.27	F	24.13	F	24.12	F	24.22
2	F	23.92	F	24.12	F	23.85	F
3	F	F	F	F	F	F	F
4	24.02	F	23.98	F	24.31	F	24.35
5	F	24.74	F	24.56	F	24.62	F
6	F	F	F	F	F	F	F
7	24.48	F	24.64	F	24.49	F	24.52
8	F	24.55	F	24.40	F	24.52	F
9	F	24.80	F	24.70	F	24.68	F
	Position						
	8	**9**	**10**	**11**	**12**	**13**	**14**
1	F	23.99	F	24.16	F	24.18	F
2	24.06	F	24.11	F	23.94	F	24.02
3	F	F	F	F	F	F	F
4	F	24.22	F	24.30	F	24.10	F
5	24.64	F	24.28	F	24.59	F	24.53
6	F	F	F	F	F	F	F
7	F	24.62	F	24.41	F	24.66	F
8	24.37	F	24.46	F	24.58	F	24.46
9	24.74	F	24.52	F	24.80	F	24.67

F: Female (negative) samples.

Numbers: C_T values for male (positive) samples.

All of the male blood samples tested positive in PCR (C_T values are listed in Table 2 and Table 3), and all female blood samples tested negative. These experiments demonstrate that the EZ1 DSP DNA Blood procedure provides no sample carryover under these conditions.

Equipment and Reagents to Be Supplied by User

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

All protocols

- Pipets* and sterile, RNase-free pipet tips
- Soft paper tissue
- Water
- 70% ethanol
- Optional: shaker–incubator* (if reagent cartridges [RCB] contain precipitates at bottom of wells)
- Optional: microcentrifuge* (if magnetic particles need to be removed from eluates)

For BioRobot EZ1 users

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

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)

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

For EZ1 Advanced and EZ1 Advanced XL users

- For sample tracking, one of the following is required:
  - PC (including monitor; QIAGEN PC, cat. no. 9016310, and monitor, cat. no. 9016308, or your own PC and monitor) with EZ1 Advanced Communicator Software (software supplied with EZ1 Advanced and EZ1 Advanced XL instruments)
  - Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)
- Optional: 80% ethanol* and 2 ml screw-capped tubes (if performing the optional 80% ethanol wash steps on the EZ1 Advanced using the V2.0 protocol card or on the EZ1 Advanced XL, see "Things to do before starting", pages 29 and 33)

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

Important Notes

Storage of blood samples

Whole blood samples treated with EDTA, ACD, 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 depend on storage conditions of the blood. Fresher blood samples may yield better results.

- For short-term storage (up to 10 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 –70°C.
- Do not use blood that shows signs of coagulation.

Precipitate in reagent cartridge (RCB)

The buffer in well 1 of the reagent cartridge (RCB) (the well that is nearest to the front of the EZ1 instrument when the reagent cartridge (RCB) is loaded) may form a precipitate upon storage. Before use, equilibrate the reagent cartridge (RCB) to room temperature. If necessary, redissolve by mild agitation at 30–40°C.

Working with EZ1 instruments

The main features of EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 or 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 DSP Cards containing ready-to-use protocols
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup
- Complete automation of nucleic acid purification

* 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.

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

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

ⓘ UV decontamination helps to reduce possible pathogen contamination of the EZ1 Advanced and EZ1 Advanced XL worktable surfaces. The efficiency of inactivation has to be determined for each specific organism and depends, for example, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

EZ1 Cards

The EZ1 DSP DNA Blood protocol is stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts the EZ1 Card into the appropriate EZ1 instrument, and the instrument is then ready to run a protocol (Figures 7 and 8).

Figure 7. Ease of protocol setup using EZ1 DSP Cards. Inserting an EZ1 Card, containing protocol, into the EZ1 instrument.

