June 2022

EZ1® DSP DNA Blood Kit
Instructions for Use (Performance Characteristics)

Version 4

IVD

For In Vitro Diagnostic Use
For use with EZ1 DSP DNA Blood Kit (48)

CE

REF 62124

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

R1 Performance Characteristics available electronically and can be found under the resource tab of the product page on **www.qiagen.com**.

Sample to Insight

General Introduction

The EZ1 DSP DNA Blood Kit is intended 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 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 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, or using the EZ2 Connect MDx, the sample input volume can be chosen from 200 or 350 µl, and the DNA elution volume can be chosen from 50, 100, or 200 µl.

The EZ1 DSP DNA Blood Kit system performance has been established in performance evaluation studies using human whole blood samples for isolation of genomic DNA. These studies have been established in conjunction with blood collected in exemplary blood collection tubes. 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.

Performance Characteristics of EZ1 Instruments

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. Performance has been established for the EZ1 DSP DNA Blood Kit in conjunction with exemplary downstream applications. However, methods for isolating nucleic acids from biological specimen are used as a front-end for multiple downstream applications. Thus, performance parameters such as the influence of exogenous interfering substances, cross-contamination, or run precision need to be established for any such workflow as part of the downstream application development. Therefore, it is the responsibility of the user to validate the whole workflow to establish appropriate performance parameters.

Basic performance and compatibility to different downstream applications

Various different primary tubes and anticoagulants can be used to collect human blood samples for the EZ1 DSP DNA Blood procedure. Basic performance for the EZ1 DSP DNA Blood Kit was evaluated using 6 single donors for gDNA extraction from 8 different blood collection tubes. Table 1 provides an overview of the sample collection tubes that have been used for evaluation of the system. The white blood cell concentration was counted for each sample, and a theoretical DNA yield was calculated for each sample. The average relative yields of DNA from blood samples using different primary tubes are shown in Figure 1.

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

Primary tube	Manufacturer	Cat. no.*	Preservative/anticoagulant
BD® Vacutainer® 9NC	BD	366007	Sodium citrate
BD Vacutainer K3E	BD	36847	K3EDTA
BD Vacutainer K2E	BD	367864	K2EDTA
S-Monovette® EDTA	Sarstedt®	02.1066.001	K2EDTA
S-Monovette LH	Sarstedt	02.1065.002	Lithium heparin
S-Monovette CPDA1	Sarstedt	01.1610.001	Citrate phosphate dextrose-adenine
Vacuette® K3E	Greiner Bio-One®	455036	K3EDTA
Vacuette 9NC	Greiner Bio-One	454382	Sodium citrate

Genomic DNA was purified from 200 or 350 µl blood samples.
* Catalog numbers are subject to change; please check with the manufacturer or supplier.

