June 2022

QIAsymphony® DSP DNA Kit Instructions for Use (Performance Characteristics)

Version 2

IVD

For In Vitro Diagnostic Use
For use with QIAsymphony DSP DNA Mini Kit and QIAsymphony DSP DNA Midi Kit

CE

REF 937236, 937255

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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 QIAsymphony DSP DNA Kits are intended to be used only in combination with the QIAsymphony SP.

The QIAsymphony DSP DNA Mini Kits provide reagents for automated purification of total DNA from human whole blood, buffy coat, tissue and formalin-fixed, paraffin-embedded (FFPE) tissue samples, as well as viral DNA from human whole blood. The QIAsymphony DSP DNA Midi Kits provide reagents for automated purification of total DNA from human whole blood and buffy coat. However, performance characteristics for every blood collection tube or tissue type have not been established and must be validated by the user.

Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for direct use in downstream applications, such as amplification reactions (PCR).The QIAsymphony SP performs all steps of the purification procedure. Up to 96 samples, in batches of up to 24, are processed in a single run.

In the following selected performance data for the different applications are shown.

Performance Characteristics

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. They have been established for the QIAsymphony DSP DNA Mini and Midi Kits 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. Performance parameters such as 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

DNA blood and buffy coat

DNA yield

Basic performance of the QIAsymphony DSP DNA Mini Kit was evaluated using different collection tubes and anticoagulants, as well as fresh and frozen human whole blood. Whole blood was collected from 3 healthy donors (white blood cell [WBC] count 4.0 to 11.0 x 10^6 cells/ml) in 3 different types of tubes: EDTA, 10 ml BD™ Vacutainer® 16 x 100 mm (K2-EDTA); citrate, 2.7 ml Sarstedt® S-Monovette® 9NC Tube 13 x 75 mm (citrate); heparin, 7.5 ml Sarstedt S-Monovette 15 x 92 mm (Li-Heparin). Blood was used either fresh (stored at 2–8°C) or frozen (stored at –20°C). Genomic DNA was purified from 200 µl samples, with 4 replicates per donor and tube type, using the QIAsymphony DSP DNA Mini Kit and blood 200 DSP protocol with an elution volume of 200 µl. DNA yields and purity were determined by spectroscopic analysis (Figure 1).

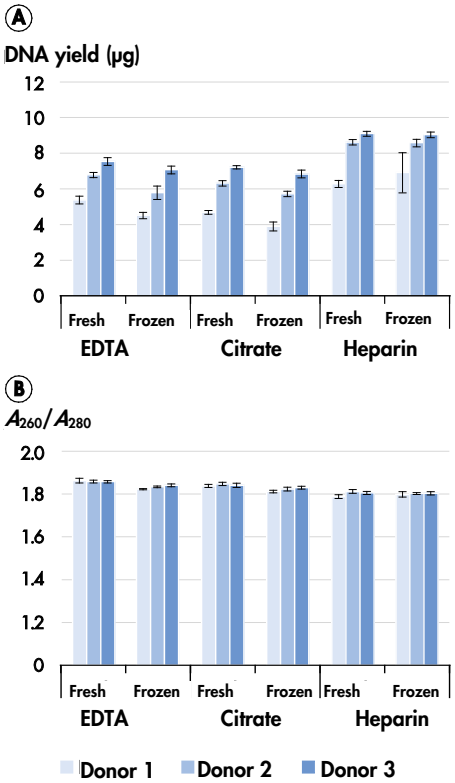


Figure 1. DNA yield and purity using different sample collection tubes and anticoagulants with fresh and frozen human whole blood. A DNA yield, bars show the absolute DNA yield with standard deviation. **B** DNA purity, bars show the DNA purity with standard deviation.

DNA integrity

Long-range PCR products (5 kb) were amplified using a LongRange PCR assay (Figure 2).

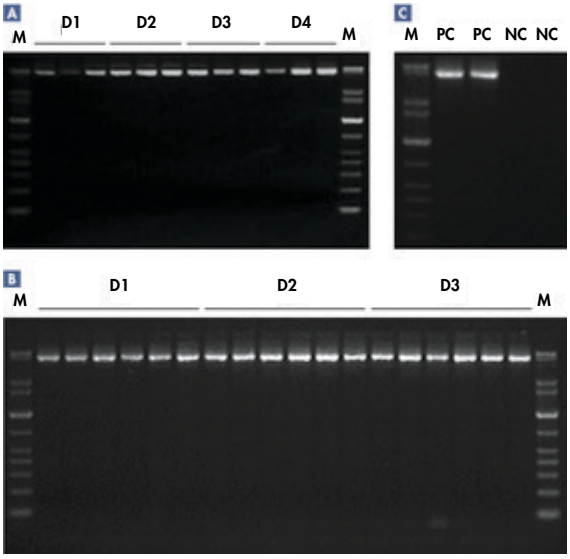


Figure 2. DNA integrity tested by long-range PCR. M, QIAGEN GelPilot 1 kb Plus Ladder. **A** Whole blood was collected from 4 healthy donors (D) in BD K2E tubes. Genomic DNA for long-range PCR was purified from 200 µl aliquots in triplicate using the QIAsymphony DSP DNA Mini Kit and blood 200 DSP protocol with an elution volume of 200 µl. D1, donor 1; D2, donor 2; D3, donor 3; and D4, donor 4. **B** Whole blood was collected from 3 healthy donors in BD K2E tubes, and buffy coat was prepared. Genomic DNA was purified from 200 µl aliquots in 6 replicates using the QIAsymphony DSP DNA Mini Kit and buffy coat 200 DSP protocol with an elution volume of 200 µl. D1, donor 1; D2, donor 2; and D3, donor 3. **C** Controls: PC, positive control; and NC, negative control.