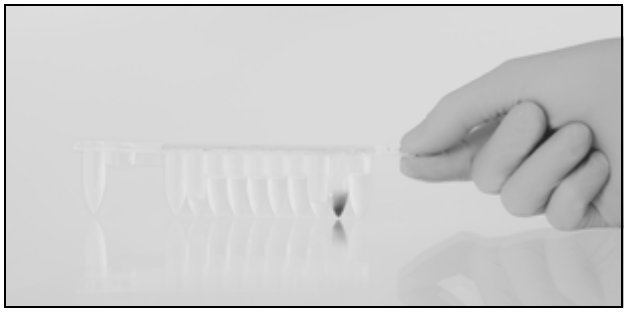
ⓘ The instrument should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted! Otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

Figure 8. 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 9, page 25). Each well of the cartridge (RCB) contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer (AVE). Since each well contains only the required amount of reagent, generation of additional waste due to leftover reagent at the end of the purification procedure is avoided.

A



B

Figure 9. Ease of instrument setup using reagent cartridges (RCB). A A sealed, prefilled reagent cartridge (RCB) of the EZ1 DSP DNA Blood Kit. B Loading reagent cartridges (RCB) into the 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, page 26).

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.

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

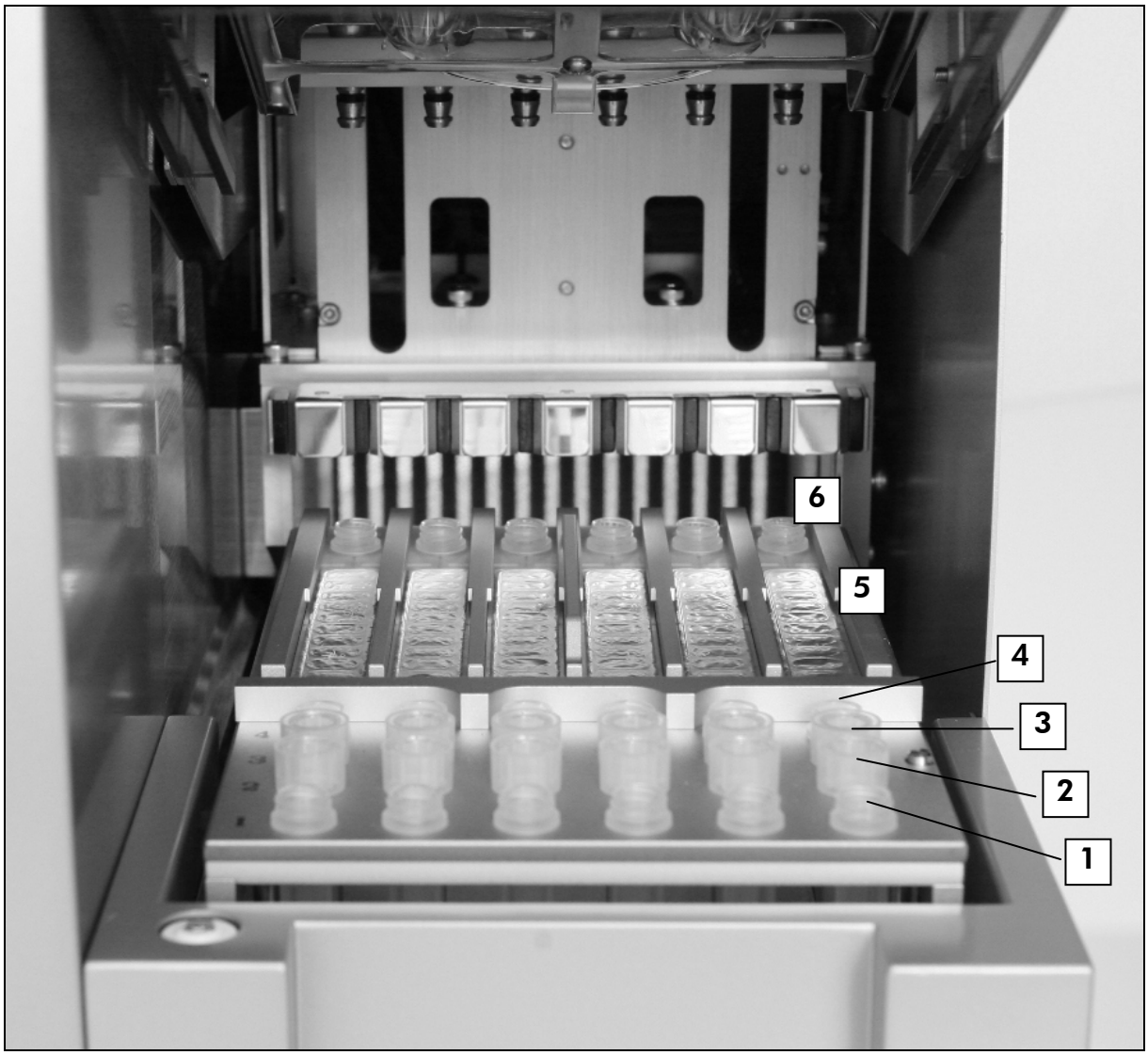


Figure 10. Worktable of an EZ1 instrument.

1. Elution tubes (ET) (1.5 ml) loaded into the first row.
2. Disposable tip holders (DTH) containing disposable filter-tips (DFT) loaded into the second row.
3. The third row is empty for the EZ1 DSP DNA Blood protocol. (Optional: If performing the optional 80% ethanol wash steps, the 2 ml tubes containing 1800 µl each of 80% ethanol are loaded into this row.)
4. Sample tubes (ST) (2 ml) loaded into the fourth row.
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 complete 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 bar code. A user ID and the Q-Card bar code can be entered manually via the

keypad or by scanning bar codes using the handheld bar code reader. Sample and assay information can also be optionally entered at the start of the protocol. At the end of 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 ordering information, see "Equipment and Reagents to Be Supplied by User" on page 20).

To receive report files on a PC, the EZ1 Advanced Communicator software needs to be installed. The software receives the report file and stores it in a folder that you define. After the PC has received the report file, you can use and process the file with a LIMS (Laboratory Information Management System) or other programs. An example of the report file is shown in Appendix D (page 68). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A to F, or the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

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

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

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

Workflow of EZ1 DSP DNA Blood operation

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 *

* EZ1 Advanced and EZ1 Advanced XL only.

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 "Important Notes" on page 22.
