June 2024

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

IVD

For In Vitro Diagnostic Use

For use with

	Σ	REF	Version
QIAsymphony DSP Circulating DNA Kit (192)	192	937556	V2
QIAsymphony DSP Circulating DNA Maxi Kit (192)	192	937566	V1
QIAsymphony DSP Circulating DNA Kit (96)	96	937555	V1

CE

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R2

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 Circulating DNA system constitutes a ready-to-use in vitro system for the qualitative purification of circulating cell-free DNA (ccfDNA) from human plasma and urine.

The QIAsymphony DSP Circulating DNA Kit is intended to be used only in combination with the QIAsymphony SP instrument.

The QIAsymphony DSP Circulating DNA Kit provides reagents for fully automated and simultaneous purification of ccfDNA from a broad range of human plasma types (with ccfDNA profile stabilizers, e.g., PAXgene® Blood ccfDNA Tube from PreAnalytiX; Cell-Free DNA BCT® from Streck® as well as without ccfDNA profile stabilizers, e.g., EDTA tubes) and human urine (with and without ccfDNA profile stabilizers). However, a performance characteristic for every blood collection tube has not been established and must be validated by the user.

The purified ccfDNA is compatible with a wide range of downstream applications, such as PCR chemistries, fluorescence-based quantification assays or NGS.

The QIAsymphony SP performs all steps of the purification procedure. Up to 96 samples, in batches of 24, are processed in a single run. Urine samples may require manual sample pretreatment.

> **Note**: Performance Characteristics highly depends on various factors and relates to the specific downstream application. It has been established for the QS DSP Circulating DNA 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, performance parameter, for example, cross contamination and 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

The basic performance for the QIAsymphony DSP Circulating DNA Kit was evaluated using 48 single donors for ccfDNA extraction from 4 mL Streck plasma as well as 4 mL stabilized urine. The ccfDNA yield has been determined with an in-house real-time PCR assay for the 18S ribosomal RNA coding sequence.

The difference in yields (log 10 copies/mL) in Figure 1 (4 mL plasma) and Figure 2 (4 mL urine) reflect the strong donor-dependent concentrations of ccfDNA typically found in the same volume of the respective sample material.

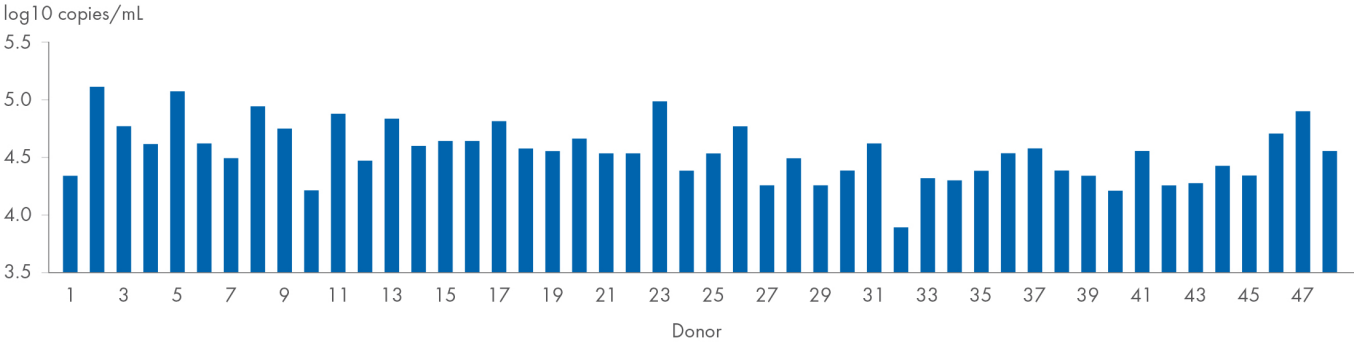

Figure 1. The ccfDNA yield from plasma from 48 single donors. Blood donation from 48 single donors was made in Cell-Free DNA BCT (Streck). CcfDNA was extracted from 4 mL plasma using the QIAsymphony DSP Circulating DNA Kit. CcfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. The results were calculated as target copies per milliliter plasma input.