Correlation of DNA yield to WBC count

The performance for the QIAsymphony DSP DNA Blood and buffy coat applications was evaluated using blood and buffy coat samples with 6 different WBC counts for each sample type. For whole blood, WBC counts ranged from 4 x 10^6 cells/ml to 11.6 x 10^6 cells/ml, and for buffy coat, counts ranged from 2.2 x 10^7 cells/ml to 5.6 x 10^7 cells/ml. DNA yields were determined by spectroscopic analysis and plotted against the WBC count (Figure 3).

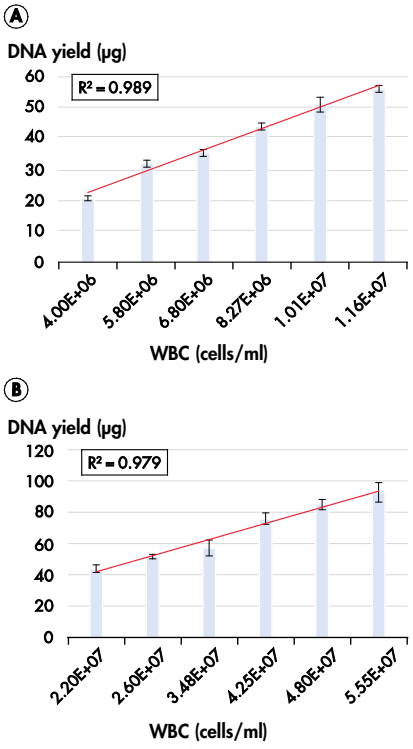


Figure 3. Correlation of DNA yield to WBC count. A Genomic DNA was purified from 1 ml human whole blood using the QIAsymphony DSP DNA Midi Kit and the blood 1000 DSP protocol with an elution volume of 500 µl. Bars show the absolute DNA yield with standard deviation. **B** Genomic DNA was purified from 400 µl buffy coat using the QIAsymphony DSP DNA Midi Kit and the buffy coat 400 DSP protocol with an elution volume of 400 µl. Bars show the absolute DNA yield with standard deviation.

Virus blood

Hit-rate studies were performed by diluting pre-quantified CMV WHO standard material in CMV-negative human whole blood. A detection rate of 100% was observed for samples with viral loads of 90 IU of CMV per milliliter (Table 1).

Table 1. Sensitivity of QIAsymphony DSP Virus Blood application

CMV (IU/ml)	Replicates	Hits	Hit (%)
350	18	18	100.00
230	32	32	100.00
115	31	31	100.00
90	32	32	100.00
60	30	24	80.00
30	30	15	50.00
15	30	10	33.33
6	21	5	23.81
2	21	2	9.52
0	15	0	0.00

Human whole blood was collected from 1 healthy CMV-negative donor in BD K2E tubes and spiked with CMV WHO standard material using different titers. Viral DNA was purified using the QIAsymphony DSP DNA Mini Kit and the virus blood 200 DSP protocol with an elution volume of 60 µl. Eluates were analyzed with a CMV real time PCR assay.

Tissue and FFPE tissue

DNA yield

The performance for the QIAsymphony DSP DNA FFPE tissue application was evaluated using 6 replicates of 1–4, 10 µm, FFPE sections of freshly cut human spleen. DNA extraction was performed using the QIAsymphony DSP DNA Mini Kit in combination with the tissue low content DSP protocol. Deparaffinization and lysis were performed using the xylene/ethanol pretreatment method. DNA was eluted in 50 µl elution buffer, and the yield of DNA was determined by spectroscopic analysis (Figure 4).

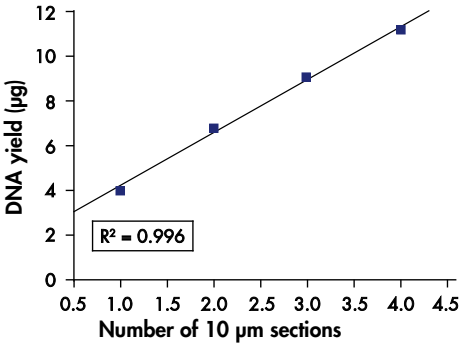


Figure 4. Correlation of DNA yield to numbers of FFPE tissue sections. Six replicates of 1–4, 10 µm, FFPE tissue sections of human spleen were deparaffinized by xylene/ethanol pretreatment. DNA extraction was performed on the QIAsymphony SP using the QIAsymphony DSP DNA Mini Kit in combination with the tissue low content DSP protocol and an elution volume of 50 µl.

