

BioRobot® EZ1™ DSP — Walkaway Nucleic Acid Purification for Molecular Diagnostics

CE-IVD-marked system



Sample & Assay Technologies

Fully automated CE-IVD-compliant solution

The BioRobot EZ1 DSP system enables fully automated purification of genomic DNA from human whole blood and viral nucleic acids from serum or plasma samples. The CE-IVD-marked system (including workstation, software, protocols, purification kits, and service) provides exact performance specifications, assuring highly reliable purification of nucleic acids for in vitro diagnostic use. We also provide IQ/OQ packages to help you to qualify your BioRobot EZ1 DSP system and comply with regulatory demands enabling seamless integration into diagnostic workflows.

The BioRobot EZ1 DSP system gives you:

- Compliance with EU Directive 98/79/EC on in vitro medical devices
- An easy-to-use standardized system for daily use in routine diagnostic applications
- Proven purification chemistries backed by QIAGEN quality
- Detailed performance data for a range of diagnostic applications
- Comprehensive instrument, application, and validation support

Easy-to-use system for efficient, standardized purification

The BioRobot EZ1 DSP enables standardized purification of nucleic acids using proven magnetic-particle technology for use in routine in vitro diagnostic applications. Reagent production is subjected to stringent quality control assuring reliable and robust performance. An easy-to-use workstation, prefilled and sealed reagent cartridges, and simple protocol selection and worktable setup help to prevent handling errors and allow the system to be operated by anyone — from the novice to the expert. Manual handling of potentially infectious samples is minimized, ensuring safety to the user and reliable processing of samples. Purified nucleic acids are ready to use in sensitive downstream diagnostic assays based on enzymatic amplification, such as PCR and RT-PCR for genotyping and viral load monitoring.

Robust, walkaway nucleic acid purification from human samples

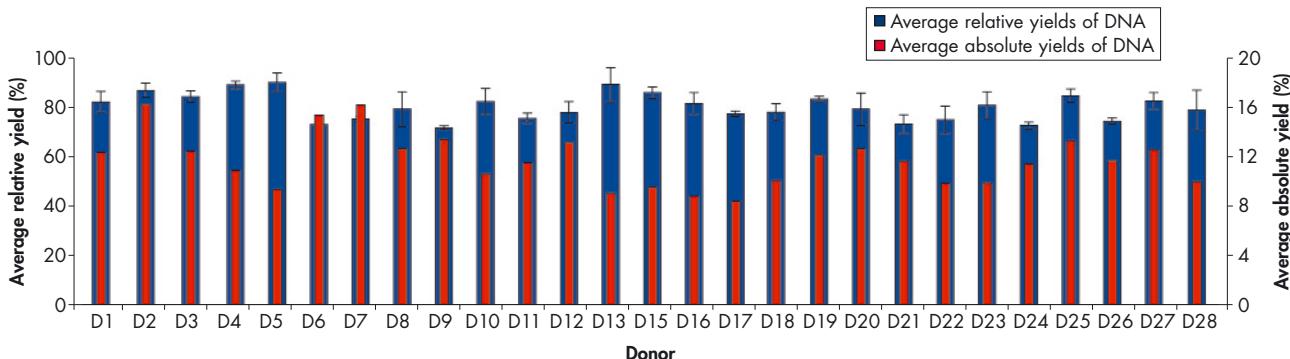
The BioRobot EZ1 DSP workstation in combination with proven EZ1 DSP Kits enables purification of genomic DNA or viral nucleic acids from 1–6 samples per run. All processing steps are performed by the workstation — from piercing the reagent cartridge to elution of highly pure nucleic acids. The streamlined procedure provides robust walkaway purification of nucleic acids with minimal hands-on time. Processing time for purification of genomic DNA from 6 samples of human whole blood is 25 minutes, and viral RNA and DNA can be purified from 6 samples of plasma or serum in 45 minutes.



Efficient purification of genomic DNA

Automated DNA purification provides high levels of reliability and standardization and helps to eliminate variation caused by handling errors. The BioRobot EZ1 DSP in combination with the EZ1 DSP DNA Blood Kit enables efficient purification of genomic DNA from human whole blood. The amount of DNA purified depends on the white blood cell content (WBC) of each blood sample. The EZ1 DSP DNA Blood system provides high relative DNA yields independent of the donor's WBC (Figure 1).