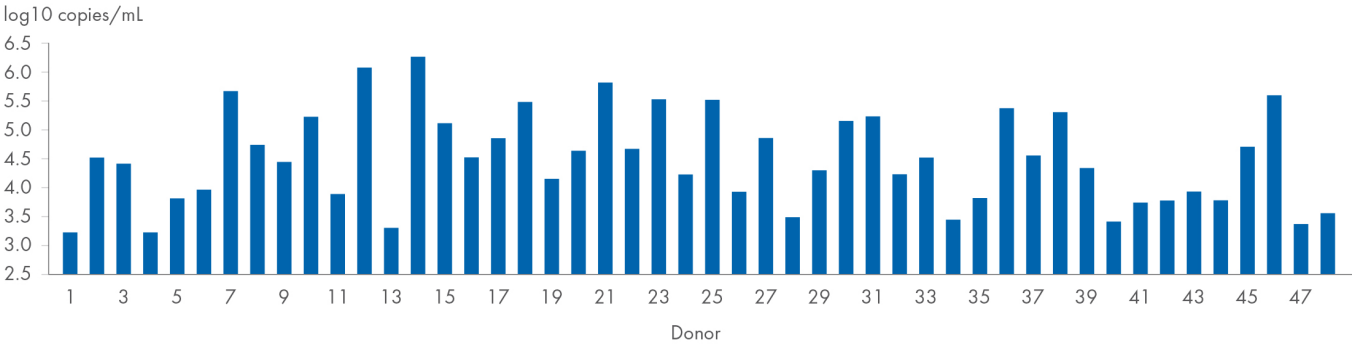

Figure 2. The ccfDNA yield from urine from 48 single donors. Urine collected from 48 single donors was stabilized using Cell-Free DNA Urine Preserve® (Streck). CcfDNA was extracted from 4mL urine using the QIAsymphony DSP Circulating DNA Kit. CcfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as target copies per milliliter urine input.

In addition, the basic performance for the QIAsymphony DSP Circulating DNA Kit was evaluated in comparison to a manual ccfDNA extraction method, the QIAamp DSP Circulating NA Kit, cat. no. 61504. For this purpose, the plasma was generated from PAXgene® Blood ccfDNA tubes (CE-IVD) from 24 single donors for ccfDNA extraction from 4 mL volume and the ccfDNA was eluted for both ccfDNA extraction kits in 75 µL. The ccfDNA yield has been determined with an in-house real-time PCR assay for the 18S ribosomal RNA coding sequence. The difference in yields (copies/mL) in Figure 3 reflects the strong donor-dependent concentrations of ccfDNA typically found in plasma.

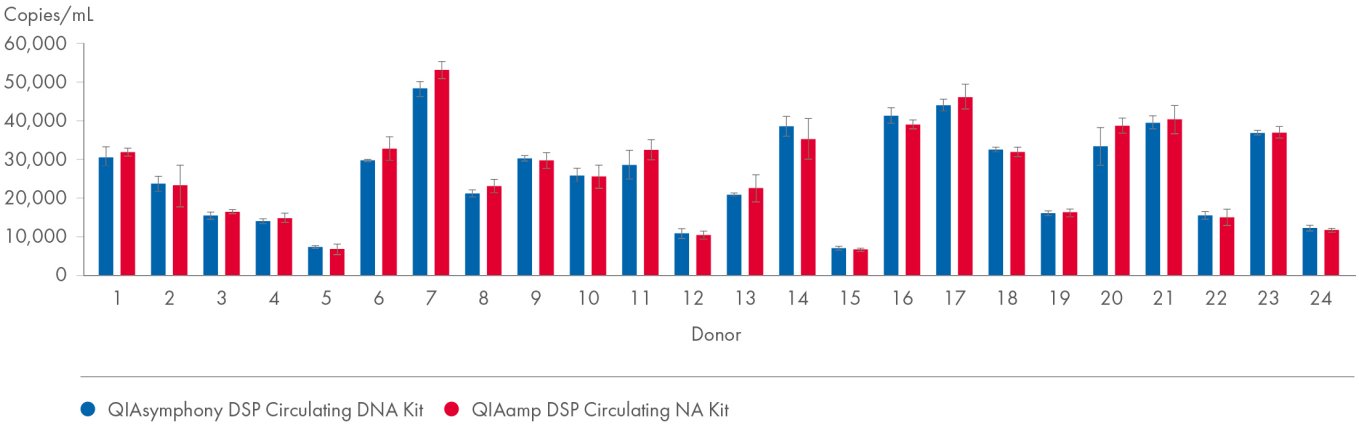

Figure 3. Equivalent ccfDNA extraction performance for the QIAsymphony DSP Circulating DNA Kit compared to the QIAamp DSP Circulating NA Kit. Plasma collected from 24 single donors was stabilized using PAXgene Blood ccfDNA tube. CcfDNA was extracted from 4 mL plasma using the QIAsymphony DSP Circulating DNA Kit and the QIAamp DSP Circulating NA Kit. CcfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as target copies per milliliter plasma input.

The performance of the automated and manual ccfDNA extraction kit is equivalent, measured in calculated copies per milliliter. The ratio of geometric mean for the QIAsymphony DSP Circulating DNA Kit and QIAamp DSP Circulating NA Kit is shown in Table 1 (The reference kit is the QIAsymphony DSP Circulating DNA Kit).