Analysis of mutational status of biomarkers by real-time PCR

Analysis of mutational status of biomarkers was performed using DNA extracted from FFPE sections of human colon and DNA extracted from human lung tissue samples.

For DNA extraction from FFPE tissue samples, 3 x 10 µm sections of human colon were used for sample preparation. DNA extraction was performed using Deparaffinization Solution for pretreatment and the tissue low content DSP protocol in combination with the 100 µl elution volume. Mutational analysis of biomarker KRAS was performed using a real time PCR assay for KRAS detection in accordance with the assay handbook. C_T values of the control assay were within the defined range, and mutation detection analysis revealed an amino acid substitution in codon 12 demonstrated by a ΔC_T value of 4.17, which is below the defined cut-off value of 8 for the detection of a 12SER mutation (Table 2).

Table 2. Results of FFPE tissue KRAS biomarker mutational analysis

Sample	Reaction	Target C_T	Internal control C_T	ΔC_T*
No template control	Control	0.00	32.75	–
	12ALA	0.00	32.65	–
	12ASP	0.00	32.69	–
	12ARG	0.00	32.86	–
	12CYS	0.00	32.35	–
	12SER	0.00	32.76	–
	12VAL	0.00	32.41	–
	13ASP	0.00	32.26	–
Standard	Control	25.95	32.73	–
	12ALA	26.39	32.29	0.44
	12ASP	26.54	32.15	0.59
	12ARG	26.35	32.14	0.40
	12CYS	26.31	32.47	0.36
	12SER	26.50	32.34	0.55
	12VAL	25.80	31.92	–0.15
	13ASP	27.09	32.54	1.14
FFPE tissue (human colon)	Control	24.94	31.98	–
	12ALA	n.d.	32.42	–
	12ASP	n.d.	32.73	–
	12ARG	n.d.	33.05	–
	12CYS	n.d.	32.74	–
	12SER	29.11	32.34	4.17
	12VAL	n.d.	32.81	–
	13ASP	n.d.	33.20	–

* ΔC_T = M C_T – C C_T, where M means mutation and C means control; n.d., not detected.

For DNA extraction from frozen tissue samples, 25 mg of human lung tissue was used for sample preparation using the tissue high content DSP protocol and an elution volume of 200 µl. Mutational analysis of the EGFR biomarker was performed using a real time PCR assay for EGFR. Analysis of control and mutation detection was done as described in the assay handbook. Results revealed a deletion within the EGFR gene, as demonstrated by a ΔC_T value of 2.47, which is below the defined cut-off value of 12 for the detection of a mutation (Table 3).

Table 3. Results of frozen tissue EGFR biomarker mutational analysis

Sample	Reaction	Target C_T	Internal control C_T	ΔC_T*
No template control	Control	0.00	31.71	–
	T790M	0.00	32.36	–
	Deletions	0.00	31.75	–
	L858R	0.00	32.05	–
	L861Q	0.00	31.77	–
	G719X	0.00	31.68	–
	S768I	0.00	32.25	–
	Ins	0.00	31.84	–
Standard	Control	28.78	31.05	–
	T790M	30.08	31.13	1.30
	Deletions	28.23	31.19	–0.55
	L858R	27.58	30.83	–1.20
	L861Q	27.80	30.86	–0.98
	G719X	27.80	30.90	–0.98
	S768I	29.28	31.41	0.50
	Ins	28.00	31.64	–0.78
Tissue (human lung)	Control	25.76	31.23	–
	T790M	n.d.	31.99	–
	Deletions	28.23	30.99	2.47
	L858R	n.d.	31.33	–
	L861Q	n.d.	31.98	–
	G719X	n.d.	32.06	–
	S768I	n.d.	31.88	–
	Ins	n.d.	31.62	–

* ΔC_T = M C_T – C C_T, where M means mutation and C means control; n.d., not detected.

Repeatability and reproducibility

DNA blood

DNA extraction was performed using the blood 200 DSP protocol with an elution volume of 200 µl. Repeatability was evaluated by a single operator performing 3 independent runs (96 samples each) on 3 different days, with each run consisting of 4 batches of 24 samples (Table 4 and Table 5).

Reproducibility was evaluated by performing 3 independent runs (96 samples each) on 3 different days, by 3 different operators on different QIAsymphony SP instruments, with each run consisting of 4 batches of 24 samples (Table 6 and Table 7).

Table 4. Results of repeatability evaluation

Run	Batch	*n*	Mean DNA yield (µg)	SD	CV
1	1	24	5.32	0.22	4.22
	2	24	4.90	0.22	4.54
	3	24	4.95	0.21	4.26
	4	24	5.05	0.18	3.60
2	1	24	5.17	0.30	5.84
	2	24	4.90	0.15	3.14
	3	24	4.82	0.20	4.13
	4	24	4.87	0.17	3.52
3	1	24	5.11	0.17	3.33
	2	24	4.84	0.24	4.91
	3	24	4.87	0.16	3.38
	4	24	4.78	0.16	3.38
Total	–	288	4.96	–	–

n, Number of replicates; SD, standard deviation; CV, coefficient of variation.

