June 2022

QIAamp® DSP DNA Blood Mini Kit Instructions for Use (Performance Characteristics)

Version 3

IVD

For In Vitro Diagnostic Use
For use with QIAamp DSP DNA Blood Mini Kit

CE

REF 61104

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

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 QIAamp DSP DNA Blood Mini Kit uses silica-membrane technology (QIAamp technology) for isolation and purification of genomic DNA from biological specimens.

The QIAamp DSP DNA Blood Mini procedures, which are designed for simultaneous processing of multiple blood samples, yield purified DNA ready for use. The procedures are suitable for use with fresh or frozen whole blood and blood that have been treated with citrate or EDTA.

The simple QIAamp DSP spin and vacuum procedures are suitable for simultaneous processing of multiple samples. Some of the QIAamp spin procedures can be fully automated on the QIAcube® Connect MDx for increased standardization and ease of use. The QIAcube Connect MDx perform automated isolation and purification of nucleic acids. It can process of up to 12 samples per single run.

Performance Characteristics

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. Performance characteristics have been established for the QIAamp DSP DNA Blood Mini 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 and 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

Basic performance of the QIAamp DSP DNA Blood Mini vacuum procedure has been determined for blood from healthy donors with white blood cell counts of 3.8 x 10^6 to 1.34 x 10^7 cells/ml (see Figure 1).

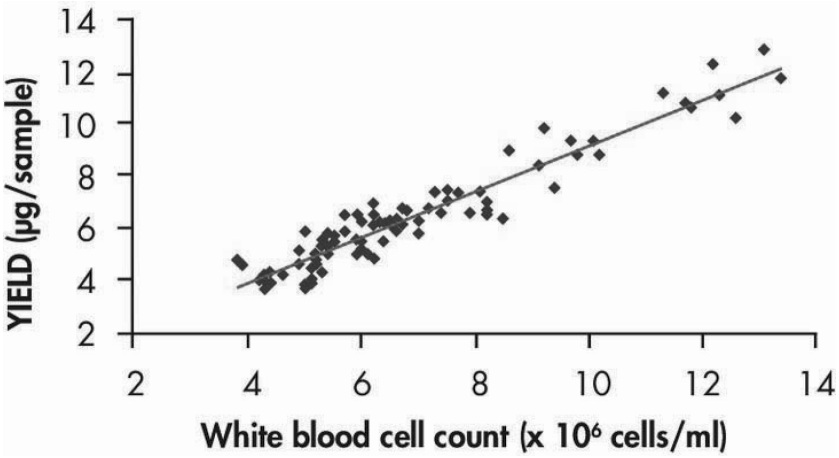

Figure 1. Observed yield using the QIAamp DSP DNA Blood Mini vacuum procedure with 200 µl elution volume. White blood cell counts of healthy donors were determined and were in the range 3.8 x 10^6 to 1.34 x 10^7 cells/ml. DNA was purified from the blood samples using the QIAamp DSP DNA Blood Mini vacuum procedure with 200 µl elution volume. Eighty-seven triplicate samples were processed.

The amount of DNA purified in the QIAamp DSP DNA Blood Mini procedures depends on the white blood cell content of each blood sample. Using the spin or vacuum procedure, genomic DNA is purified from 200 µl blood samples from healthy donors. Various different primary tubes and anticoagulants can be used to collect blood samples for the QIAamp DSP DNA Blood Mini procedures (Table 1).

Table 1. Average relative yields of DNA from blood samples collected using various primary tubes and anticoagulants

Primary tube	Manufacturer	Cat. no.	Nominal volume	Average yield*
BD™ Vacutainer® 9NC	BD	366007	9 ml	6.4 µg
BD Vacutainer K3E	BD	36847	10 ml	6.6 µg
BD Vacutainer K2E	BD	367864	6 ml	6.4 µg
S-Monovette® EDTA	Sarstedt®	02.1066.001	9 ml	6.5 µg
S-Monovette CPDA1	Sarstedt	01.1610.001	8.5 ml	6.3 µg
Vacuette® K3E	Greiner Bio-One®	455036	9 ml	6.5 µg
Vacuette 9NC	Greiner Bio-One	454382	2 ml	6.3 µg

Genomic DNA was purified from 200 µl blood samples from healthy donors (4.0 to 9.0 x 10^6 cells/ml).
* For each primary tube, the average yield is determined from 11 triplicate samples.