Table 1. Ratio of geometric mean QIAamp DSP Circulating NA Kit / QIAsymphony DSP Circulating DNA Kit (*N* = 213)

Parameter	Value
Estimated ratio of geometric mean in calculated copies/ml	1.074
Lower 95% confidence limit	1.048
Upper 95% confidence limit	1.100

Run precision

Coefficients of variations (CVs) were determined for the extraction of human ccfDNA from EDTA plasma. For precision analysis, ccfDNA was quantified using an in-house real-time PCR assay for the 18S ribosomal coding sequence. In total, 10 QIAsymphony runs were performed each in 4 batches (8 replicates per batch). The precision data are shown in Table 2.

Table 2. Analysis of precision estimates

Precision	CV (%)
Within batch	11.67
Repeatability	13.14
Intermediate precision	13.14
Total precision	14.12

Equivalent performance of 2 mL and 4 mL protocols

Equivalent performance of protocols for 2 mL and 4 mL sample input was evaluated for the QIAsymphony DSP Circulating DNA Kit using endogenous ccfDNA extracted from a human EDTA plasma pool. In total, 8 independent QIAsymphony runs were performed each in 4 batches with 8 replicates per batch. The linear range of the QIAsymphony DSP Circulating DNA Kit procedure has been determined for the 18S coding sequence with an in-house real-time PCR assay (Figure 4). The ratio of difference for the 2 and 4 mL protocols is shown in Table 3 (The reference protocol is 4 mL sample input).

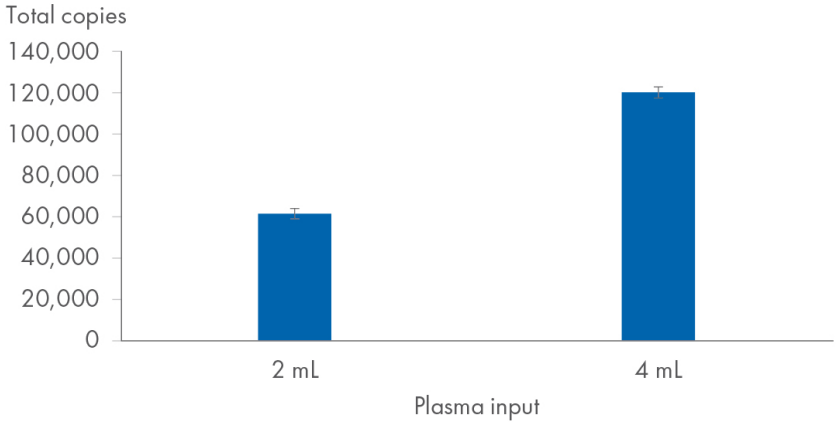

Figure 4. Equivalent performance using the 2 and 4 mL sample input protocol. The linear range of the ccfDNA protocol was determined using the 2 and 4 mL protocols. The ccfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as total copies per protocol.

Table 3. Difference between 2 and 4 mL protocols (*N* = 256)

Parameter	Value
Estimated ratio of geometric mean in calculated copies/ml	1.01
Lower 95% confidence limit	0.92
Upper 95% confidence limit	1.11
Total precision	14.12

The performance of protocols for 2 and 4 mL sample input is equivalent, measured in calculated copies per milliliter.

Linear ccfDNA extraction efficiency from 1–10 mL sample volume

Equivalent performance of protocols for 1–10 mL sample input was evaluated for the QIAsymphony DSP Circulating DNA Kit using endogenous ccfDNA extracted from a human plasma and urine pool. Plasma was generated from Streck Cell-Free DNA BCT®, and urine was stabilized using Streck® Urine Preservative. Stabilized plasma and urine were pooled from minimum 10 donors and stored at –20°C until use. CfDNA was extracted from 1, 2, 4, 6, 8, and 10 mL volume using the QIAsymphony DSP Circulating DNA Kit in combination with circDNA protocols for 1 mL up to 10 mL sample volume. For each input volume, 12 replicates were extracted. The linear range of the QIAsymphony DSP Circulating DNA Kit procedure has been determined for the 18S coding sequence with an in-house real-time PCR assay (Figure 5).