Table 5. Precision data for repeatability evaluation

	SD	CV
Batch to batch within same run	0.25	4.95
Overall repetition accuracy	0.26	5.18

SD, standard deviation; CV, coefficient of variation.

Table 6. Results of reproducibility evaluation

Run	Batch	*n*	Mean DNA yield (µg)	SD	CV
1	1	24	5.32	0.22	4.22
	2	24	4.90	0.22	4.54
	3	24	4.95	0.21	4.26
	4	24	5.05	0.18	3.60
2	1	24	5.73	0.22	3.81
	2	24	5.56	0.26	4.63
	3	24	5.40	0.20	3.63
	4	24	5.46	0.21	3.89
3	1	24	5.73	0.26	4.62
	2	24	5.54	0.24	4.40
	3	24	5.41	0.18	3.34
	4	24	5.49	0.17	3.16
Total	–	288	5.38	–	–

n, Number of replicates; SD, standard deviation; CV, coefficient of variation.

Table 7. Precision data for reproducibility evaluation

	SD	CV
Batch to batch within same run	0.25	4.73
Overall repetition accuracy	0.38	7.03

SD, standard deviation; CV, coefficient of variation.

Comparative performance

DNA Blood

Performance was analyzed for the QIAsymphony DSP DNA blood system in comparison to the EZ1® DSP DNA blood system and the QIAamp® DNA Blood Mini Kit manual preparation procedure. DNA was purified from different blood samples, analyzed for DNA yield (Figure 5).

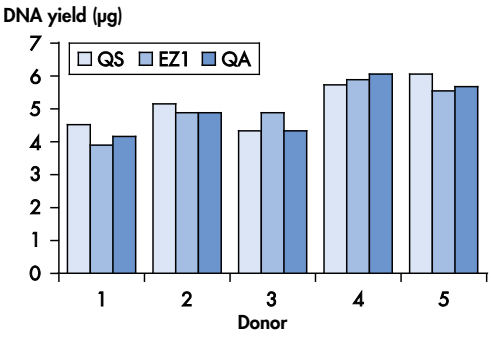


Figure 5. Comparison of DNA yields between different blood DNA purification systems. Whole blood was collected from 5 healthy donors in BD K2E tubes. For all methods, 200 µl sample input volumes and elution volumes of 200 µl were used. QS, QIAsymphony DSP DNA Mini Kit and blood 200 DSP protocol; EZ1, EZ1 Advanced XL using EZ1 DSP DNA Blood Kit; QA, QIAamp DNA Blood Mini Kit. The bars show the absolute DNA yield for each sample.

Tissue and FFPE tissue

The performance of the QIAsymphony DSP DNA Mini Kit was compared to the performance of the manual QIAamp DSP DNA FFPE Tissue Kit and the QIAamp DSP DNA Mini Kit using FFPE tissue and fresh and frozen tissues, respectively, as sample material. Manual and automated sample preparations, as well as quantification of the DNA yields, were performed simultaneously. DNA yields after extraction from fresh/frozen and FFPE tissue samples using the QIAsymphony DSP DNA Mini Kit, QIAamp DSP DNA Mini Kit, (tissue) and the QIAamp DSP DNA FFPE Tissue Kit (FFPE tissue) are shown in Figure 6.

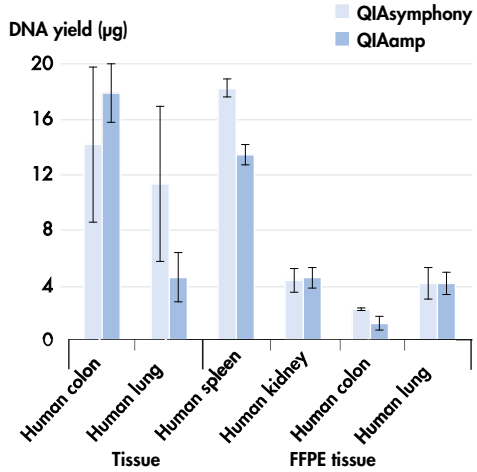


Figure 6. DNA extraction from tissue and FFPE tissue samples. For fresh/frozen tissue, human lung and colon samples were cut into 6 x 25 mg pieces. Three pieces of each tissue type were used for sample preparation using the QIAsymphony SP in combination with the tissue high content DSP protocol. DNA extraction from remaining samples was performed using the QIAamp DSP DNA Mini Kit. DNA was eluted in 200 µl, and DNA yield was determined by spectroscopic analysis. For DNA extraction from FFPE tissue, 12 replicates containing 3 x 10 µm FFPE tissue sections from various human organs were prepared. Six samples were used for sample preparation using the QIAsymphony SP in combination with the Deparaffinization Solution pretreatment and the tissue low content DSP protocol. DNA extraction from remaining samples was performed using the QIAamp DSP DNA FFPE Tissue Kit. DNA was eluted in 50 µl, and DNA yield was determined by spectroscopic analysis. Bars show the absolute DNA yield with standard deviation.