A



B

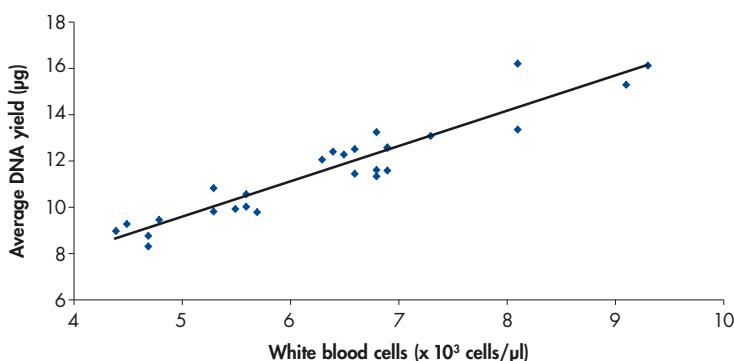
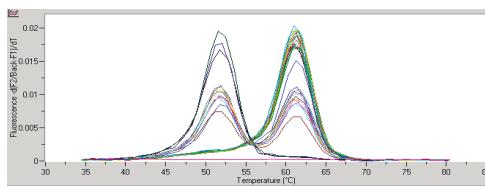


Figure 1. Average absolute and relative DNA yields from different donors. Whole blood was collected from 27 donors in triplicate. Genomic DNA was purified from 350 µl of each sample using the EZ1 DSP DNA Blood system. Theoretical DNA yield was determined by white blood cell counts. **A** Mean absolute and 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.

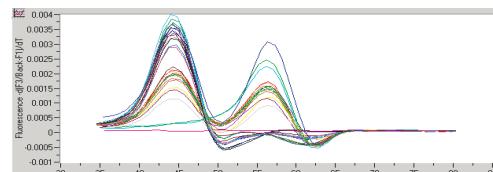
High-quality DNA for sensitive and specific analyses

Sensitive downstream applications demand the use of highly pure DNA for reliable results. DNA purified using the EZ1 DSP DNA Blood system is ready for immediate use in a broad range of sensitive diagnostic assays, such as real-time PCR using CE-IVD-marked *artus®* PCR Kits (Figure 2).

A



B



Unparalleled removal of inhibitors

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities than single-step enzyme-catalyzed reactions. DNA purified using the EZ1 DSP DNA Blood system performs well in PCR even when large amounts of eluate are used, demonstrating the high purity of the DNA (Figure 3).

Figure 2. Melting curve analysis of amplification products at nucleotides 677 and 1298 of the MTHFR gene. DNA was purified from whole blood of 30 donors using the EZ1 DSP DNA Blood system. Eluates were analyzed using the CE-IVD-marked *artus* MTHFR LC PCR Kit with melting curve analysis on the LightCycler® Instrument. **A** Analysis at nucleotide 677. **B** Analysis at nucleotide 1298.

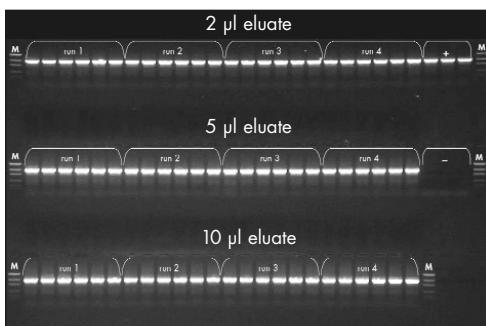


Figure 3. Effects of eluate volume used in PCR on PCR performance. Blood was collected from a healthy donor in BD K2E tubes and pooled before use. Genomic DNA was purified from twenty-four 350 µl aliquots in 4 runs of 6 replicates each using the EZ1 DSP DNA Blood system. The indicated amount of eluate was used in a 50 µl PCR with primers for a 1100 bp single-copy human gene fragment. +: Positive control. -: Negative control. M: Low DNA mass ladder.