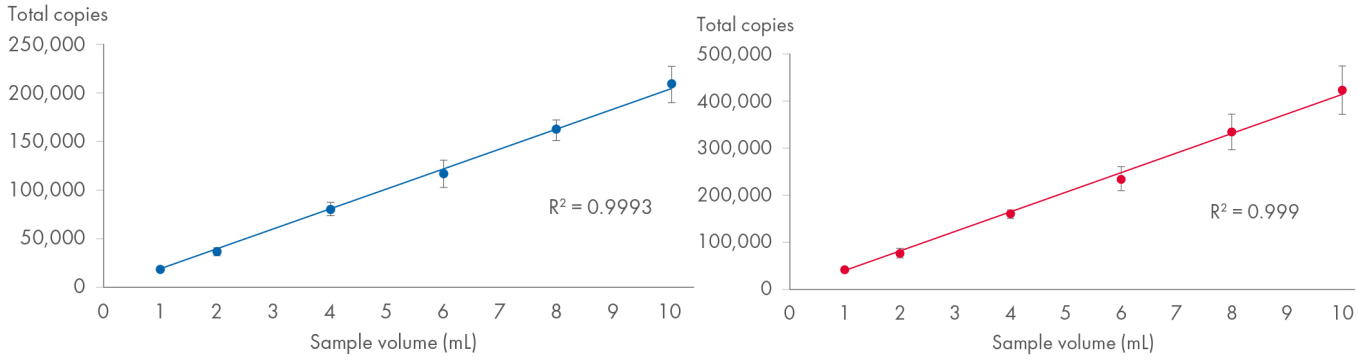

Figure 5. Linear ccfDNA extraction efficiency from 1–10 mL sample volume. The linear range of the ccfDNA protocol was determined using the 1, 2, 4, 6, 8, and 10 mL protocols. CcfDNA was extracted from stabilized plasma (left figure, blue dots) and stabilized urine (right figure, red dots). The ccfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as total copies per protocol.

Size distribution

To evaluate the size distribution of sample output, ccfDNA from a sample input of 4 mL was extracted using the QIAsymphony DSP Circulating DNA Kit, eluted in 75 µL and then 1 µL of eluate was subjected to size analysis with the Agilent® 2100 Bioanalyzer using an Agilent High Sensitivity DNA Chip. A total of 5 independent replicates were performed. One representative DNA profile is shown for plasma in Figure 6A and for urine in Figure 7.

The electropherogram for plasma in Figure 6A shows the frequently observed peak at approximately 165 bp, ranging from 145 to 196 bp, which is in the range of the length of the histone-bound DNA in the nucleosome. The electropherogram for urine in Figure 7 shows that the predominant peak at approximately 160 bp is broader, ranging from approximately 145 to 250 bp. In addition, for urine

a second peak ranging from approximately 20 to 100 bp (at the level of the lower marker peak) is present indicating a ccfDNA fraction with a higher degree of fragmentation. Moreover, Figure 7 shows a high number of long DNA fragments from approximately 2 kb. High abundance of such genomic DNA fragments is often found in urine sample most likely due to genomic DNA release from cells present in urine.

Next to the peak at approximately 165 bp for the histone-bound DNA (mono-nucleosome), extraction of cfDNA from large sample volumes reveals in addition peaks for the multi-nucleosomes at approximately 350 bp and >500 bp Figure 6B). For this purpose, ccfDNA from 1–10 mL plasma, generated from PAXgene Blood ccfDNA Tubes, was extracted using the QIAsymphony DSP Circulating DNA Kit, eluted in 75 µL and then 1 µL of eluate was subjected to size analysis with the Agilent® Cell-free DNA Screen Tape.