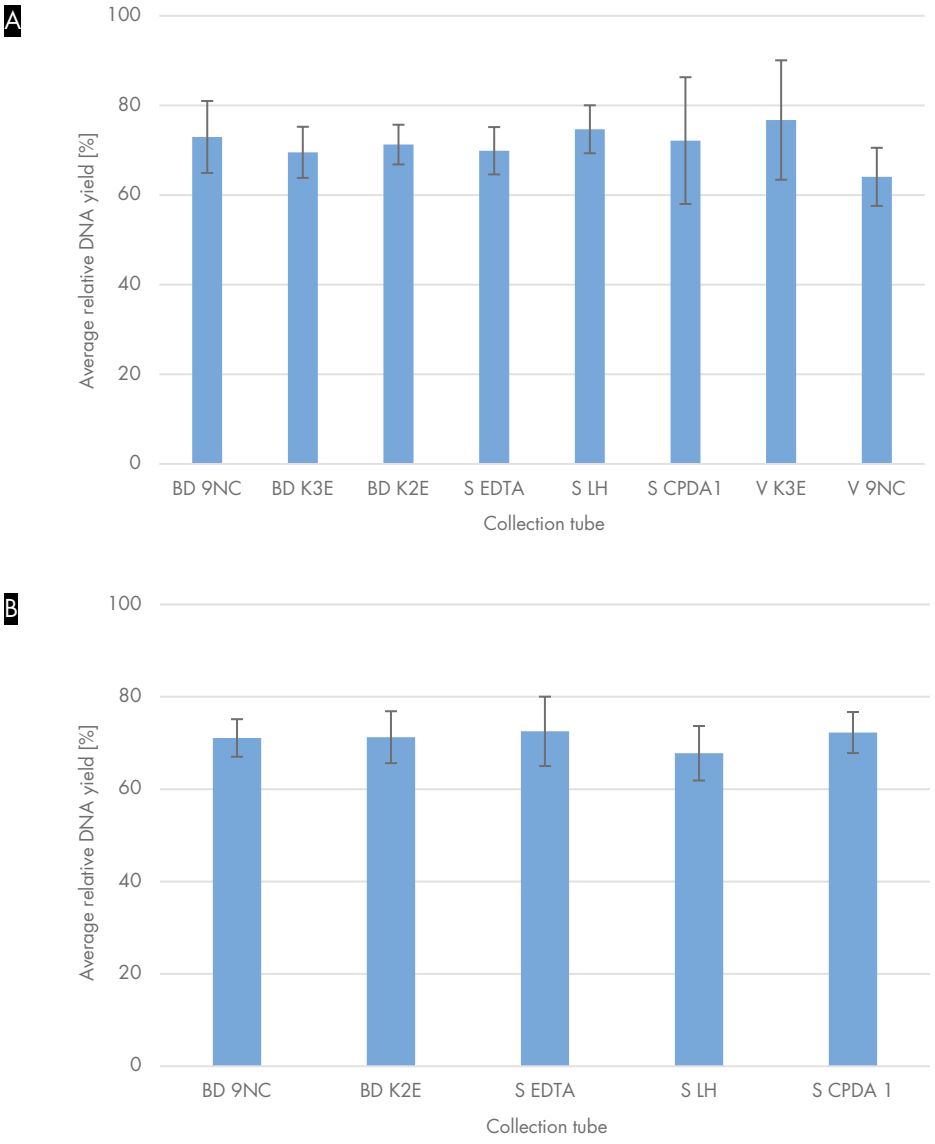

Figure 1. Basic performance using different collection tubes and anticoagulants. Whole blood was collected from healthy donors in different types of tubes with replicates of 3 per donor and tube. The tubes used are listed in Table 1 (BD: Becton Dickinson, S: S-Monovette, V: Vacuette). **A**: Blood was 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 6 donors in 5 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.

To determine the integrity of genomic DNA, eluates from different blood collection tubes were analyzed by agarose gel electrophoresis (Figure 2).

Figure 2. Basic performance using different collection tubes and anticoagulants. The eluates from the different blood collection tubes were analyzed by agarose gel electrophoresis to determine the integrity of the genomic DNA. 1: BD K2E, 2: BD 9NC, 3: S EDTA, 4: S LH, 5: S CPDA1. Shown are the results of 4 different donors.

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).