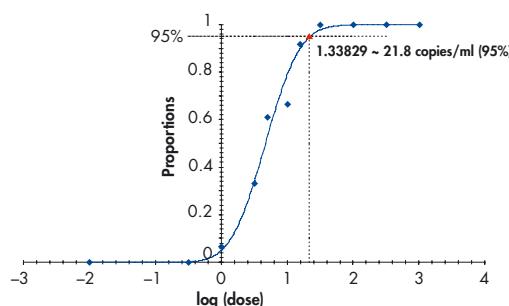


Figure 4. Probit analysis for detection of CMV DNA using the EZ1 DSP Virus system and the artus CMV RG PCR Kit. Viral nucleic acids were purified using the EZ1 DSP Virus system, and the artus CMV RG PCR Kit was used for detection of CMV DNA on the Rotor-Gene 3000. The 95% probit value was 21.8 copies/ml.

High analytical sensitivity with lower detection limits

The BioRobot EZ1 DSP in combination with the EZ1 DSP Virus Kit provides simultaneous purification of viral RNA and DNA from human plasma or serum. Efficient purification of viral RNA and DNA results in sensitive analytical detection in PCR, as demonstrated using CE-IVD-marked *artus* PCR Kits for detection of CMV DNA (Table 1 and Figure 4) and HBV DNA (Table 2 and Figure 5) on a variety of real-time thermal cyclers.

Table 1. Detection limit of CMV DNA using the EZ1 DSP Virus system and *artus* CMV PCR Kits

Input titer (copies/ml)	Hits (LightCycler)	Hits (Rotor-Gene)	Hits (ABI PRISM)
1000	18/18	18/18	18/18
316	15/15	15/15	15/15
100	23/24	24/24	24/24
31.6	30/33	27/27	32/33
15.8	10/18	11/12	16/18
10	12/36	21/36	26/36
7.9	8/18	7/18	13/18
5.0	4/18	11/18	9/18
3.16	2/18	6/18	7/18
1	0/18	1/18	5/18
0.316	1/18	0/18	2/18
0.1	1/18	1/18	2/18
0	0/6	0/6	0/6
95% probit value (copies/ml)	67.2	21.8	38.3
Confidence interval (copies/ml)	41.8–142	14.5–44.1	21.5–89.8

The detection limit was determined by the 95% probit value for the EZ1 DSP Virus system. The detection limit was determined by processing a dilution series of prequantified CMV cell-culture supernatant. The virus was diluted in CMV-negative human EDTA plasma pool. Each dilution was analyzed in at least 2 independent runs with at least 6 replicates per dilution. 400 µl plasma was used for sample preparation on the BioRobot EZ1 DSP with elution in 60 µl. *artus* CMV PCR Kits were used for detection of CMV DNA. The samples were analyzed on a LightCycler 1.2 Instrument (Roche), a Rotor-Gene™ 3000 (Corbett-Research), and an ABI PRISM® 7000 SDS (Applied Biosystems).

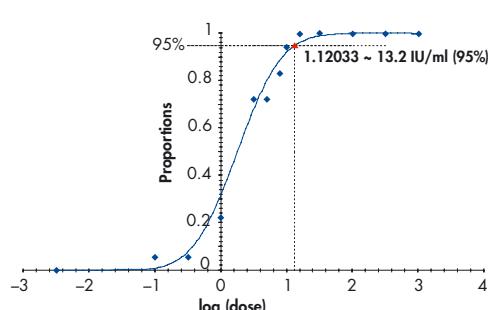


Figure 5. Probit analysis for detection of HBV DNA using the EZ1 DSP Virus system and the *artus* HBV TM PCR Kit. Viral nucleic acids were purified using the EZ1 DSP Virus system, and the *artus* HBV TM PCR Kit was used for detection of HBV DNA on the ABI PRISM 7000 SDS. The 95% probit value was 13.2 IU/ml.

Linear yields of highly pure viral DNA and RNA

Purification of viral nucleic acids provides linear yields, allowing accurate quantitative analysis for both high and low viral titers (Figure 6). Purified viral nucleic acids give high-performance results in sensitive diagnostic assays, even when starting material contains elevated levels of endogenous inhibitors, demonstrating the robustness of the purification procedure (Table 3).