Sample input/eluate output range

DNA blood

Different sample input and eluate output ranges for the DNA blood application were compared using samples from blood donors with a WBC count range from 5.0 to 8.0 x 10^6 cells/ml.

Whole blood was collected from 8 healthy donors in BD K2E tubes. DNA was purified from 6 replicates, each using the QIAsymphony DSP DNA Mini/Midi Kit and the DNA blood 200 DSP protocol with 200 µl elution volume, DNA blood 400 DSP protocol with 400 µl elution volume and the DNA blood 1000 DSP protocol with 500 µl elution volume (Figure 7).

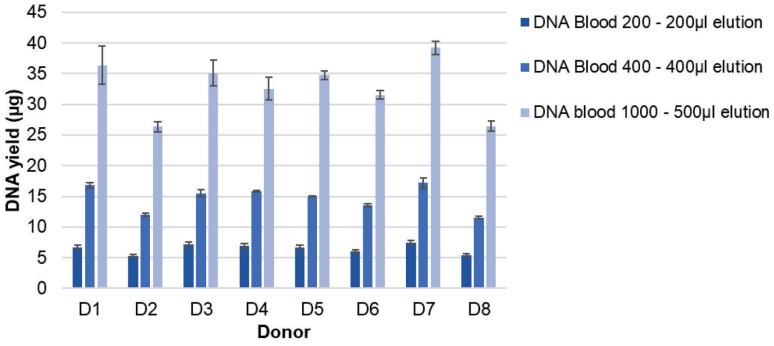


Figure 7. Comparison of different sample inputs and elution volumes for the blood DNA purification systems. Whole blood was collected from 8 healthy donors in BD K2E tubes. DNA extraction was performed using the DNA blood 200 protocol with 200 µl elution volume, the DNA blood 400 protocol with 400 µl elution volume, and the DNA blood 1000 protocol with 500 µl elution volume. DNA yield was determined by spectroscopic analysis. The bars show the absolute DNA yield (mean value with standard deviation) for each donor.

Virus blood

Whole blood was collected from 3 healthy donors, with a WBC count range from 4.0 to 11.0 x 10^6 cells/ml, in BD K2E tubes and spiked with CMV standard material (titer 3.7 log copies/ml). Viral DNA was purified from 7 replicates, each using the QIAsymphony DSP DNA Mini Kit and the virus blood 200 DSP protocol with 4 different elution volumes (Figure 8).

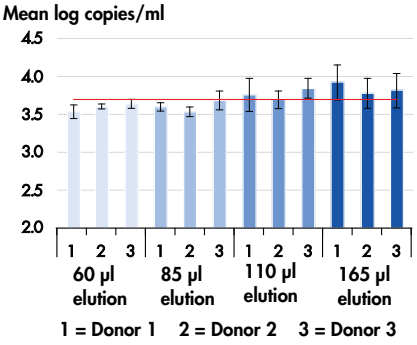


Figure 8. Comparison of viral DNA quantification for different elution volumes. Eluates from each donor sample and elution volume (60, 85, 110, and 165 µl) were analyzed with a CMV real time PCR assay. The red line represents the target titer and bars show mean log copies per milliliter with standard deviation.

Eluate stability

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

DNA blood and buffy coat

Eluate stability for the DNA blood application was tested using eluates from QS runs performed with the DNA Blood 200 protocol with 200 µl elution volume and with the DNA Blood 1000 protocol with 500 µl elution volume. Eluates were stored in 2ml Sarstedt Tubes at room temperature, 2–8°C, –20°C, and –80°C. DNA yield and purity was determined by spectroscopic analysis. DNA integrity was analyzed by gel electrophoresis and a LongRange PCR assay (Figure 9).

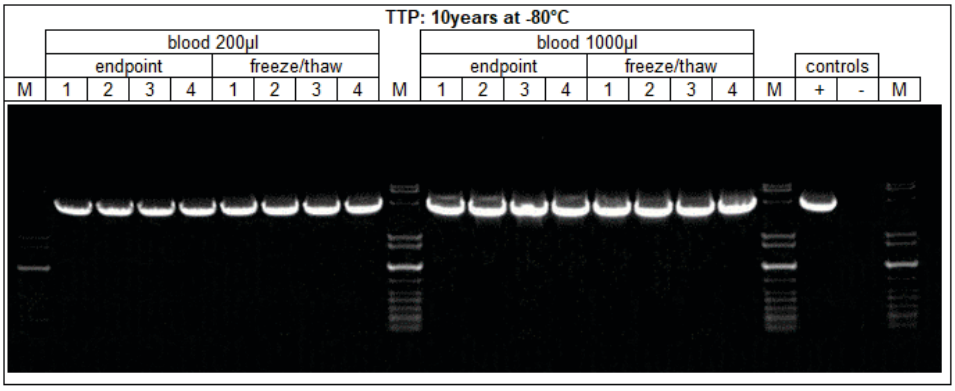


Figure 9. Eluate stability for DNA blood. The DNA was purified using the DNA Blood 200 µl and 1000 µl protocols. Eluates have been stored at –80°C in 2 ml Sarstedt Tubes. Four replicates were analyzed. DNA integrity was tested by long-range PCR. The figures shows the results after storage for 10 years. M, QIAGEN GelPilot 1 kb Plus Ladder.