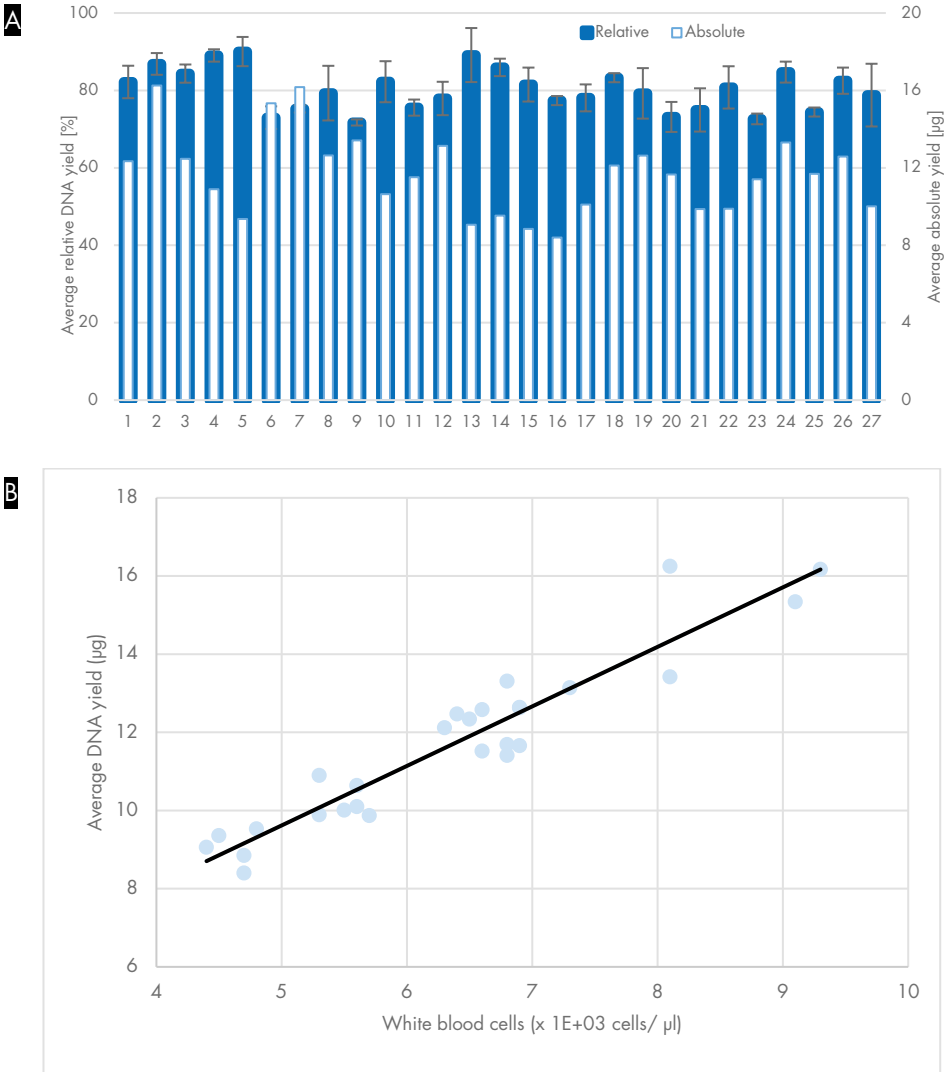

Figure 3. Average absolute and relative DNA yields from different donors. Whole blood was collected from 27 donors in triplicate. Genomic DNA was purified from 350 µl of each sample using the EZ1 DSP DNA Blood system. **A**: Theoretical DNA yield was determined by white blood cell counts. Mean absolute (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.

Genomic DNA eluates purified from whole blood samples using the EZ1 DSP DNA Blood system were analyzed and showed compatibility with different downstream applications like endpoint PCR, agarose gel electrophoresis as well as photometric measurement and quantitative real-time PCR (qPCR) (see Cross-contamination section, page 9).

Freezing–thawing of samples

Fresh or frozen human whole blood samples can be used with the EZ1 DSP DNA Blood system. The effects of freezing and thawing blood samples on DNA purification has been determined (see Figure 4).