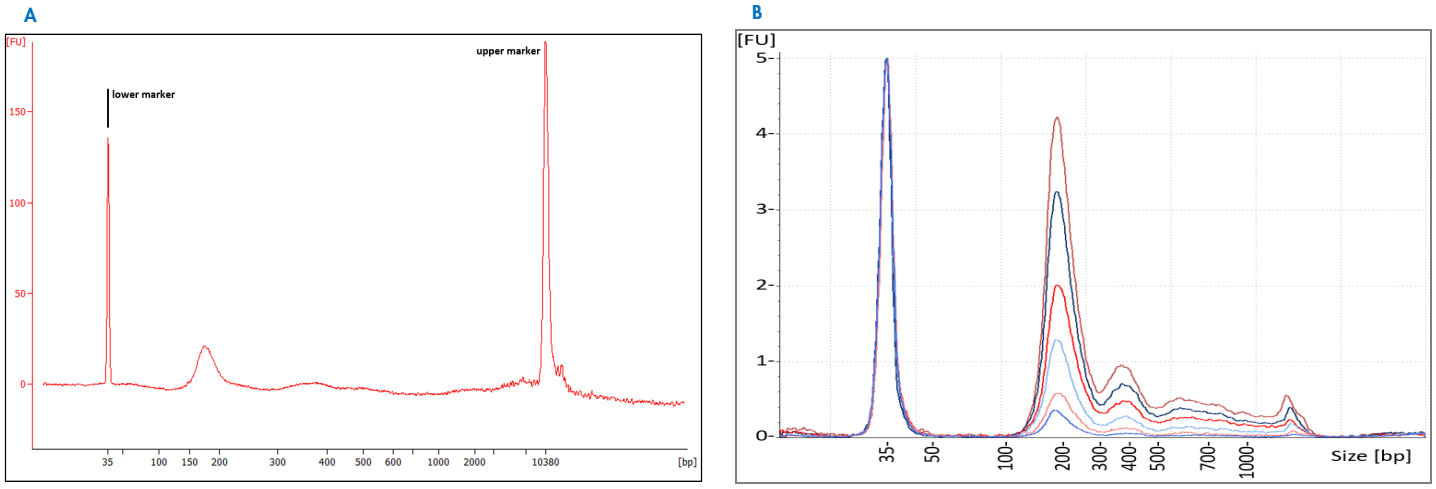

Figure 6. Size distribution of ccfDNA from plasma (Bioanalyzer profile). (A) The ccfDNA was extracted from 4 mL EDTA plasma using the QIAsymphony DSP Circulating DNA Kit; 1 µl eluate was subjected to an Agilent High Sensitivity DNA Chip analysis. x axis: base pair size (bp); y axis: fluorescence units (FU). **(B)** The ccfDNA was extracted from 1 mL, 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL plasma, generated from PAXgene® Blood ccfDNA tubes, using the QIAsymphony DSP Circulating DNA Kit; 1 µL eluate was subjected to an Agilent Cell-free DNA Screen Tape analysis. The six size profiles in different colors illustrate the increase in sensitivity for detection of the ccfDNA size distribution depending on the plasma input volume from 1–10 mL used for extraction. x axis: base pair size (bp); y axis: fluorescence units (FU), peak at 35 bp: lower marker.

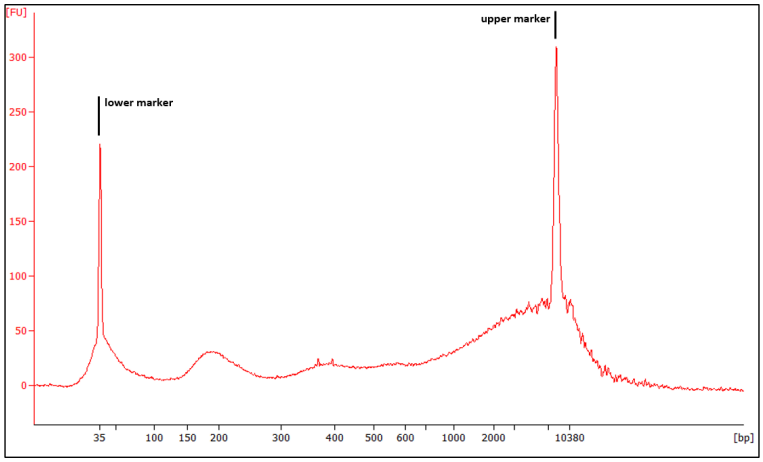

Figure 7. Size distribution of ccfDNA from urine (Bioanalyzer profile). The ccfDNA was extracted from 4 mL urine using the QIAsymphony DSP Circulating DNA Kit; 1 µL eluate was subjected to an Agilent High Sensitivity DNA Chip analysis. x axis: base pair size (bp); y axis: fluorescence units (FU).

Eluate stability

Eluate stability for the QIAsymphony DSP Circulating DNA Kit was evaluated, using extracted ccfDNA from a human EDTA plasma pool. Eluates were stored in 2 different elution rack formats: QIAGEN® EMTR (Elution Microtubes CL 96; cat. no. 19588) and 1.5 mL Eppendorf® LoBind® Snap Cap Safe-Lock tubes. Eluates were analyzed in replicates of 8. Stability of DNA in eluates was determined with an in-house real-time PCR assay for the 18S ribosomal RNA coding sequence.

Eluate stability at 2–8°C was not affected by duration of the storage period up to one month, or by storage format (Figure 8). Stability of DNA in LoBind tubes was not affected by storage at –15°C to –30°C that included 3 freeze–thaw cycles after 7 days, one month and two months (Figure 9).