Eluate stability for the buffy coat application was tested using eluates from QS runs performed with the BC 400 µl protocol and 200 µl elution volume. Eluates were stored in 2 ml Sarstedt Tubes and Elution Micro Tube Racks at room temperature, 2–8°C, –20°C, and –80°C. Moreover, eluates were subjected to freeze/thaw testing for up to 3 cycles (Figure 10). DNA yield and purity were determined by spectroscopic analysis. DNA integrity was analyzed by gel electrophoresis and a LongRange PCR assay (50 µl reaction).

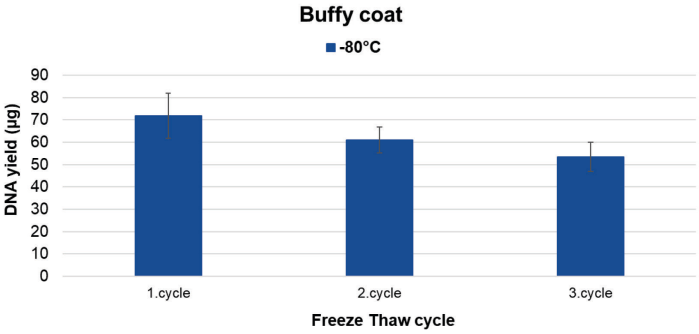


Figure 10. Eluate freeze/thaw cycles for buffy coat. The DNA was purified using the DNA BC 400 µl protocol. Buffy coat was generated from EDTA blood. Eluates have been stored in 2 ml Sarstedt Tubes. The yield of DNA was determined at the test time points by using the same eluate at 3 freeze/thaw cycles. DNA yield was determined by spectroscopic analysis. The bars show the absolute DNA yield (mean value with standard deviation).

Virus blood

Eluate stability for the virus blood application was tested using eluates from QS runs performed with the Virus Blood 200 protocol with 60 µl elution volume. K_2 EDTA blood spiked with commercial CMV standard (titer 2.7 log copies/ml) was used as sample material. Eluates were stored in 2 ml Sarstedt Tubes at 2–8°C, –20°C, and –80°C. Eluates were analyzed using a CMV real time assay (Figure 11). In the following, results of several test time points are shown.

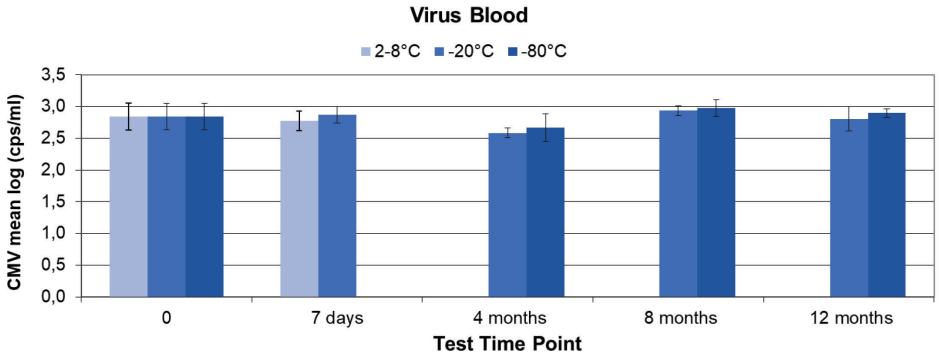


Figure 11. Eluate stability for virus blood application. EDTA blood samples spiked with commercial CMV standard were purified with the Virus Blood 200 protocol. Eluates have been stored at several temperatures in Elution Micro tube racks and 2 ml Sarstedt Tubes. Per Test time point 4 replicates were analyzed. The bars show the CMV titer (mean log value with standard deviation).

Tissue

Eluate stability for the tissue application was tested using the Tissue HC 200 µl protocol and 200 µl elution volume. Fresh bovine liver was used as sample material. Eluates were stored in 2 ml Sarstedt Tubes and Elution Micro Tube Racks at room temperature, 2–8°C, –20°C, and –80°C. DNA yield and purity were determined by spectroscopic analysis (Figure 12). DNA integrity was analyzed by gel electrophoresis.