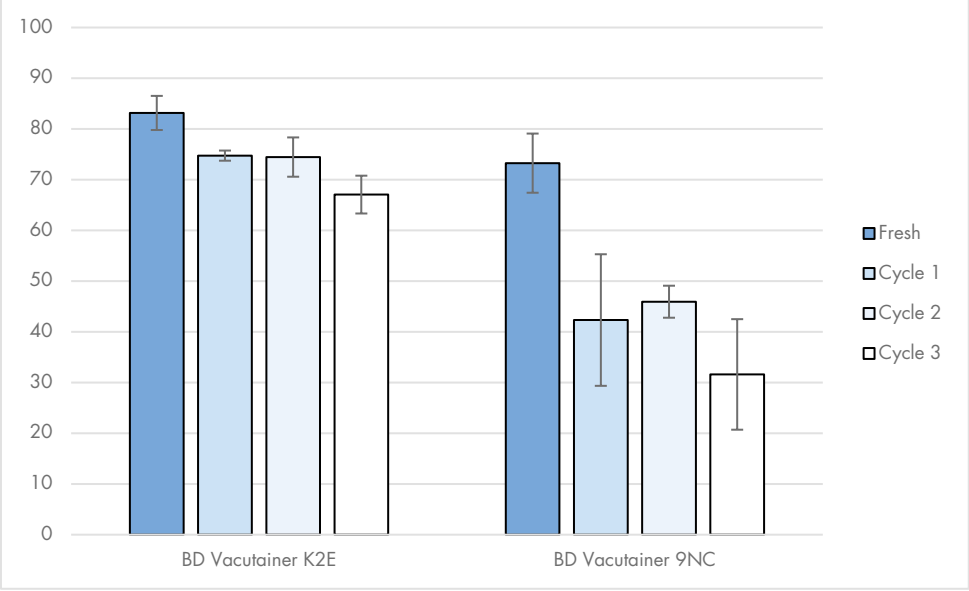

Figure 4. Influence of freeze-thaw cycles on DNA yields. Whole blood was collected from 3 healthy donors in the indicated tubes with 6 replicates each. The tubes used are listed in Table 1. Genomic DNA was purified from 350 µl of each sample using the EZ1 DSP DNA Blood system, and mean values of relative 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).

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 freezing–thawing, tubes with EDTA as an anticoagulant are recommended.

Precision

DNA yields from 350 µl human whole blood and 200 µl elution were compared for different runs using the EZ1 DSP DNA Blood system on the EZ1 Advanced and EZ1 Advanced XL. In total, 8 purification runs were performed with one operator, on one device (per instrument type) and on two different days. The intra-run precision data are shown as standard deviations of the DNA yields (Figure 5).

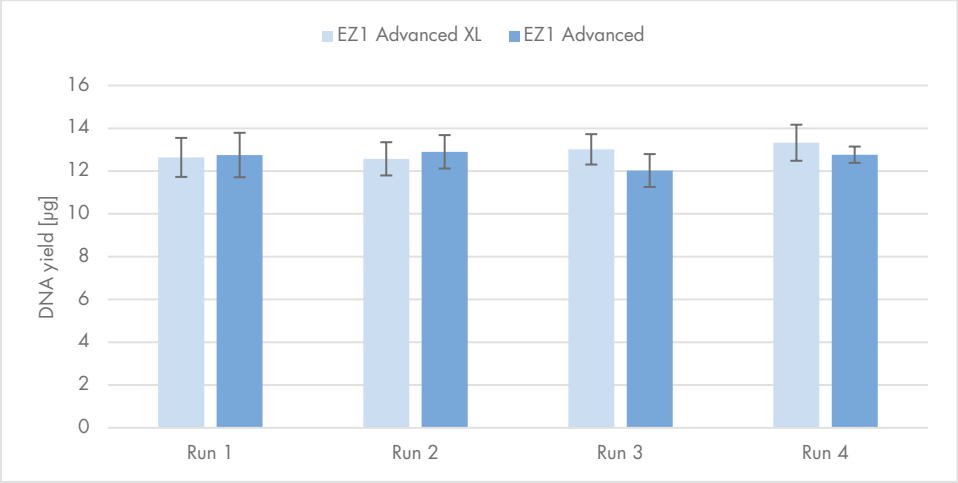

Figure 5. Intra-run precision using the EZ1 DSP DNA Blood system. Blood was collected from a healthy donor in BD K2E tubes and pooled before use. Genomic DNA was purified from 350 µl aliquots in 4 runs of 6 replicates each on the EZ1 Advanced, and in 4 runs of 14 replicates each on the EZ1 Advanced XL using the EZ1 DSP DNA Blood system. Mean total DNA yield and standard deviation are shown for each run.

Coefficients of variations (CVs) were determined for the extraction of human DNA from whole blood. The precision data are shown in Table 2.

Table 2. Analysis of precision estimates – intra-run variability

Precision	CV (%) (EZ1 Advanced XL)	CV (%) (EZ1 Advanced)
Intra run (Run 1)	7.21	8.15
Intra run (Run 2)	6.18	6.06
Intra run (Run 3)	5.45	6.39
Intra run (Run 4)	6.33	2.99

The intra-run variability for the EZ1 Advanced XL instrument was determined to be equivalent to the intra-run variability on the EZ1 Advanced instrument when using the EZ1 DSP DNA Blood kit.

In addition, inter-run variability was determined for both instruments (Table 3).

Table 3. Analysis of precision estimates – inter-run variability

Precision	CV (%) (EZ1 Advanced XL)	CV (%) (EZ1 Advanced)
Inter-run (Run 1-4)	6.58	6.39

Sample input/eluate output

Genomic DNA was purified from 200 and 350 µl whole blood samples from healthy donors using the EZ1 DSP DNA Blood procedure on the EZ1 Advanced XL with three different elution volumes. The differences in DNA concentration of the eluates are shown in Figure 6.