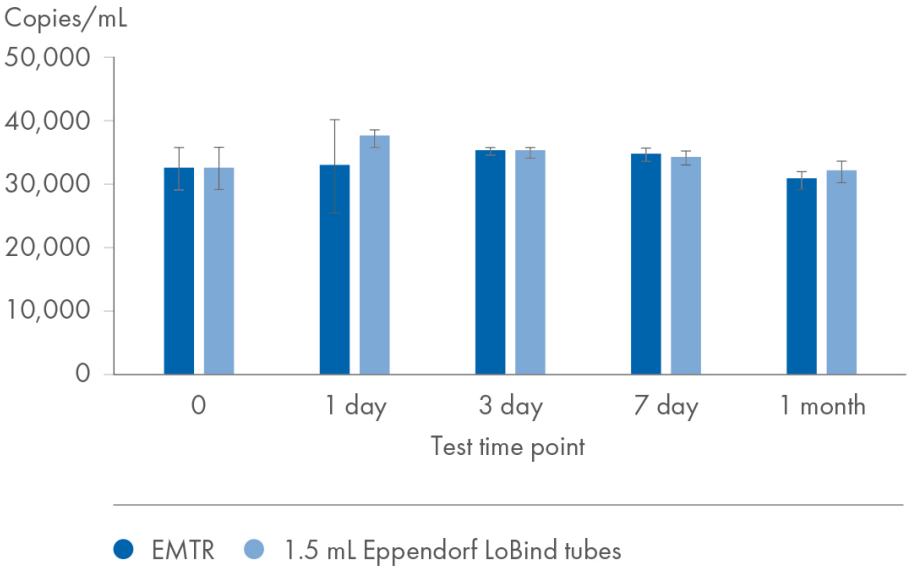

Figure 8. Stability of ccfDNA in eluates stored at 2–8°C in 2 tube formats. The ccfDNA was extracted from EDTA plasma using the QIAsymphony DSP Circulating DNA Kit and stored at 2–8°C for different test time points. The yield of ccfDNA was quantified using an in-house real-time PCR assay for the 18S coding sequence. The results were calculated as target copies per milliliter plasma input.

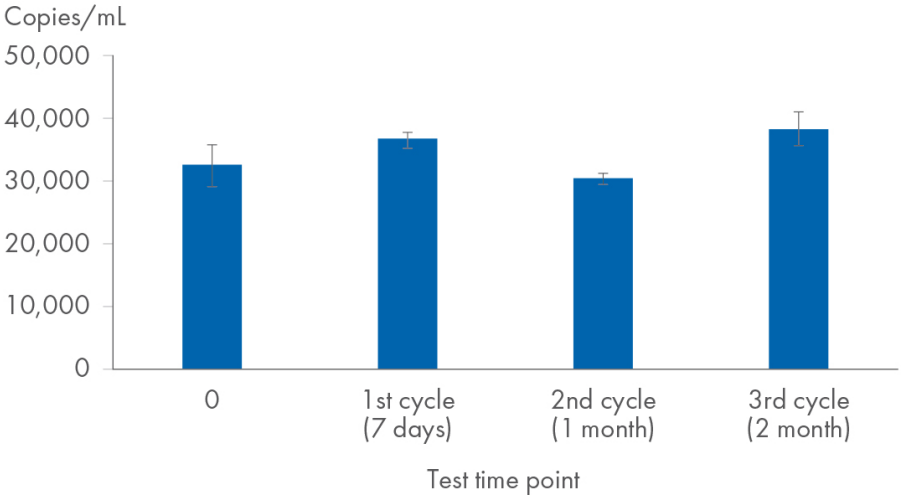

Figure 9. Stability of ccfDNA in eluates stored at –15°C to –30°C including 3 freeze–thaw cycles. The ccfDNA was extracted from EDTA plasma using the QIAsymphony DSP Circulating DNA Kit and stored at –15°C to –30°C in 1.5 mL Eppendorf LoBind tubes. The yield of ccfDNA was determined at 3 test time points by using the same eluate at

3 freeze–thaw cycles. The yield of ccfDNA was quantified using an in-house real-time PCR assay for the 18S coding sequence. The results were calculated as target copies per milliliter plasma input.

Interfering substances

Human plasma and urine were spiked with different potential interfering substances (see Table 4) to test their impact on the ccfDNA extraction performance of the QS DSP Circulating DNA Kit and subsequent compatibility to exemplary downstream assays. Eluates were analyzed with an in-house real-time PCR for the 18S coding sequence and with the Qubit® Fluorometer using a High Sensitivity dsDNA assay.

Table 4. Test concentrations of potential interfering substances

Interfering substances	Plasma	Urine
Bilirubin	200 mg/liter*	200 mg/liter*
Hemoglobin	2 g/liter[1]	-
BSA and Gamma-Globin	Up to 120 g/liter*	1 g/liter†
Triglycerides	5 g/liter*	-
Glucose	10 g/liter*	10 g/liter*
Blood	-	1%†
pH	-	pH 4 and pH 9†

* CLSI EP7-A2 Vol. 25 No. 27
† FDA Draft Guidance (11.05.2011)

None of the substances listed in Table 4 are interfering, with following exceptions: plasma samples with high concentrations of gamma-globulin (>30 g/liter) may lead to reduced recovery of circulating cell-free DNA.