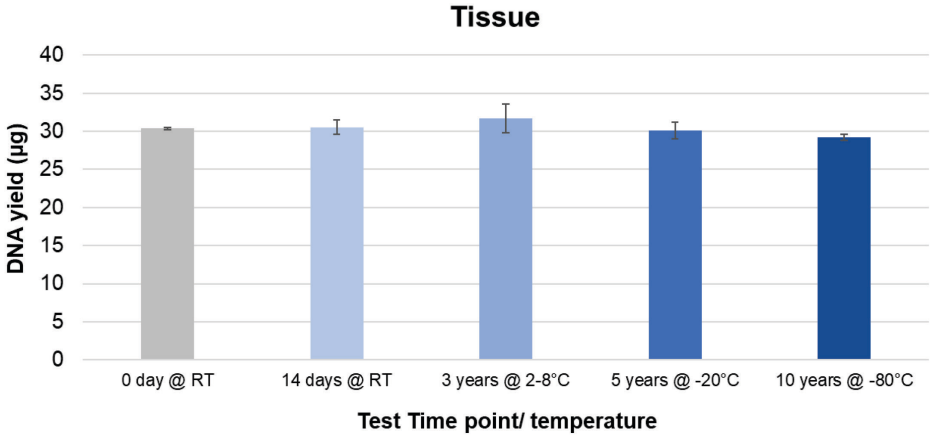
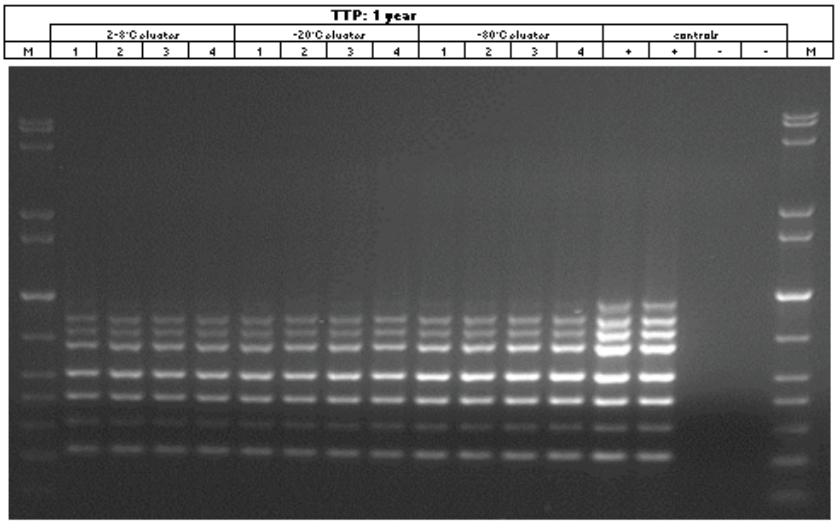


Figure 12. Eluate stability for tissue. The DNA was purified using the DNA Tissue HC protocol with 200 µl elution volume. Fresh bovine liver was used as sample material. Eluates have been stored at several temperatures in Elution Micro tube racks and 2 ml Sarstedt Tubes. Per test time point, 4 replicates were analyzed. DNA yield was determined by spectroscopic analysis. The bars show the absolute DNA yield (mean value with standard deviation).

FFPE tissue

Eluate stability for the FFPE tissue application was tested using the Tissue LC 200 µl protocol and 100 µl elution volume. Commercial human FFPE tissue was used as sample material. Eluates were stored in 2 ml Sarstedt Tubes and Elution Micro Tube Racks at room temperature, 2–8°C, –20°C, and –80°C. Eluates were analyzed with an Inhouse human 8-plex PCR assay (Figure 13).In the following, results of two test time points are shown.

A:



B:

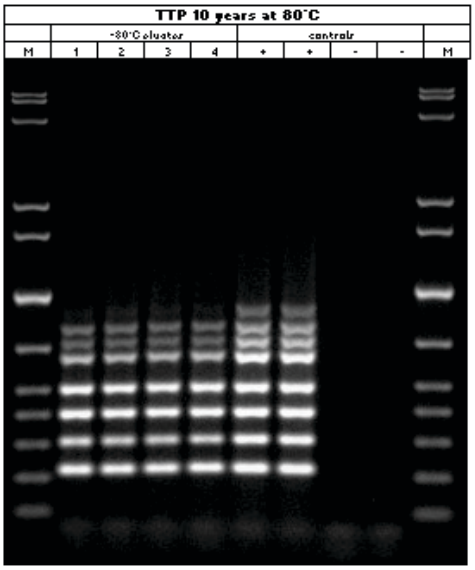


Figure 13. Eluate stability for FFPE tissue. The DNA was purified using the DNA Tissue LC protocol. Commercial FFPE tissue was used as sample material. Eluates have been stored at several temperatures in Elution Micro tube racks and 2 ml Sarstedt Tubes. Per test time point, 4 replicates were analyzed. Eluates were analyzed by Inhouse human 8-plex PCR assay.

Interfering substances

The influence of inhibitory substances, which may be present in whole blood, on the performance of the DNA blood application, virus blood application, and tissue application was tested by addition of the following substances:

Table 8. Potential interfering substances tested for the different applications

Interfering substances	Concentration	Blood	Virus blood	Tissue
Bilirubin	200 mg/L	√	√	√
Hemoglobin	200 g/L	√	√	
Triglycerides	30 g/L	√	√	√
Protein	120 g/L	√	√	√

Note: "√" indicates which sample materials were tested for the respective potential interfering substance.)

