February 2017

QIAsymphony® DSP Circulating DNA Kit

Performance Characteristics

IVD

CE

MAT 937556

Sample to Insight

QIAGEN

Contents

The QIAsymphony DSP Circulating DNA system constitutes a ready-to-use in vitro system for the qualitative purification of human 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 human ccfDNA from a broad range of human plasma types (EDTA or citrate anti-coagulated as well as plasma from ccfDNA stabilized blood collection tubes) and human urine (stabilized and non-stabilized). 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. 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.

Performance Characteristics

Basic performance

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

The difference in yields (log10 copies/ml) in Figure 1 (4 ml stabilized plasma), Figure 2 (4 ml EDTA plasma) and Figure 3 (4 ml stabilized urine) reflects the strong donor-dependent concentrations of ccfDNA typically found in the same volume of the respective sample material. The ccfDNA yield between stabilized and EDTA plasma shows a high correlation for the 48 single donors using plasma from two different types of BCTs (Figure 1 and Figure 2).

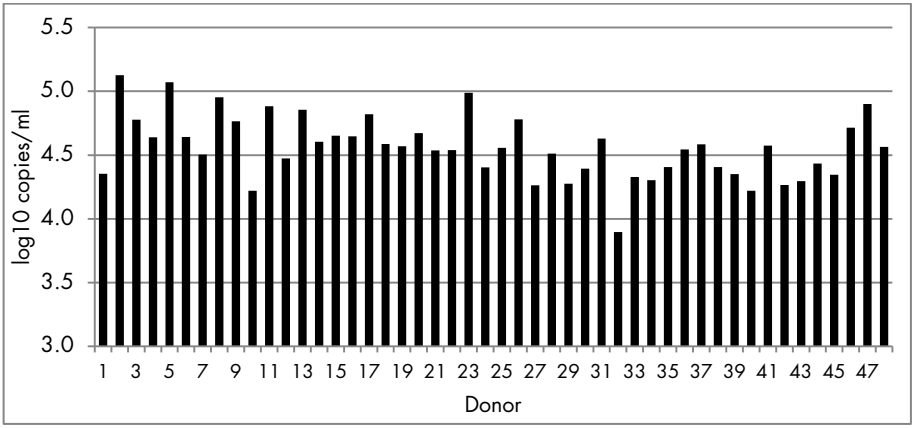

Figure 1. The ccfDNA yield from plasma from 48 single donors: ccfDNA stabilized blood collection tubes. Blood donation from 48 single donors was made in ccfDNA stabilized blood collection tubes. The ccfDNA was extracted from 4 ml plasma using the QIAsymphony DSP Circulating DNA Kit and ccfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as target copies per ml plasma input.

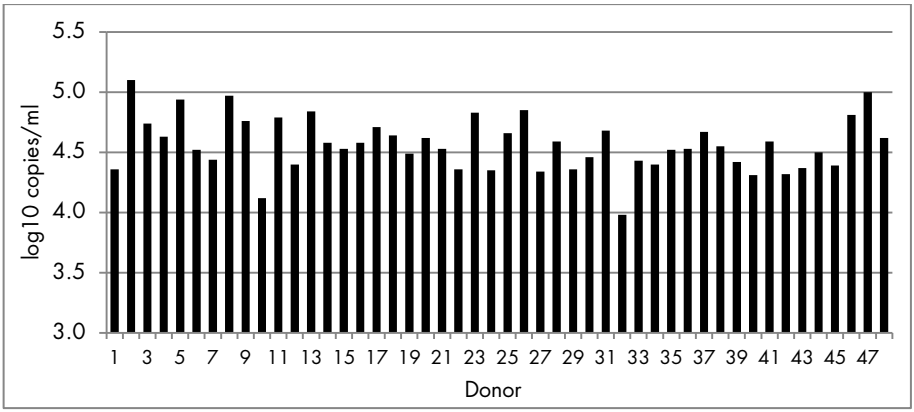

Figure 2. The ccfDNA yield from plasma from 48 single donors: EDTA blood collection tubes. Blood donation from 48 single donors was made in EDTA blood collection tubes. The ccfDNA was extracted from 4 ml plasma using the QIAsymphony DSP Circulating DNA Kit and ccfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as target copies per ml plasma input.