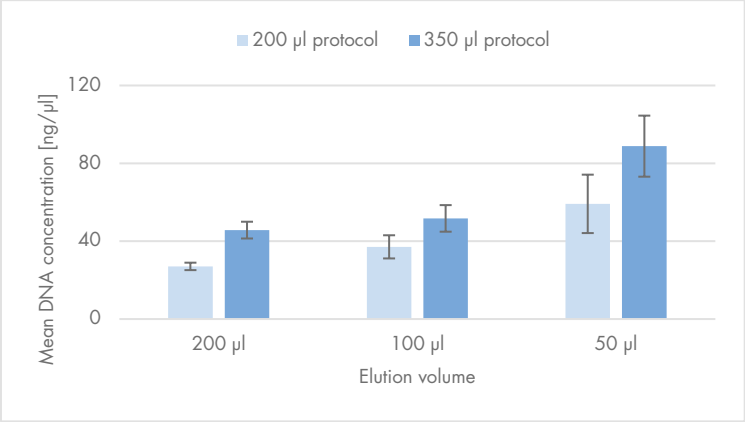

Figure 6. Mean DNA concentration obtained with different elution volumes. Whole blood was collected from 3 donors. Genomic DNA was purified from 200 and 350 µl of each sample and eluted in 200, 100, and 50 µl, each in triplicate, using the EZ1 DSP DNA Blood system on the EZ1 Advanced XL. Mean DNA concentration 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.

Depending on the complete workflow (sample preparation in combination with specific downstream application), there may be a most beneficial combination of sample input and elution volume that can help to optimize, for example, the final DNA yield and concentration or to further minimize the potential influence of residual interfering substances. Different downstream applications even for the same sample material might require different sample input/eluate output combinations. Therefore, it is the user's responsibility to validate the whole workflow within their specific application to establish appropriate performance parameters.

Eluate stability

Eluate stability for the EZ1 DSP DNA Blood Kit was evaluated using extracted genomic DNA from whole blood samples collected in BD Vacutainer K2E tubes. Eluates were stored at different temperatures and different time periods and were tested for integrity (agarose gel electrophoresis) and suitability for PCR (in-house assay).

The results demonstrated stability of genomic DNA in EZ1 eluates for 24 months when stored at 2–8°C or –20°C and for 36 months when stored at –20°C or –80°C.

Interfering substances

Interferents present in the specimen are of major importance as they may affect the performance of the automated isolation of nucleic acids. In addition, the extraction method itself may deliver interfering substances to a different level into the eluate, which may affect purity and compatibility of eluates in downstream applications. Therefore, potentially interfering substances were spiked in whole blood samples to test their impact on the EZ1 DSP DNA Blood procedure and subsequent compatibility to exemplary downstream assays. Eluates were tested for integrity (agarose gel electrophoresis), PCR ability (in-house assay), and purity (photometric measurement).

Table 4. Test concentrations of potential interfering substances

Interfering substances	Final test concentration
Bilirubin	200 mg/l
Hemoglobin	200 g/l
Albumin (BSA)	120 g/l
Triglycerides	30 g/l

None of the substances listed in Table 4 showed any interference with the downstream applications used.

Note: Testing was done using exemplary downstream applications for an assessment of the quality of the extracted nucleic acids. However, different downstream applications may have different requirements with respect to purity (i.e., absence of potential interfering substances), so the identification and testing of relevant substances also needs to be established as part of the downstream application development for any workflow involving the EZ1 DSP DNA Blood Kit.

Cross-contamination

The risk of cross-contamination of the EZ1 DSP DNA Blood system was analyzed by performing 12 runs on the EZ1 Advanced (2.0 protocol, 350 µl input, 200 µl elution) and 9 runs on the EZ1 Advanced XL (200 µl input, 200 µl elution) with alternating checkerboard patterns. To detect sample-to-sample carryover, the runs were performed with male (positive) and female (negative) blood samples in alternating positions. 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.