For hemoglobin (200 g/l) and protein (120 g/l), existing levels in the blood sample were determined and additional hemoglobin or protein was added to achieve the indicated concentrations, 200 or 120 g/l, respectively. For bilirubin (200 mg/l) and triglycerides (30 g/l), the total amount of each substance was added to the samples to achieve the indicated concentrations.

For tissue, the total amount of each substances was added directly to the lysates, no determination for the bilirubin, triglyceride, or protein concentration of the used tissue sample was done.

Any potential interfering substances (e.g., drugs) and corresponding concentration is very specific to the downstream application and possible previous medical treatments of a patient and needs to be investigated during verification of such downstream application using the QIAsymphony DSP DNA Mini and Midi Kits.

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 or concentration of potential interfering substances), so the identification and testing of relevant substances and respective concentrations also needs to be established as part of the downstream application development for any workflow involving the QIAsymphony DSP Mini and Midi Kits.

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

DNA blood and buffy coat

For the DNA blood applications testing was performed using the DSP DNA 1000 protocol, which covers the highest sample input volume, using 200 and 500 µl elution volumes.

Eluates were analyzed by spectroscopic analysis for DNA yield and purity. PCR compatibility was tested by using a real-time PCR as well as an endpoint PCR assay.

None of the substances listed in Table 9 are interfering; however, blood samples with high concentrations of triglycerides (>30 g/l) may lead to reduced gDNA yield.

Virus blood

For the virus blood application, testing was performed using the DSP Virus Blood 200 protocol with 60 µl elution volume. CMV-negative blood samples were spiked with 500 copies/ml (low concentration) and 1x10 E+04 copies/ml (high concentration, Figure 14) of a commercial CMV standard.

Eluates were analyzed with a CMV Real-time PCR assay.

None of the substances listed in Table 9 are interfering; however, blood samples with high concentrations of triglycerides (>30 g/l) may lead to a reduced purification of viral DNA.

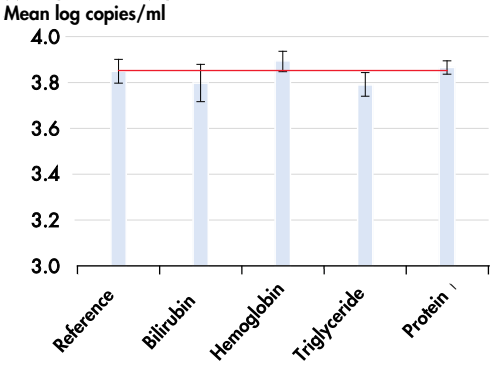


Figure 14. Inhibitory substance test. Whole blood was collected from 1 healthy donor in BD K2E tubes and spiked with CMV standard material (titer 4.0 log copies/ml). Five samples were tested by addition of potential inhibitors, and viral DNA was purified from 4 replicates of each sample using the QIAsymphony DSP DNA Mini Kit and the virus blood 200 DSP protocol with an elution volume of 165 µl. Eluates were analyzed with a CMV real time PCR assay. The red line represents the determined titer for reference samples, which were not spiked with any inhibitory substance, and bars show mean log copies per milliliter with standard deviation.

Tissue

For DNA tissue (fresh and frozen), testing was performed using the DSP DNA HC protocol, using 200 µl elution volume.

Eluates were analyzed by spectroscopic analysis for DNA yield and purity. PCR compatibility was tested by using a real-time PCR assay.

None of the substances listed in Table 9 were identified to have a negative impact on the sample preparation.

FFPE tissue

For FFPE tissue, testing was performed using the DSP DNA LC protocol, using 50 µl elution volume.

The substances (refer to Table 9) were added directly to the lysate.

Table 9. Potential interfering substances tested for the different applications

Interfering substances	Concentration in lysate
Xylene	Up to 11%
Ethanol	Up to 11%
Deparaffinization solution	Up to 11%
Paraffin	0.1 µM section

Eluates were analyzed by spectroscopic analysis for DNA yield and purity. PCR compatibility was tested by using a real-time PCR as well as an Inhouse human 8-plex PCR assay.

None of the substances listed in Table 9 were identified to have a negative impact on the sample preparation.

Cross-contamination

DNA blood

The risk of cross contamination of the QIAsymphony DNA Blood application was analyzed by performing four 96 sample runs on the QIAsymphony SP instrument with alternating checkerboard batches (positive and negative samples alternating), interrupted by completely negative batches. Male blood (containing a WBC count of ≥1.0 x 10^7 cells/ml and female blood containing a WBC count between 4.0 x 10^6 and 9 x 10^6 cells/ml) was used as a model system. Sample preparation was performed using the blood 1000 µl protocol, which covers the highest sample volume. A potential contamination of the negative female samples during the extraction runs was evaluated by subsequent analysis of the eluates using a real-time PCR for the Y-chromosome.

No cross contamination was detected for a sample to sample, or batch to batch, 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

Revision History

Revision	Description
R1, June 2022	Version 2, Revision 1 • Update to version 2 for compliance to IVDR • Sections for Interfering substances, Cross contamination, Eluate stability and Compatibility to downstream applications 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.