- 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 6 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 6). Do not use damaged reagent cartridges (RCB) or other kit components, since their use may lead to poor kit performance.
- The yield of genomic DNA depends on the number of white blood cells in the sample.

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. If necessary, redissolve by warming at 30–40°C and then place at room temperature.
- The protocol includes an option to perform washes with 80% ethanol instead with the buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, 2 ml tubes containing 1800 µl each of 80% ethanol should be placed in row 3 of the worktable (see Figure 10, page 26). For preparation of 80% ethanol sufficient for 14 samples, add 6 ml nuclease-free water to 24 ml 100% ethanol.* Follow the instructions given in the onscreen messages.

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

Procedure

1. **Equilibrate up to 14 whole blood samples at room temperature.**

   ⓘ 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.

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.**
6. **Press "1" or "2" to start worktable setup for the 200 µl DSP Protocol or 350 µl Protocol, respectively.**
7. **Choose the elution volume: press "1" to elute in 50 µl; "2" to elute in 100 µl; "3" to elute in 200 µl.**
8. **Choose 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 1–14 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.**
11. **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.

    ⓘ 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.**
13. **Close the instrument door.**
14. **Press "START" to start the protocol.**

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

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

16. **Open the instrument door.**
17. **Remove the elution tubes containing the purified DNA from the first row. Discard the sample-preparation waste.***
18. **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. **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.

20. **To run another protocol, press "START", carry out steps 1 and 2 of the protocol, and then follow the protocol from step 5. Otherwise press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

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

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

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)", page 35.

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 "Important Notes" on page 22.
- 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 6 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 6). Do not use damaged reagent cartridges (RCB) or other kit components, since their use may lead to poor kit performance.
- The yield of genomic DNA depends on the number of white blood cells in the sample.

