Performance Characteristics

QIAamp® DSP Circulating NA Kit, Version 1 REF 61504

Version management

CE IVD ⚠	Check availability of new electronic labeling revisions at **www.qiagen.com** under the product number and product resources before test execution.

General introduction

The QIAamp DSP Circulating NA Kit is a system that uses silica-membrane technology (QIAamp technology) for isolation and purification of circulating, cell-free (ccf) DNA and RNA from human blood plasma samples.

The product is intended to be used by professional users, such as technicians and physicians, who are trained in molecular biological techniques.

The QIAamp DSP Circulating NA Kit is intended for in vitro diagnostic use.

Yield of purified nucleic acids (NA)

Plasma samples may exhibit a high variance in yield of purified nucleic acids. Therefore, the users should optimize the plasma input and elution volume for their specific target and downstream application in their laboratory.

If the kit is being used in conjunction with a QIAGEN® downstream application, refer to the relevant handbook for instructions.

Analysis of Downstream Applications

Nucleic acids isolated with the QIAamp DSP Circulating NA Kit are ready for use in different downstream applications. To evaluate the performance, nucleic acids from single-donor human blood plasma were isolated using three different blood collection tubes (BD Vacutainer® K2EDTA Tube, Becton Dickinson®; PAXgene® Blood ccfDNA Tube, PreAnalytiX; and Streck® Cell-Free DNA Blood Collection Tube (BCT)®, Streck; n=24 donors each). Eluates from 1 ml plasma input were tested using

quantitative PCR (qPCR, Figure 1A), digital droplet PCR (ddPCR, Figure 1B), as well as reverse transcription qPCR (RT-qPCR) for RNA (only BD Vacutainer K2EDTA Tube plasma, Figure 2).

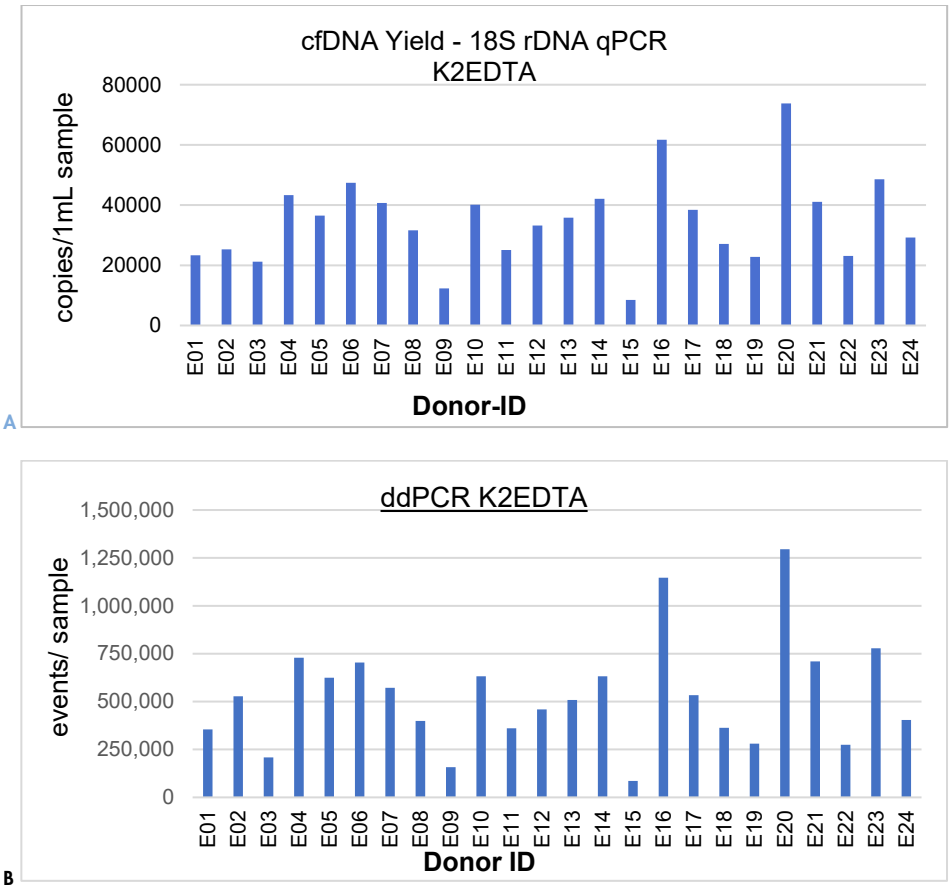

Figure 1. Comparison of single donor plasma (1 ml input) between qPCR and ddPCR (Bio-Rad®)