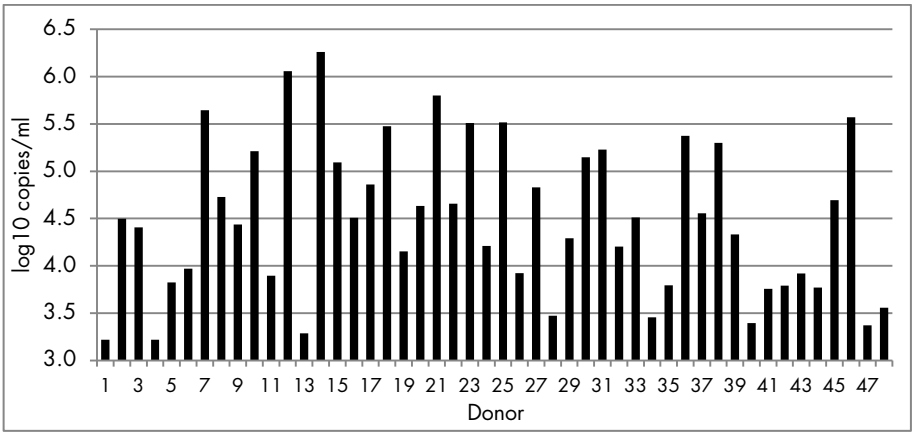

Figure 3. The ccfDNA yield from stabilized urine from 48 single donors. Urine from 48 single donors was stabilized immediately after collection. The ccfDNA was extracted from 4 ml urine using the QIAsymphony DSP Circulating DNA Kit and ccfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as target copies per ml urine input.

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 1.

Table 1. 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. A total of 8 independent QIAsymphony runs was performed, each run in 4 batches with 8 replicates per batch. The linear range of the QIAsymphony DSP Circulating DNA Kit procedure was determined for the 18S coding sequence with an in-house real-time PCR assay (Figure 4). The ratio of difference for the 2 ml and 4 ml protocols is shown in Table 2. (The reference protocol is 4 ml sample input).

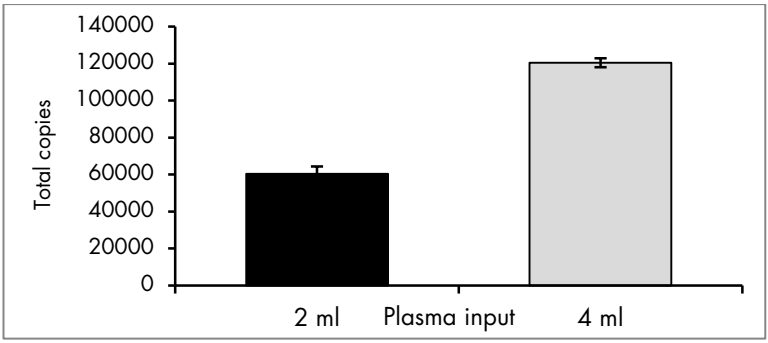

Figure 4. Equivalent performance using the 2 ml and 4 ml sample input protocol. The linear range of the ccfDNA protocol was determined using 2 ml and 4 ml protocols. The ccfDNA yield was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as total copies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

Table 2. Difference between 2 ml 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

The performance of protocols for 2 ml and 4 ml sample input is equivalent, measured in calculated copies/ml.

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 was performed. One representative DNA profile is shown for plasma in Figure 5 and for stabilized urine in Figure 6.

The electropherogram for plasma in Figure 5 shows the frequently observed peak at ~160 bp, ranging from 145 bp 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 6 shows that the predominant peak at ~160 bp is broader, ranging from ~145 bp to 250 bp. In addition, for urine a second peak ranging from ~20 bp 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 6 shows a high number of long DNA fragments from ~ 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.

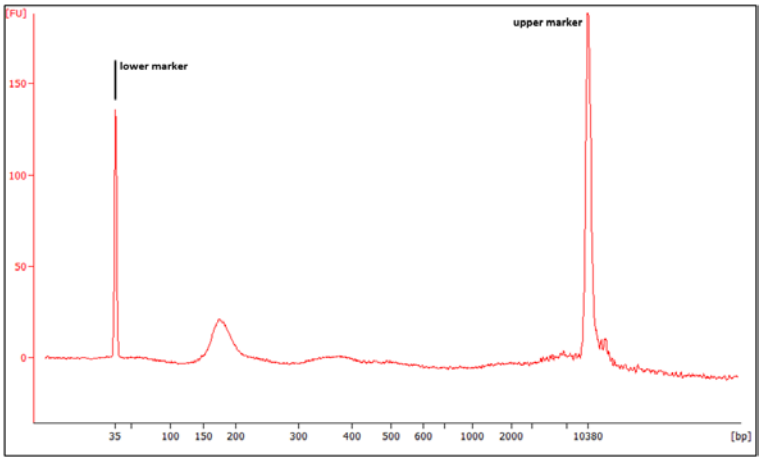

Figure 5. Size distribution of ccfDNA from plasma (Bioanalyzer profile). The ccfDNA was extracted from 4 ml EDTA plasma using the QIAsymphony DSP Circulating DNA Kit; 1 µl eluate was subjected to an Agilent High Sensitivity DNA Chip analysis. X-axis: base pair size (bp); Y-axis: fluorescence units (FU).