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. If necessary, redissolve by warming at 30–40°C and then place at room temperature.
- The protocol includes an option to perform washes with 80% ethanol instead with the buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, 2 ml tubes containing 1800 µl each of 80% ethanol should be placed in row 3 of the worktable (see Figure 10, page 26). For preparation of 80% ethanol sufficient for 6 samples, add 3 ml nuclease-free water to 12 ml 100% ethanol.* Follow the instructions given in the onscreen messages.

Procedure

1. **Equilibrate up to 6 whole blood samples at room temperature.**

   ⓘ 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.

2. **Insert the EZ1 Advanced DSP DNA Blood Card (V2.0) 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 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.**
6. **Press "1" or "2" to start worktable setup for the 200 µl DSP Protocol or 350 µl Protocol, respectively.**
7. **Choose the elution volume: press "1" to elute in 50 µl; "2" to elute in 100 µl; "3" to elute in 200 µl.**
8. **Choose 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.**

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

10. **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.**
11. **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.

    ⓘ 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.
12. **Follow the onscreen instructions for further worktable setup.**
13. **Close the instrument door.**
14. **Press "START" to start the protocol.**
15. **When the protocol ends, the display shows "Protocol finished". Press "ENT" to generate the report file.**

    The EZ1 Advanced can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
16. **Open the instrument door.**
17. **Remove the elution tubes containing the purified DNA from the first row. Discard the sample-preparation waste.***
18. **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. **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.
20. **To run another protocol, press "START", carry out steps 1 and 2 of the protocol, and then follow the protocol from step 5. Otherwise press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

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

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

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 32.

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 "Important Notes" on page 22.
- 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 6 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 6). Do not use damaged reagent cartridges (RCB) or other kit components, since their use may lead to poor kit performance.
- The yield of genomic DNA depends on the number of white blood cells in the sample.

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. If necessary, redissolve by warming at 30–40°C and then place at room temperature.

Procedure

1. **Equilibrate up to 6 whole blood samples at room temperature.**

   ⓘ 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.

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.

   ⓘ 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.

8. **Close the instrument door.**
9. **Press "START" to start the protocol.**
10. **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.

11. **Open the instrument door.**

12. **Remove the elution tubes containing the purified DNA from the first row. Discard the sample-preparation waste.***
13. **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
14. **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.
15. **To run another protocol, press "START", carry out steps 1 and 2 of the protocol, and then follow the protocol from step 5. Otherwise press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

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

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

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 "Important Notes" on page 22.
- 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 6 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 6). Do not use damaged reagent cartridges (RCB) or other kit components, since their use may lead to poor kit performance.
- The yield of genomic DNA depends on the number of white blood cells in the sample.

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. If necessary, redissolve by warming at 30–40°C and then place at room temperature.

Procedure

1. **Equilibrate up to 6 whole blood samples at room temperature.**

   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.

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 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 and protocol variable selection.**

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

   ⓘ 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.
8. **Close the instrument door.**
9. **Press "START" to start the protocol.**

   When the protocol ends, the display shows "Protocol finished".
10. **Open the instrument door.**
11. **Remove the elution tubes containing the purified DNA from the first row. Discard the sample-preparation waste.***
12. **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.
13. **To run another protocol, press "START", carry out steps 1 and 2 of the protocol, and then follow the protocol from step 5. Otherwise press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

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

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

Troubleshooting Guide

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

	Comments and suggestions
General handling	
Error message in instrument display	ⓘ Refer to the user manual supplied with your EZ1 instrument.
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) Insufficient reagent aspirated	ⓘ After inverting the reagent cartridges (RCB) to resuspend the magnetic particles, ensure that you tap the cartridges (RCB) to deposit the reagents at the bottom of the wells.
c) Frozen blood samples not mixed properly after thawing	ⓘ Thaw frozen blood samples in an incubator* or water bath* at 30–40°C with mild agitation to ensure thorough mixing.
d) Precipitates visible at the bottom of the wells of the reagent cartridges (RCB)	ⓘ Place the reagent cartridges (RCB) into a shaker–incubator, and incubate at 30–40°C with mild agitation for up to 2 hours. Do not use the reagent cartridges (RCB) if the precipitates do not redissolve.