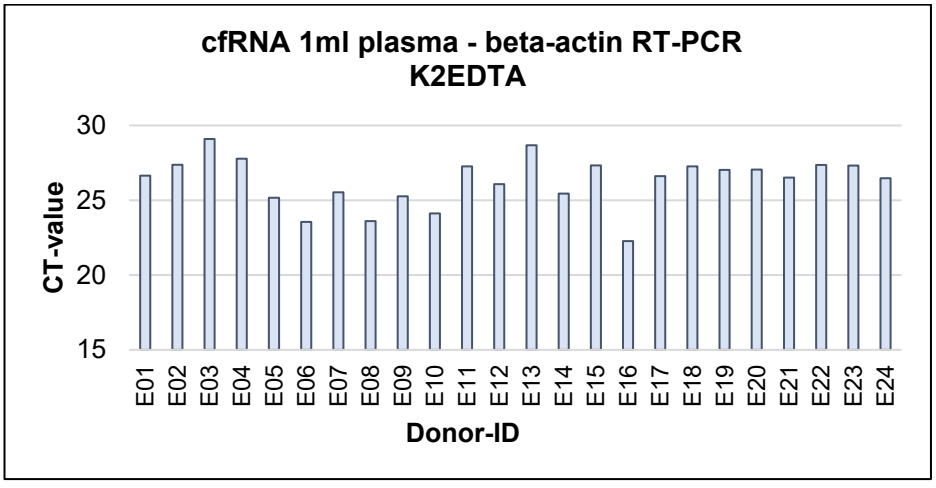

Figure 2. Detection of cell-free RNA in single donor plasma (1 ml input) using a Reverse Transcription-qPCR assay for the human beta-actin gene (293 bp fragment length).

For Next Generation Sequencing (NGS) analysis, eluates from 5 ml plasma input volume (BD Vacutainer K2EDTA Tube, PAXgene Blood ccfDNA Tube, and Streck Cell-Free DNA BCT; n=8 donors each) were generated. The total DNA yield for 5 ml plasma ranged between 50–150 ng DNA detected with the Qubit® HS dsDNA assay. NGS analysis was done using the GeneRead® QIAact Actionable Insights Tumor Panel and the GeneReader™ system. All samples were successfully enriched, and libraries were generated. >98% of the generated reads were mapped to the human genome, and >99.8% of positions in the regions of interest had a base coverage of ≥500x.

For both nucleic acid species (DNA and RNA), successful application of downstream technologies was shown (Figure 3).

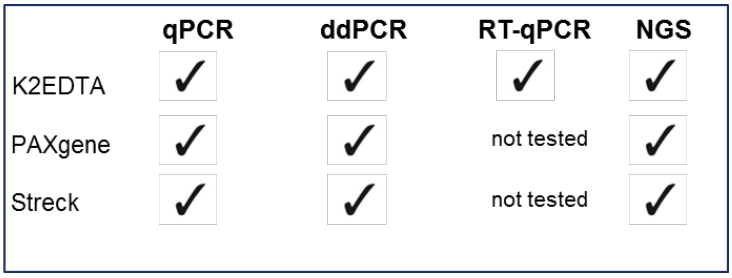

Figure 3. Successful use of isolated nucleic acids with different downstream applications.

The user should optimize the plasma input and elution volume for their target molecule and any subsequent procedures used in their laboratory or refer to the specific performance of the relevant downstream application.

Eluate stability

Eluate stability will depend on the content and type of isolated nucleic acids, elution volume, and storage conditions. We recommend that users establish the eluate stability as needed for their particular requirements.

Eluate stability was tested for DNA and eluates derived from human plasma generated from BD Vacutainer K2EDTA Tube (Becton Dickinson) and stabilizing blood collection tubes (PAXgene Blood ccfDNA Tube and Streck Cell-Free DNA BCT). Eluates were stored at –30 to –15°C and –90 to –65°C. No deterioration was observed for up to 12 months. Eluates stored at 2–8°C and at room-temperature (15–25°C) were stable for up to 48 hours. All conditions were assessed using qPCR targeting the human 18S rDNA gene.

Eluate stability was tested for RNA, and eluates derived from human plasma generated from BD Vacutainer K2EDTA Tubes (Becton Dickinson). Eluates were stored at –30 to –15°C and –90 to –65°C. No deterioration was observed for up to 6 months. Eluates stored at 2–8°C were stable for up to 48 hours. All conditions were assessed using RT-qPCR targeting the human beta-actin gene.

If the kit is being used in conjunction with QIAGEN downstream applications, refer to the relevant kit handbook for instructions.

Precision of NA isolation

Precision was evaluated using human plasma, and conditions were assessed using qPCR targeting the human 18S rDNA gene.

The experimental setup comprised 12 purification runs with 12 replicates each (total 144 purifications). Purification runs were arranged with three different operators on three different days with three different instruments using three different lots of the QIAamp DSP Circulating NA Kit. The standard deviation (SD) and coefficient of variation (CV) were determined for each single parameter and for the overall variability (total) of the QIAamp DSP Circulating NA Kit (Table 1).