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 QIAsymphony DSP Circulating DNA kit.

Cross-contamination

The risk of cross-contamination of the QIAsymphony DSP Circulating DNA system was analyzed for protocols with 1 mL, 4 mL, and 10 mL sample volume that include one, two and five separate sample transfer steps of each 1 mL or 2 mL volume. Three 96 sample runs (1 mL and 4 mL) and six 48 sample runs (10 mL) were performed on the QIAsymphony SP instrument with alternating checkerboard batches (positive and negative samples alternating). For 1 mL and 4 mL sample volume, female plasma (negative sample) and female plasma spiked with sheared male gDNA of a concentration of 1.0E+05 copies of SRY1 gene per milliliter plasma (positive sample) were used as sample materials for a model system. For 10 mL sample volume, plasma (negative sample) and plasma spiked with a 1000 bp DNA fragment from the GFP gene of a concentration of 1.0E+05 copies per milliliter plasma (positive sample) were used as sample materials for a model system.

A potential contamination of the negative plasma samples during the extraction runs was evaluated by subsequent analysis of the eluates using a real-time PCR for the Y-chromosome specific gene SRY1 (1 mL and 4 mL protocol) and for the GFP specific sequence (10 mL protocol).

No cross-contamination was detected for a sample to sample, batch to batch, or run to run carry over.

Equivalent ccfDNA extraction for the three QIAsymphony DSP Circulating DNA Kits

The equivalent performance for the QIAsymphony DSP Circulating DNA Kit (192), cat. no. 937556, QIAsymphony DSP Circulating DNA Kit (96), cat. no. 937555 and QIAsymphony DSP Circulating DNA Maxi Kit (192), cat. no. 937566 was evaluated using 24 single donors for ccfDNA extraction from 2 mL or 6 mL Streck plasma. The ccfDNA yield has been determined with an in-house real-time PCR assay for the 18S ribosomal RNA coding sequence (Figure 10).

The difference in yields (copies/mL) reflect the strong donor-dependent concentrations of ccfDNA typically found in the same plasma volume.

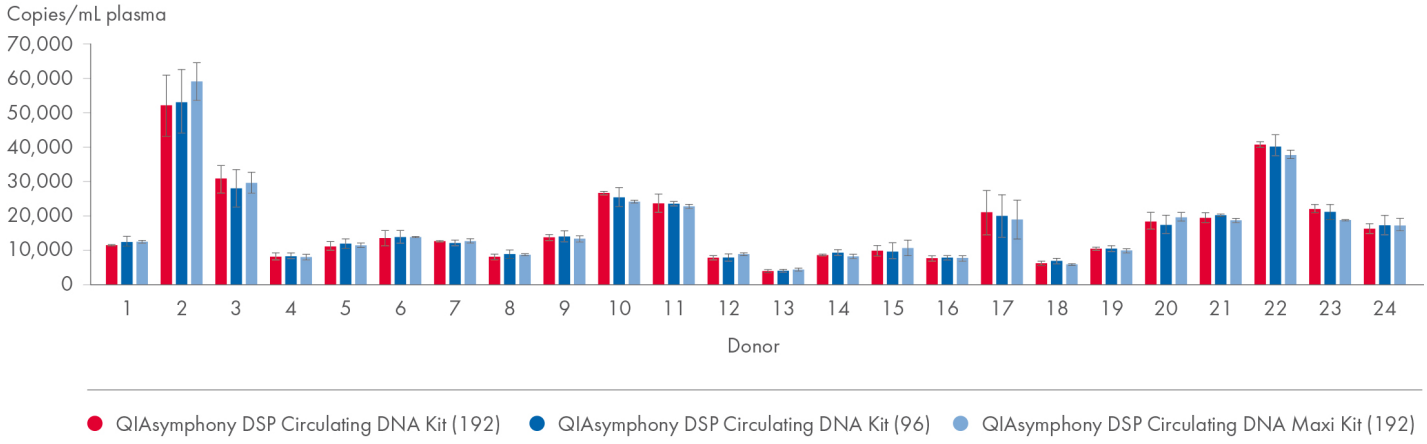

Figure 10. Equivalent ccfDNA extraction efficiency for the three QIAsymphony DSP Circulating DNA Kits. Blood donation from 24 single donors was made in Cell-Free DNA BCT (Streck). CcfDNA was extracted from 2 mL plasma using the QIAsymphony DSP Circulating DNA Kit (192) and QIAsymphony DSP Circulating DNA Kit (96) and from 6 mL plasma using the QIAsymphony DSP Circulating DNA Maxi Kit (192). For each kit and donor ccfDNA was extracted from three replicates resulting in total nine data points per donor. CcfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. The results were calculated as target copies per milliliter plasma input.