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

	Comments and suggestions
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 65).
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 65).
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 32) or the EZ1 Advanced XL DSP DNA Blood Card (see page 29).
Low A_{260}/A_{280} ratio for purified nucleic acids	
Absorbance reading at 320 nm not subtracted from the absorbance readings obtained at 260 nm 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 nm and 280 nm

Appendix A: Display Messages

The messages displayed by the software protocol during worktable setup, during the protocol run, and after the protocol run are listed in Tables 6–9. The numbers of the messages listed in the tables correspond to the numbers of the messages displayed by the software.

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

Table 6. 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 bar code 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 14 ENT: Next

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
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

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
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
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: 200ul DSP Blood 2: 350ul DSP Blood Choose 1 or 2

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
23	Guidance	Select elution volume: 1: 50ul 2: 100ul 3: 200ul
24	Guidance	Pure ethanol wash? 1: No 2: Yes Choose 1 or 2
25	Guidance	You have chosen: [xxx]ul blood, EtOH [xxx]ul elution ENT: Next, ESC: Back
26	Guidance	Load cartridges at same positions as samples ENT: Next, ESC: Back
27	Guidance	Load elution tubes (ET) (1.5ml) 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 2ml tubes with 1800ul 80% EtOH into third row ENT: Next, ESC: Back
30	Guidance	Load 2ml tubes (ST) with sample into fourth row ENT: Next, ESC: Back

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
31	Guidance	Loading finished Close door and press START ESC: Back
32	Guidance	Please close door! ENT: Next
33	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

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
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

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
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 decontamina- tion. Enter 20 to 60 ENT: Next

Table continued on next page.

Table 6. Continued

Message number	Message type	EZ1 Advanced XL message text
60	Guidance	UV decontami- nation 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 7. 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 bar code 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

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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: 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

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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: 200ul DSP Blood 2: 350ul DSP Blood Choose 1 or 2
23	Guidance	Select elution volume: 1: 50ul 2: 100ul 3: 200ul
24	Guidance	Pure ethanol wash? 1: No 2: Yes Choose 1 or 2
25	Guidance	You have chosen: [xxx]ul blood, EtOH [xxx]ul elution ENT: Next, ESC: Back

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
26	Guidance	Load cartridges at same positions as samples ENT: Next, ESC: Back
27	Guidance	Load elution tubes (ET) (1.5ml) 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 2ml tubes with 1800ul 80% EtOH into third row ENT: Next, ESC: Back
30	Guidance	Load 2ml 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

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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
53	Status	Report file sent ENT: Next
54	Status	Report file could not be sent ENT: Resend

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
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 decontamina- tion. Enter 20 to 60 ENT: Next
60	Guidance	UV decontami- nation 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

Table continued on next page.

Table 7. Continued

Message number	Message type	EZ1 Advanced message text (V2.0 protocol)
63	Status	Decontamination UV lamps cooling Please stand by
64	Guidance	Perform regular maintenance after each run ESC: Main menu

Table 8. 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 bar code
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]

Table continued on next page.

Table 8. Continued

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
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]
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: 350ul Elution Volume: 200ul Next=Any
17	Guidance	Load cartridges at same positions as samples Next=Any, Prev=Esc
18	Guidance	Load elution tubes (ET) (1.5ml) 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

Table continued on next page.

Table 8. Continued

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
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
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

Table continued on next page.

Table 8. Continued

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
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

Table continued on next page.

Table 8. Continued

Message number	Message type	EZ1 Advanced message text (V1.0 protocol)
45	Guidance	Perform UV run? ENT: Yes ESC: No
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 9. 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]ul Elution Volume: [ElutionVolume]ul 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

Table continued on next page.