Table 1. Precision results

Precision			
Parameter	**Mean Copies/ml**	**SD**	**CV (%)**
Run to run	25894	461	1.78
Operator to Operator		1392	5.38
Instrument to Instrument		228	0.88
Day to Day		2096	8.09
Lot to Lot		969	3.74
Total		3120	12.05

Linearity

Data have been generated for 1–5 ml plasma input volume from blood stored in BD Vacutainer K2EDTA Tubes, PAXgene Blood ccfDNA Tubes, and Streck Cell-Free DNA BCTs. For all BCTs, a linear increase of DNA yield was observed (see Figure 4); for BD Vacutainer K2EDTA Tubes, this was also the case for RNA.

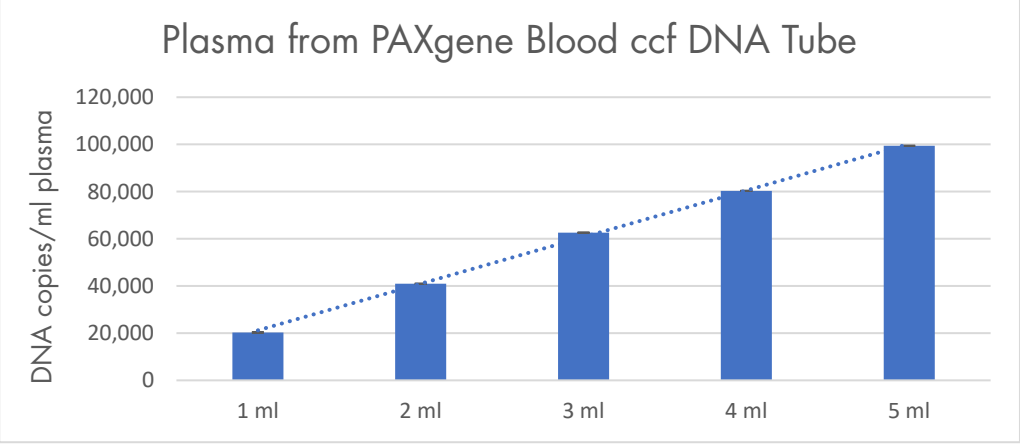

Figure 4. Linear increase of total DNA yield (DNA copies/ml plasma input) for different plasma input volumes. Data for plasma generated from PAXgene Blood ccfDNA Tube shown, equivalent results for plasma derived from BD Vacutainer K2EDTA Tube (DNA/RNA) and Streck Cell-Free DNA BCT.

Protocol Equivalency (Breeze/Classic Protocols)

Equivalency in performance between the breeze protocol and the classic protocol was determined by showing that the corresponding 95% confidence limit of difference in mean Ct value (RNA) or mean copies/ml (DNA) was within ± 2 x STD, with STD being the observed precision of the classic protocol (reference condition). Three kit lots were used, and three operators performed the experiments.

The total precision (STD) of the Ct values generated for the breeze protocol was less than the upper limit of the two-sided 95% prediction interval for the total precision (STD) of the classic protocol, wherein the prediction interval was calculated within the study using the data from the classic protocol (n=143) and using the number of data points for the breeze protocol (n=144) in the study.

Interfering substances

Potentially interfering substances can originate from different sources, e.g., natural metabolites, substances introduced during patient treatment, or substances ingested by the patient. For the QIAamp DSP Circulating NA Kit hemoglobin, triglycerides, EDTA, caffeine, albumin, conjugated bilirubin, and unconjugated bilirubin were tested as endogenous components. No interference was found when applying qPCR as downstream application. Furthermore, no interference derived from components of the QIAamp DSP Circulating NA Kit (Proteinase K, Buffer ACL, Buffer ACB, Buffer ACW1, Buffer ACW2, and Ethanol) during sample processing and nucleic acid extraction was observed.

Due to the complexity of potential interfering substances and different sensitivity of specific downstream applications, we recommend that users assess the effect of interfering substances specific for their own workflow and validate a method for controlling interference in their specific diagnostic downstream application.

For more information on interfering substances in specific QIAGEN® downstream applications, refer to the relevant kit handbooks.

Cross-contamination

To assess the level of cross-contamination, 10^5 copies of HBV-virus were spiked into 5 ml or 2 ml human blood plasma (positive samples) and were isolated adjacent to virus-free samples (negative samples) in a checker-board setup alternating with extraction runs containing only negative samples (to assess intra- and inter-extraction run cross-contamination). The study aimed to mimic the situation wherein samples containing a high level of a nucleic acid target molecules can cross-contaminate other samples during the extraction procedure. NA purification was conducted using one lot of reagents. Cross-contamination was assessed using the *artus*® HBV RG CE PCR Kit. The results showed no cross-contamination within the entire system.

Notes

Revision History

Date	Changes
R1 09/2019	Initial release
R2 08/2021	Revised storage condition for eluate stability tested for DNA: Storage of –30 to –15°C and –90 to –65°C is possible for up to 12 months. Revised storage condition for eluate stability tested for RNA: Storage of –30 to –15°C and –90 to –65°C is possible for up to 6 months. Corrected the error in the performance sheet: RNA storage has not been tested at room temperature (15–25°C). Corrected the precision study data under SD and CV columns in Table 1.

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.