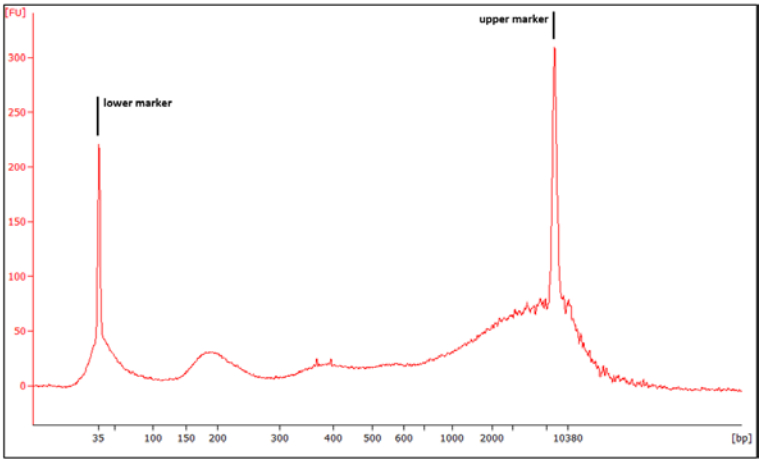

Figure 6. Size distribution of ccfDNA from urine (Bioanalyzer profile). The ccfDNA was extracted from 4 ml stabilized urine using the QIAsymphony DSP Circulating DNA Kit; 1 µl eluate was subjected to an Agilent High Sensitivity DNA Chip analysis. X-axis: base pair size (bp); Y-axis: fluorescence units (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 7). Stability of DNA in LoBind tubes was not affected by storage at –15 to –30°C that included 3 freeze-thaw cycles after 7 days, one month and two months (Figure 8).

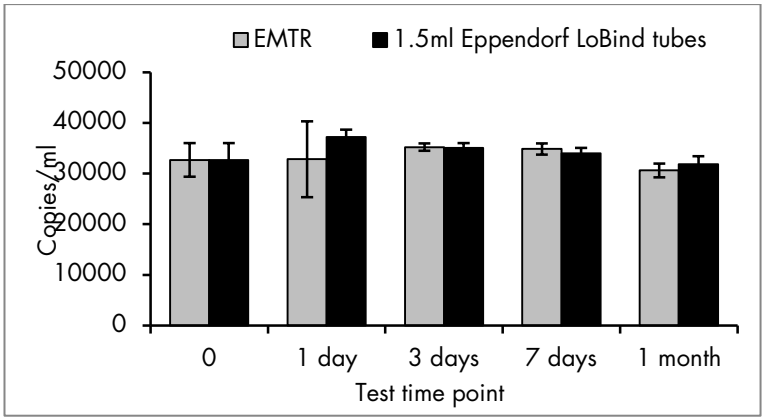

Figure 7. Stability of ccfDNA in eluates stored at 2–8°C in 2 tube formats. The ccfDNA was extracted from EDTA plasma using the QIAsymphony DSP Circulating DNA Kit and stored at 2–8°C for different test time points. The yield of ccfDNA was quantified using an in-house real-time PCR assay for the 18S coding sequence. Results were calculated as target copies per ml plasma input.

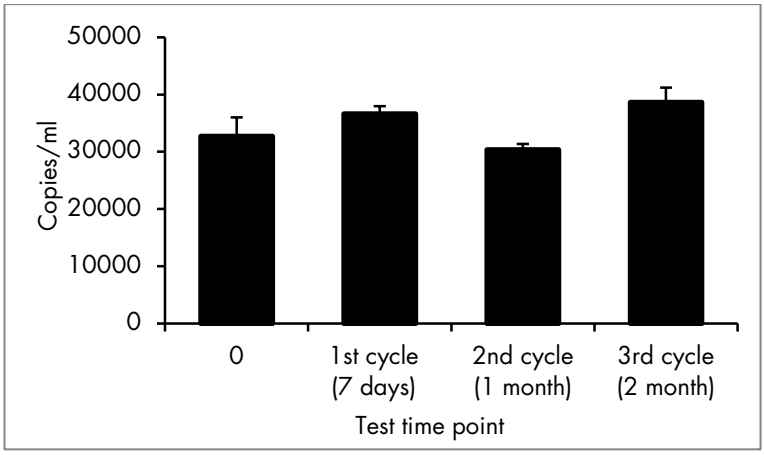

Figure 8. Stability of ccfDNA in eluates stored at –15 to –30°C including 3 freeze-thaw cycles. The ccfDNA was extracted from EDTA plasma using the QIAsymphony DSP Circulating DNA Kit and stored at –15 to –30°C in 1.5 ml Eppendorf LoBind tubes. The yield of ccfDNA was determined at 3 test time points 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. Results were calculated as target copies per ml plasma input.

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.

02/2017 HB-2309-D01-001