Table 9. Continued

Message number	Message type	BioRobot EZ1 DSP message text
15	Status	Adding Lysis Buffer
16	Status	Mixing Lysate
17	Status	Adding Magnetic Beads
18	Status	DNA binding to Magnetic Beads Magnetic Separation
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: Storage, Quantification, and Determination of Purity of DNA

Storage of DNA

Purified DNA may be stored at 2–8°C or at –20°C for up to 24 months. For extended archiving, eluates should be stored at –70°C.

Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 µg of DNA per milliliter (A_{260}=1 → 50 µg/ml). Use 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 system:

- Apply the tube containing the DNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) 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, carefully withdraw 10–50 µl of isolated DNA and dilute to a final volume of 100 µl in buffer of neutral pH.
- Measure the absorbance at 320 nm 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.

Concentration of DNA sample = 50 µg/ml x (A_{260} – A_{320}) x dilution factor

Total amount of DNA isolated = concentration x volume of sample in milliliters

* 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.

† If the samples are not diluted, use water to calibrate the spectrophotometer.

Purity of DNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm, i.e., $(A_{260} – A_{320})/(A_{280} – A_{320})$. Pure DNA has an $A_{260} – A_{280}$ ratio of 1.7–1.9. Use buffer of slightly alkaline pH (e.g., 10 mM Tris·Cl, pH 7.5) to dilute the samples and to calibrate the spectrophotometer.* If the samples are not diluted, use water to calibrate the spectrophotometer.

* 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.

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 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:** _____

Instrument serial number: _____

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

Appendix D: Example of an EZ1 Advanced Report File

This appendix shows a typical report file generated on the EZ1 Advanced. The values for each parameter will differ from the report file generated on your EZ1 Advanced. Report files generated on the EZ1 Advanced XL show equivalent information and differ only in the channel number.

Report File EZ1 Advanced:
Serial No. EZ1 Advanced:,"6987"
User ID:,"555"
Firmware version:,"V 1.0.0"
Installation date of instrument:,"Oct 05, 2007"
Weekly maintenance done on:,"Jly 29, 2009"
Yearly maintenance done on:,"Mar 24, 2009"
Date of last UV-run:,"Mar 31, 2009"
Start of last UV-run:,"10:59"
End of last UV-run:,"10:59"
Status of last UV-run:,"o.k."

Protocol name:,"DSP DNA Blood Version 2.0"
,"DSP DNA Blood 350"

Date of run:,"Aug 05, 2009"
Start of run:,"07:58"
End of run:,"08:28"
Status run:,"o.k"
Error Code:,"---"
Sample input Volume [ul]:," 350"
Elution volume [ul]:," 200"

Channel A:
Sample ID:,"1"
Reagent Kit number:,"9900801"
Reagent Lot number:,"0133203571"
Reagent Expiry date:,"1209"
Assay Kit ID:,"1"
Note:,"1"

Channel B:
Sample ID:,"2"
Reagent Kit number:,"9900801"
Reagent Lot number:,"0133203571"
Reagent Expiry date:,"1209"
Assay Kit ID:,"2"
Note:,"2"

Channel C:
Sample ID:,"3"
Reagent Kit number:,"9900801"
Reagent Lot number:,"0133203571"
Reagent Expiry date:,"1209"
Assay Kit ID:,"3"
Note:,"3"

Channel D:
Sample ID:,"4"
Reagent Kit number:,"9900801"
Reagent Lot number:,"0133203571"
Reagent Expiry date:,"1209"
Assay Kit ID:,"4"
Note:,"4"

Channel E:
Sample ID:,"5"
Reagent Kit number:,"9900801"
Reagent Lot number:,"0133203571"
Reagent Expiry date:,"1209"
Assay Kit ID:,"5"
Note:,"5"

Channel F:
Sample ID:,"6"
Reagent Kit number:,"9900801"
Reagent Lot number:,"0133203571"
Reagent Expiry date:,"1209"
Assay Kit ID:,"6"
Note:,"6"

[Checksum A0C47444]

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

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