Table 2. Detection limit of HBV DNA using the EZ1 DSP Virus system and artus HBV PCR Kits

Input titer (IU/ml)	Hits (LightCycler)	Hits (Rotor-Gene)	Hits (ABI PRISM)
1000	6/6	12/12	6/6
316	18/18	18/18	18/18
100	18/18	18/18	18/18
31.6	18/18	18/18	18/18
15.8	10/18	18/18	18/18
10	13/36	34/36	34/36
7.9	9/18	15/17	15/18
5	4/18	13/18	13/18
3.16	5/36	23/36	26/36
1	1/18	5/15	4/18
0.316	0/18	2/17	1/18
0.1	0/18	1/18	0/18
0	0/18	0/18	0/18
95% probit value (IU/ml)	45.7	14.4	13.2
Confidence interval (IU/ml)	28–102	9.5–26.5	9.0–23.1

The detection limit was determined by the 95% probit value for the EZ1 DSP Virus system using HBV WHO international virus standard. The detection limit was determined by processing a dilution series of HBV. The virus was diluted in HBV-negative normal human EDTA plasma pool. Each dilution step was prepared in at least 3 independent runs with at least 6 replicates per dilution. 400 µl plasma was used for sample preparation on the BioRobot EZ1 DSP with elution in 60 µl. artus HBV PCR Kits were used for detection of HBV DNA. The samples were analyzed on a LightCycler 1.2 Instrument (Roche), a Rotor-Gene 3000 (Corbett Research), and an ABI PRISM 7000 SDS (Applied Biosystems).

Table 3. Robust purification procedure provides unparalleled removal of inhibitors

Inhibitors	Concentration	Mean log copies/ml	Standard deviation
Untreated	–	2.87	0.04
Bilirubin	200 mg/liter	2.83	0.02
Hemoglobin	5 g/liter	2.80	0.09
Protein	90 g/liter	2.77	0.06
Liposyn	30 g/liter	2.80	0.06

Exclusion of sample carryover

High process reliability is critical for clinical laboratories that purify nucleic acids for routine testing. Lack of sample-to-sample carryover is a prerequisite for reliable results. To evaluate the risk of sample-to-sample carryover during and between runs, the EZ1 DSP Virus procedure was subjected to rigorous testing using an alternating checkerboard setup of negative and highly positive (1.0×10^8 IU/ml) parvovirus B19 DNA samples. All of the highly positive samples were detected positive using the CE-IVD-marked artus Parvo B19 RG PCR Kit. All negative samples, in the checkerboard runs and the all-negative runs, were unresponsive (Table 4). This demonstrates that the EZ1 DSP Virus procedure provided no sample carryover under these conditions.

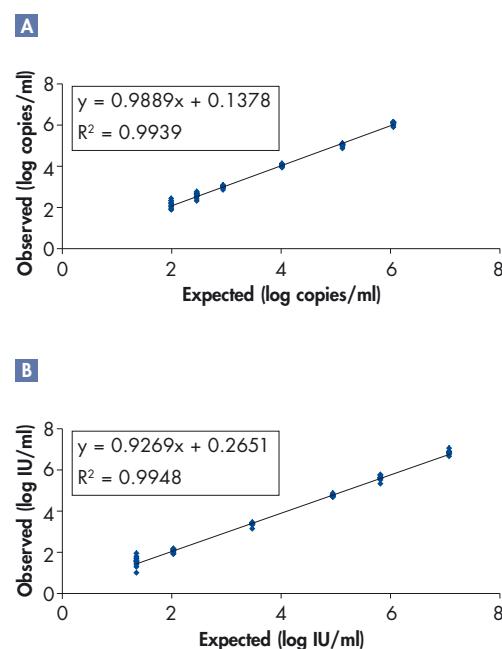


Figure 6. Linear range of yields using the EZ1 DSP Virus protocol. The linear range of the EZ1 DSP Virus protocol was determined using viral dilution series and Abbott RealTime viral load assays. □ for HIV-1 and ■ for HCV. RealTime Internal Controls (17 µl each) were added directly to each HIV-1 or HCV sample before extraction. Viral nucleic acids were extracted from 400 µl samples and eluted in 90 µl elution buffer (AVE). PCR was carried out on the Abbott m2000rt.

HBV (1000 copies/ml [log 3]) was added to panels of recalcified plasma containing elevated levels of endogenous inhibitors. A 3.4 µl aliquot of RealTime HBV Internal Control was combined with carrier RNA for each sample, and viral nucleic acids were extracted from 400 µl samples, in replicates of 5, and eluted in 90 µl elution buffer (AVE). The Abbott RealTime HBV assay* was used for detection of HBV. PCR was carried out on the Abbott m2000rt.

* Product in development.