Performance Characteristics of EZ2 Connect MDx

Performance Characteristics for the EZ2 Connect MDx have been established in comparison to the EZ1 Advanced XL using the EZ1 DSP DNA Blood Kit. Kit-related performance characteristics like eluate stability or basic performance are valid for all instrument systems listed in the instructions for use of the EZ1 DSP DNA Blood Kit since the kit as part of the system does not change for the different automated platforms.

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. Performance has been established for the EZ1 DSP DNA Blood Kit in conjunction with exemplary downstream applications. However, methods for isolating nucleic acids from biological specimen are used as a front-end for multiple downstream applications. Thus, performance parameters such the influence of exogenous interfering substances, as cross contamination or run precision need to be established for any such workflow as part of the downstream application development. It is therefore the responsibility of the user to validate the whole workflow to establish appropriate performance parameters.

Basic performance and compatibility to different downstream applications

Basic performance data generated using the EZ1 Advanced XL, EZ1 Advanced, or BioRobot EZ1 apply to the EZ2 Connect MDx instrument too (see page 3). Sample composition and the kit are identical for the instrument systems for use with the EZ1 DSP DNA Blood Kit. Furthermore, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal or improved basic performance of the system. During equivalency testing, compatibility to different downstream applications (including qPCR) was confirmed as well.

However, as only exemplary downstream methods were used, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Freezing–thawing of samples

Freezing–thawing data of sample material generated using the EZ1 Advanced XL, EZ1 Advanced, or BioRobot EZ1 apply to the EZ2 Connect MDx instrument too (see page 6). Freezing–thawing of samples is done before nucleic acid extraction, and the related degree of sample degradation thus is independent of the downstream extraction procedure. Furthermore, sample composition and kit chemistry are identical for the instrument systems for use with the EZ1 DSP DNA Blood Kit. Also, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal or improved performance of the system. The instructions for sample handling apply to all automated systems for use with the kit.

However, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Precision

DNA yields from 200 µl human whole blood and 100 µl elution volume were compared for different runs using the EZ1 DSP DNA Blood system on the EZ2 Connect MDx and EZ1 Advanced XL. In total, 12 purification runs were performed with three different operators, on three different devices (per instrument type) and on three different days. The intra-run precision data are shown as standard deviations of the DNA yields (Figure 7).