Eluted genomic DNA is ready for use in different downstream assays.

Sample input/eluate output range and DNA purity

Different elution volumes can be selected for genomic DNA isolation from 200 µl whole blood. For the manual procedure, elution volumes range from 50 to 200 µl. For the fully automated spin workflow, 100 and 200 µl are possible elution volumes and for the partly automated spin workflow (after manual lysis) 100–200 µl (in 10 µl increments) are possible elution volumes. Elution in smaller volumes increases the final DNA concentration in the eluate but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.

The effect of different elution volumes on the overall DNA concentration has been assessed. Figure 2 shows an increase in DNA concentration in the eluates when decreasing the elution volume.

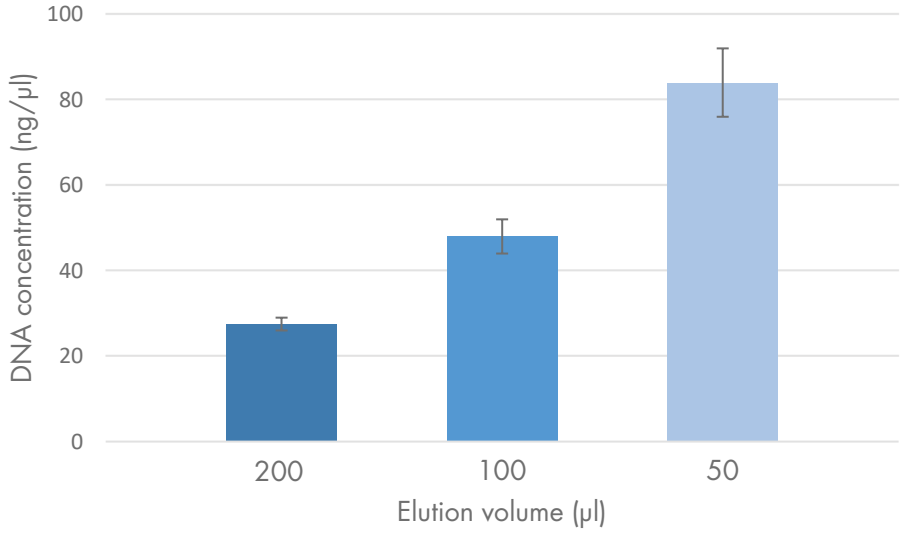

Figure 2. DNA concentration obtained after DNA isolation from whole blood using the QIAamp DSP DNA Blood Mini Kit with different elution volumes. Each bar on the graph represents the results from 32 replicates (mean ± standard deviation).

Additionally, as an indicator for DNA purity, the ratio between absorbance at 260 and 280 nm was measured for the different tested elution volumes. No difference was observed between different elution volumes and overall the average ratio indicated low protein contamination.

Precision

Coefficients of variations (CVs) were determined for the automated extraction of human genomic DNA from whole blood using the QIAamp DSP DNA Blood Mini Kit on the QIAcube Connect MDx. Total DNA yield was determined by OD measurement.

Repeatability (intra-run variability within one purification run) and intermediate precision (inter-run variability across different purification runs with different operators, on different instruments, and on different days) were determined. The precision data are shown in Table 2.

Table 2. Analysis of precision estimates

Precision	CV (%)
Intermediate precision	1.65
Repeatability	6.09
Total precision	6.24

For the manual vacuum procedure, mean yields and CVs were determined and evaluated to assess intermediate precision, repeatability, and reproducibility. In addition DNA integrity and performance in an in-house real-time PCR assay were analyzed.