Table 4. Cross-contamination test setup and C_T values for detection of parvovirus B19 DNA

Run	Position					
	1	2	3	4	5	6
1	15.47	X	15.41	X	15.36	X
2	X	15.48	X	15.53	X	15.32
3	X	X	X	X	X	X
4	15.35	X	15.20	X	15.27	X
5	X	15.21	X	15.13	X	15.43
6	X	X	X	X	X	X
7	15.62	X	15.48	X	15.23	X
8	X	15.31	X	15.83	X	15.62
9	X	X	X	X	X	X

Nine runs on the BioRobot EZ1 DSP were performed to evaluate the risk of cross-contamination events during and between EZ1 DSP Virus procedures. The test was performed using a quantified parvovirus B19 patient sample. The viral load of positive samples used for the carryover tests was 1.0×10^8 IU/ml. For dilution of positive samples and as negative control samples, a human parvovirus B19 negative EDTA plasma pool was used.

To detect sample-to-sample carryover, 2 runs were performed with an alternating checkerboard setup of negative and highly positive samples. Every third run was performed using all negative samples to monitor possible run-to-run carryover. This sample setup was repeated three times resulting in a total of nine runs. Parvovirus B19 DNA was detected and quantitated using the CE-IVD-marked *artus* Parvo B19 RG PCR Kit on the Rotor-Gene 3000. The analytical detection limit of the *artus* Parvo B19 RG PCR Kit is determined to be 0.2 IU/ μ l in the eluate ($p = 0.05$). This indicates that there is a 95% probability that 0.2 IU/ μ l in the eluate will be detected.

Mean C_T value of all samples = 15.40 ± 0.18 (CV = 1.14%)

X: Unresponsive after 45 PCR cycles.

Service and application support

QIAGEN Instrument Service offers a wide range of flexible Service Support products, giving you peace of mind and letting you enjoy complete coverage and cost control. With ISO 9001/ISO 13485 certification and an international team of highly qualified and experienced Support Specialists, we deliver the high-quality service that you deserve and that your applications demand.

Coverage tailored to your needs

With a Service Support Agreement, you get priority access to our expert service ensuring maximum productivity from your investment. To meet your demands, we offer a wide range of flexible agreements — from all-inclusive coverage and a 24-hour (1 working day) response time for laboratories that need to deliver high-quality results within fixed time frames to basic coverage for laboratories that require defined budgeting of maintenance costs.

IQ/OQ service and documentation

QIAGEN Qualification Services help you to qualify your system for correct installation (IQ) and operation (OQ) in your laboratory. We can perform the IQ/OQ on-site to save time and costs. IQ/OQ packages consist of professional documentation and the IQ/OQ is performed by Instrument Service Specialists to ensure compliance with regulatory demands.

Visit www.qiagen.com/goto/EZ1DSP to discover more about walkaway nucleic acid purification for molecular diagnostics!

Ordering Information

Product	Contents	Cat. no.
BioRobot EZ1 DSP*	Robotic workstation for automated purification of nucleic acids using EZ1 DSP Kits, 1-year warranty on parts and labor	9001360
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 DSP DNA Blood Card	Preprogrammed card for EZ1 DSP DNA Blood protocol	9017713
EZ1 DSP Virus Kit (48)	For 48 viral nucleic acid preps: Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, Carrier RNA	62724
EZ1 DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol	9017707
IQ/OQ Services, BioRobot EZ1	Validation support service for the BioRobot EZ1 and BioRobot EZ1 DSP. The validation support includes IQ/OQ documentation and performance of the qualification protocols with travel and labor costs covered	9240826
Warranty PLUS 2, BioRobot EZ1 DSP	3-year warranty, 1 preventive maintenance visit per year, 48-hour (2 working days) priority response, all labor, travel, and repair parts plus consumables required for preventive maintenance	9240811
Warranty PLUS 2 Premium, EZ1 DSP	3-year warranty, 1 preventive maintenance visit per year, 24-hour priority response (1 working day), all labor, travel, and repair parts plus consumables required for preventive maintenance	9240813

* The BioRobot EZ1 DSP is not available in all countries; please inquire.

The BioRobot EZ1 DSP workstation, EZ1 DSP Kits and Cards, *artus* CMV PCR Kits, *artus* HBV PCR Kits, *artus* MTHFR PCR Kits, and *artus* Parvo B19 PCR Kits are intended for in-vitro diagnostic use in Europe.

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Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

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