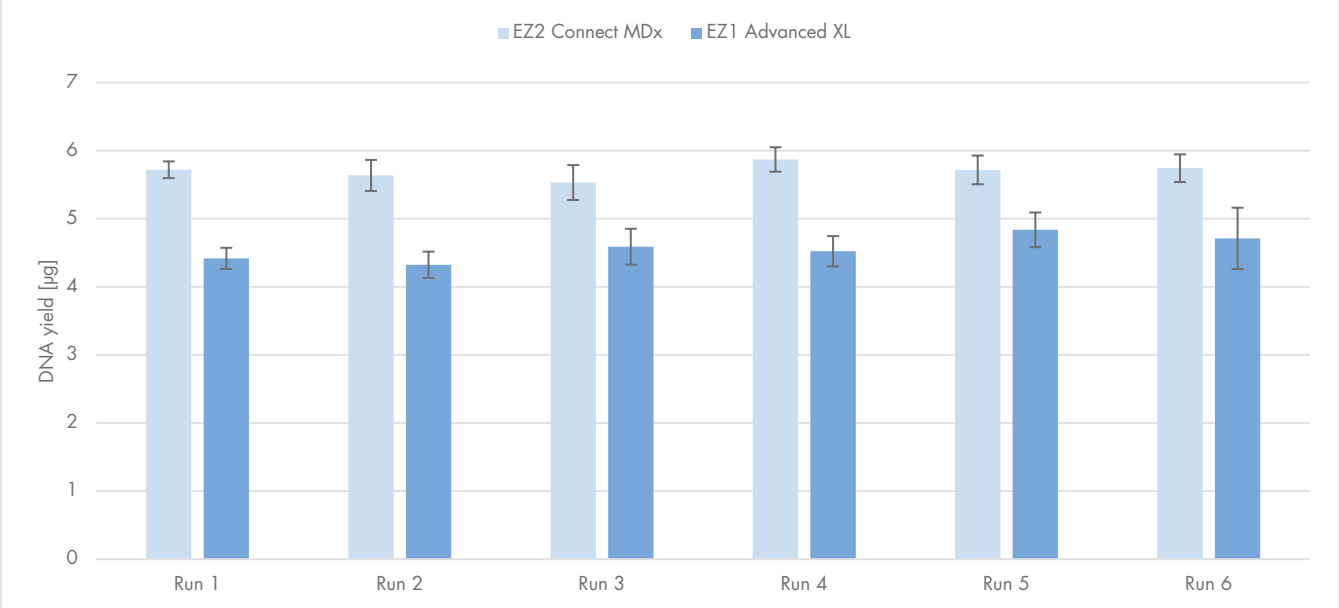

Figure 7. Intra-run precision using the EZ1 DSP DNA Blood system. Blood was collected from a healthy donor in BD K2E tubes and pooled before use. Genomic DNA was purified from 200 µl aliquots in 6 runs of 14 replicates each on the EZ1 Advanced XL, and from 200 µl aliquots in 6 runs of 24 replicates each on the EZ2 Connect MDx using the EZ1 DSP DNA Blood system. Mean total DNA yield and standard deviation are shown for each run.

Coefficients of variations (CVs) were determined for the extraction of human DNA from whole blood. The precision data are shown in Table 5.

Table 5. Analysis of precision estimates- intra-run variability

Precision	CV (%) (EZ2 Connect MDx)	CV (%) (EZ1 Advanced XL)
Intra run (Run 1)	2.14	3.52
Intra run (Run 2)	4.04	4.47
Intra run (Run 3)	4.64	5.75
Intra run (Run 4)	3.06	4.91
Intra run (Run 5)	3.69	5.26
Intra run (Run 6)	3.54	9.55

The intra-run variability for the EZ2 Connect MDx instrument was determined to be equivalent to the intra-run variability on the EZ1 Advanced XL instrument when using the EZ1 DSP DNA Blood kit in equivalency tests.

In addition, inter-run variability was determined for the EZ2 Connect MDx instrument (Table 6).

Table 6. Analysis of precision estimates – inter-run variability

Precision	CV (%) (EZ2 Connect MDx)	CV (%) (EZ1 Advanced XL)
Inter-run (Run 1-6)	4.02	7.07

Sample input/eluate output

The EZ1 DSP DNA Blood system on the EZ2 Connect MDx offers the possibility to combine different sample input volumes (either 200 or 350 µl) with different eluate output volumes (50, 100, or 200 µl). Overall performance testing of the extraction procedures used on the EZ2 Connect MDx system showed equal or improved performance of the system in relation to the EZ1 Advanced XL.

Depending on the complete workflow (sample preparation in combination with specific downstream application), there may be a most beneficial combination of sample input and elution volume that can help to optimize, for example, the final DNA yield and concentration or to further minimize the potential influence of residual interfering substances. Different downstream applications even for the same sample material might require different sample input/eluate output combinations. Therefore, it is the user's responsibility to validate the whole workflow within their specific application to establish appropriate performance parameters.

Accuracy

Using three different concentrations of white blood cells (WBC), 6 purification runs on the EZ2 Connect MDx and EZ1 Advanced XL were performed. DNA yields from 200 µl sample input and 200 µl elution volume were determined by spectrophotometric measurement and compared between the different instruments.