Sample stability

Note: Sample stability highly depends on various factors and relates to the specific downstream application. It has been evaluated 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.

The effects of freezing and thawing on EDTA-treated blood samples on DNA purification using the QIAamp DSP DNA Blood Mini Kit has been determined. No significant decrease of yield (see Figure 3) or performance in downstream assays was observed.

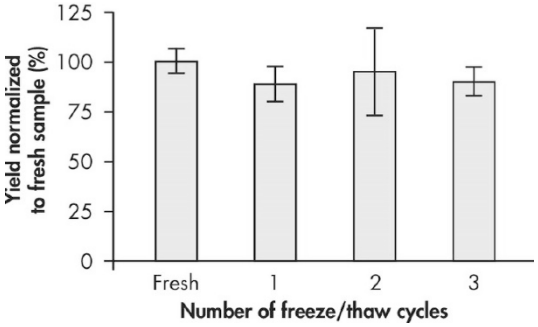

Figure 3. Effects of freezing and thawing blood samples. EDTA-treated blood was frozen and thawed up to 3 times, and then subjected to DNA purification using the QIAamp DSP DNA Blood Mini Kit. The calculated DNA yields are normalized to the yield from fresh sample (100%). Each bar on the graph represents the results from 32 replicates (mean ± standard deviation).

Eluate stability

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been evaluated for the QIAamp DSP DNA Blood Mini 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.

Eluate stability for the QIAamp DSP DNA Blood Mini Kit was evaluated after extraction of nucleic acid from human blood with spectrophotometry and an in-house real-time PCR assay. Eluted DNA can be stored at 2–8°C for up to 4 weeks. For long-term storage, we recommend storage at –20°C.

Interfering substances

Different potential exogenous and endogenous interfering substances present in patient whole blood were spiked into blood samples to test their impact on exemplary downstream assays after gDNA isolation with the QIAamp DSP DNA Blood Mini Kit.

Common relevant potential interfering substances for hemolysis (human hemoglobin), lipemia (triglycerides), and jaundice (bilirubin unconjugated) were evaluated. Besides, the interfering effect of three times higher anticoagulant K2-EDTA, K3-EDTA, and Na2-EDTA concentration than already present in the collection tube was assessed. No significant negative impact was observed for these potential interferents and for approximately 20 additional potential interferents such as drugs typically used, for example, for cancer treatment, thus, likely to be found in patient samples.

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 concentration also needs to be established as part of the downstream application development for any workflow involving the QIAamp DSP DNA Blood Mini Kit.

Any potentially 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 QIAamp DSP DNA Blood Mini Kit.

Note: According to ISO 20186-2:2019(E), 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, we recommend usage of blood samples treated with EDTA or citrate as anticoagulant for plasma preparation.

Cross-contamination

The risk of cross-contamination for the automated purification of nucleic acid on the QIAcube Connect MDx was analyzed by performing five 12 sample runs with alternating checkerboard batches (positive and negative samples alternating) using an exemplary QIAamp workflow (QIAamp DSP Virus Spin together with plasma and serum samples of 1.00E+07 copies/ml of a DNA virus). A potential contamination of the negative samples during the extraction runs was evaluated by subsequent analysis of the eluates using an in-house real-time PCR assay. No cross-contamination was detected for sample to sample or run to run carryover.

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 3, Revision 1 • Update to version 3 for compliance to IVDR • Transfer and update of performance characteristics from kit handbook into this document: o Transfer of Yield of purified DNA section and Performance in downstream assay section in Basic performance and compatibility to different downstream applications section o Addition of Sample input/eluate output range and DNA purity section o Addition of Precision section o Update of Eluate stability section o Addition of Sample stability section o Addition of Interfering substances section o Addition of Cross-contamination section o Addition of Symbols section o Addition of Revision history section

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.