The performance of the three QIAsymphony DSP Circulating DNA applications is equivalent, measured in calculated copies per milliliter. The ratio of difference for the QIAsymphony DSP Circulating DNA Kit (192), the QIAsymphony DSP Circulating DNA Maxi Kit (192), and QIAsymphony DSP Circulating DNA Kit (96) is shown in Table 5.

Table 5. The back transformed difference and two-sided 95% confidence interval to give the ratio of geometric mean (*N* = 216)

Difference Calculated	Estimate	Lower Two-sided 95% Confidence Limit	Upper Two-sided 95% Confidence Limit
QIAsymphony DSP Circulating DNA Kit (96) / QIAsymphony DSP Circulating DNA Kit (192)	1.012	0.969	1.057
QIAsymphony DSP Circulating DNA Maxi Kit (192) / QIAsymphony DSP Circulating DNA Kit (192)	1.002	0.960	1.047
QIAsymphony DSP Circulating DNA Kit (96) / QIAsymphony DSP Circulating DNA Maxi Kit (192)	1.009	0.964	1.056

Compatibility to different downstream applications

Exemplary downstream applications were utilized during the development of the QIAsymphony DSP Circulating DNA kit to demonstrate that the isolated nucleic acids are compatible to a wide range of different downstream application technologies, including Real Time-PCR (see Figures 1–5 and Figures 8–10), Qubit Fluorometer (protein assay and high sensitivity dsDNA assay), Library (see Figure 11), and Next Generation Sequencing (NGS).

The electropherogram in Figure 11 shows an example for a successful adapter ligation and subsequent amplification of ccfDNA. Next to the prominent peak at 300 bp for the nucleosomal ccfDNA (approx. 165 plus approx. 70 bp for each adapter), also the di-nucleosomal peak at approx. 470 bp is visible.

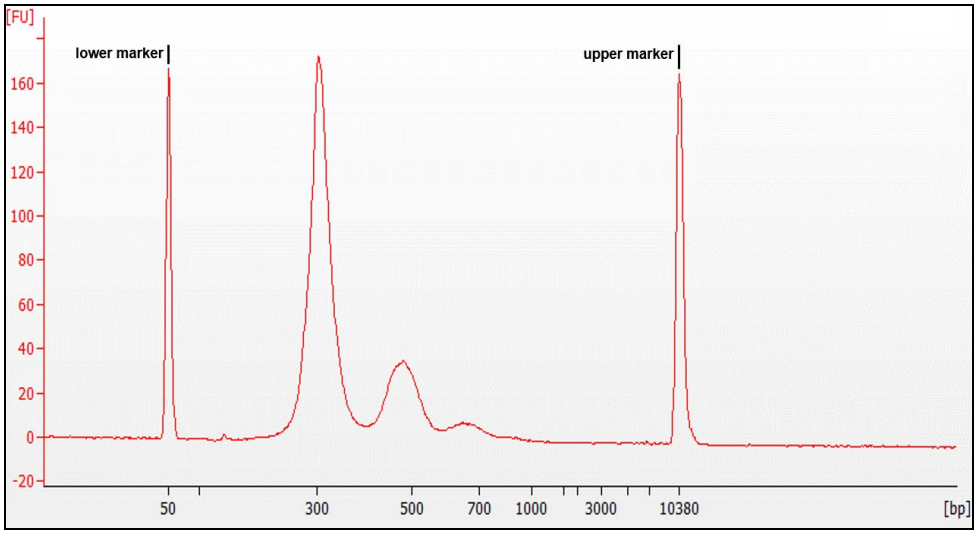

Figure 11. DNA Library of ccfDNA (single donor) extracted with the QIAsymphony DSP Circulating DNA Kit. The ccfDNA was extracted from Streck plasma using the 4 mL protocol and subsequently 35 µL eluate was transferred into the NEBNext® Ultra DNA Library Prep Kit (Biolabs). After amplification and AMPure XP cleanup 1µL eluate was analyzed with the Agilent 7500 DNA Kit.

Symbols

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

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 • Section for Interfering substances, Cross contamination and Compatibility to downstream applications added
R2, June 2024	• Document version was removed from revision history • Update to add performance data for the QIAsymphony DSP Circulating DNA Maxi Kit (192) and QIAsymphony DSP Circulating DNA Kit (96) in combination with BioScripts for 6 mL, 8 mL, and 10 mL sample volume. • Add performance data for the BioScript for 1 mL sample volume

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.