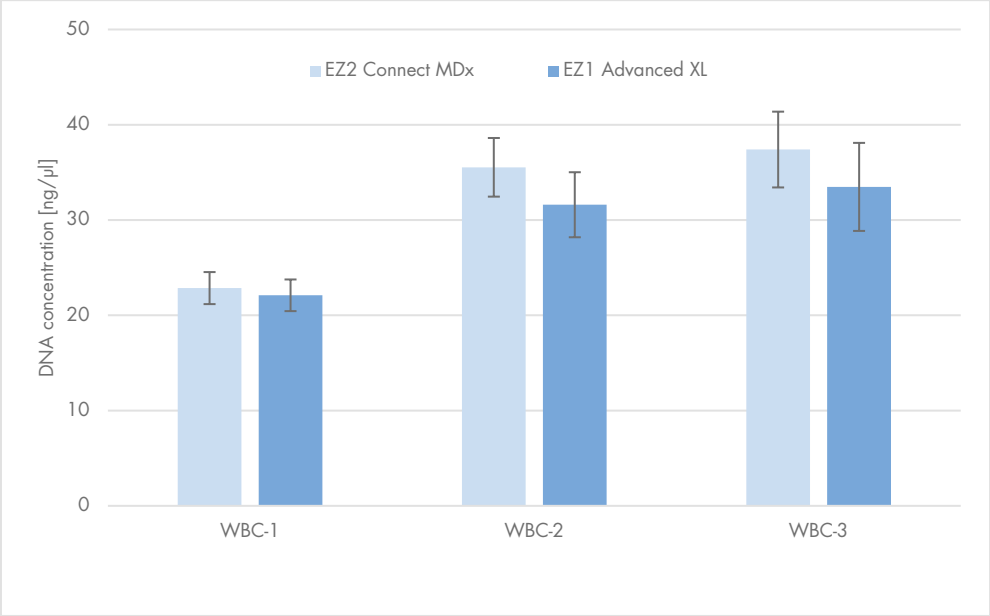

Figure 8. Mean DNA concentration obtained with different WBC concentrations Whole blood was collected from different donors, pooled and adjusted to the required WBC concentrations with buffy coat. Genomic DNA was purified from 200µl of each sample and eluted in 200 µl, using the EZ1 DSP DNA Blood system on the EZ1 Advanced XL and EZ2 Connect MDx. Mean DNA concentration is shown for each WBC concentration.

Table 7. Summary of accuracy test results

WBC	Instrument	Day	DNA concentration			
			Mean (ng/µl)	Median (ng/µl)	SD	% CV
WBC-1	EZ1	1	21.92	22.50	1.662	7.58
		2	22.28	22.05	1.785	8.01
	EZ2	1	23.00	23.00	1.490	6.48
		2	22.71	22.45	1.975	8.70
WBC-2	EZ1	1	33.23	33.30	3.565	10.73
		2	29.98	31.03	2.635	8.79
	EZ2	1	35.75	36.05	3.066	8.58
		2	35.32	35.15	3.341	9.46
WBC-3	EZ1	1	34.48	34.70	3.418	9.91
		2	32.47	31.35	5.717	17.61
	EZ2	1	38.04	37.50	4.260	11.20
		2	36.76	36.63	3.935	10.70

The statistical analysis showed equal performance of the EZ2 Connect MDx compared to the EZ1 Advanced XL instrument.

Eluate stability

Eluate stability data generated using the EZ1 Advanced XL, EZ1 Advanced, or BioRobot EZ1 apply to the EZ2 Connect MDx instrument too (see page 8). Sample and kit composition are identical for the instrument systems for use with the EZ1 DSP DNA Blood Kit. Furthermore, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal or improved performance of the system. The instructions for eluate handling apply to all automated systems for use with the kit.

However, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Interfering substances

The influence of interfering substances was determined using the EZ1 Advanced XL, EZ1 Advanced, or BioRobot EZ1. These data apply to the EZ2 Connect MDx instrument too (see page 8). Sample and kit composition are identical for the instrument systems for use with the EZ1 DSP DNA Blood Kit. The sample input/eluate output volumes are identical so that no impact on type or concentration of interfering substances in the eluates is expected. Furthermore, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal or improved performance of the system. The instructions for sample and eluate handling apply to all automated systems for use with the kit.

However, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Cross-contamination

The risk of cross contamination of the EZ1 DSP DNA Blood Kit used on the EZ2 Connect MDx was analyzed by performing 10 runs (350 µl input, 50 µl elution) with alternating checkerboard patterns. To detect sample-to-sample carryover, the runs were performed with male (positive) and female (negative) blood samples in alternating positions. Every second 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.

All of the male blood samples tested positive in PCR, and all female blood samples tested negative. No cross-contamination was detected for a sample-to-sample or run-to-run carry over.

Symbols

The following symbols appear in this document. For a full list of symbols used in the instructions for use or on the packaging and labeling, please refer to the handbook.

Symbol	Symbol definition
CE	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
IVD	In vitro diagnostic medical device
REF	Catalog number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Manufacturer
	Important note

Revision History

Revision	Description
R1, June 2022	Version 4, Revision 1 • Generation of document for new kit version. Data for EZ2 Connect MDx added

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.