PurePrep Body Fluid Kit

Application Note | Scalable automated DNA extraction from fresh and frozen whole blood

INTRODUCTION

In genomics, different sample volumes are needed to obtain minimum required DNA amounts. These amounts depend on the specific measurement technology to answer a research question or to detect analytical targets with a diagnostic test. Analytical methods using whole blood range from a few microlitres for a relatively small number of DNA markers, up to ten millilitres for genome-wide analysis. Furthermore, the intended use of the extracted genomic DNA may require larger amounts for long term storage and re-analysis.

PurePrep Body Fluid Kit allows for a fast and cost-effective extraction of DNA from whole blood and saliva samples. This application note describes the sample scalability and automated use of **PurePrep Body Fluid Kit** for genomic DNA extraction from up to 10 mL whole blood on magnetic particle processing instruments and provides an extensive quantity and quality analysis of the extracted DNA. Volumes of 200 µL, 1 mL, 2 mL, 4 mL, 5 mL and 10 mL whole blood were used for DNA extraction. Four different processing methods were evaluated for use of 200 µL sample; manual, the PurePrep 32 System (PP32), PurePrep 96 System (PP96) and KingFisher™ 96 Magnetic Particle Processor (KF96) by Thermo Scientific™. Automated DNA extraction from 4 mL sample was evaluated on the PurePrep 24 System (PP24). Extracted DNA samples were subjected to multiple analytical methods to demonstrate suitability for typical applications involving nucleic acid amplification. Total yield, concentration, purity, DNA integrity, and PCR compatibility were evaluated.

MATERIALS AND METHODS

Fresh EDTA blood was collected from healthy donors according to standard laboratory practice and stored at 2-8°C for up to 7 days. DNA extraction was performed with fresh and frozen whole blood. Frozen EDTA blood was prepared by overnight storage at -20°C.

Sample volumes of 200 µL were aliquoted into the 96 DeepWell plates for automated extraction on the PurePrep 32, PurePrep 96 and KingFisher™ 96 systems or in 2 mL microtubes for manual DNA extraction (reference procedure). Aliquots of 1 mL, 2 mL, 5 mL and 10 mL were aliquoted into 50 mL tubes for manual DNA extraction and aliquots of 2 mL were added to two PurePrep 24 DeepWell plates for automated DNA extraction from a total of 4 mL sample.

Reagent volumes were adjusted proportionally for each sample volume used, taking into account the specified working volumes of the equipment and consumables used. For automated DNA extraction, all plates were filled with reagents and loaded onto the instrument, software programs were loaded, and the DNA extraction protocol was started. For manual DNA extraction, all reagents were added and removed at specific steps of the extraction procedure, and magnetic separation was performed with a magnetic separator suitable for the consumable used (i.e. MM-Separator M12 + 12 for microtubes and MM-Separator M50 for 50 mL centrifuge tubes). In the table below, an overview of the reagent volumes for different sample volumes is shown.

Whole blood	200 µL	1 mL	2 mL	5 mL	10 mL
Lysis Buffer U1	200 µL	1 mL	2 mL	5 mL	10 mL
Proteinase K (20 mg/mL)	10 µL	50 µL	100 µL	250 µL	500 µL
MagSi-BF9	20 µL	100 µL	200 µL	500 µL	1 mL
Binding Buffer U1	400 µL	2 mL	4 mL	10 mL	20 mL
Wash Buffer I	2 x 800 µL	2 x 4 mL	2 x 8 mL	2 x 20 mL	2 x 40 mL
Wash Buffer II	800 µL	4 mL	8 mL	20 mL	40 mL
Elution Buffer	150 µL	200 µL	400 µL	1 mL	2 mL

Table 1. *Reagent volumes used for different whole blood sample volumes*

DNA concentrations of the eluates were measured with the Qubit™ dsDNA BR Assay Kit and purity was assessed by UV-VIS with the NanoDrop™ One according to manufacturer's instructions (Thermo Scientific™). The quality of the DNA was assessed by automated gel electrophoresis on the 4150 TapeStation with a Genomic DNA ScreenTape and by Real-Time PCR on the AriaMx Real-Time PCR system (Agilent) with universal primers targeting the ALB gene (Pongers-Willemse et al, 1998), using 2 µL of DNA sample in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

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RESULTS

The DNA yield obtained from different sample volumes used are presented in Fig. 1 and Table 2. An increase in DNA concentration proportional to the amount of input sample is measured, showing the efficiency and scalability of the extraction procedure for different sample amounts.

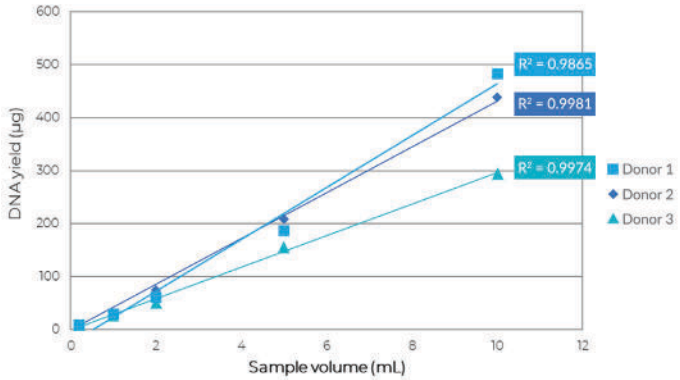

Figure 1. DNA yields from 200 µL, 1 mL, 2 mL, 5 mL and 10 mL whole blood of 3 donors determined with the Qubit™ dsDNA BR Assay Kit and the obtained correlation efficients between sample volume and yields. Data presented are mean values (n=3).

Whole blood	200 µL	1 mL	2 mL	5 mL	10 mL
DNA yield (µg)	7.50	27.70	61.80	184.00	404.70

Table 2. Average DNA yields obtained from different sample volumes from 3 donors

Purity

The DNA purity obtained from different sample volumes used are presented in Fig. 2. All A260/A280 purity ratios are ≥1.88 and all A260/A230 ratios are ≥1.80, indicating highly pure DNA without protein or salt contamination.

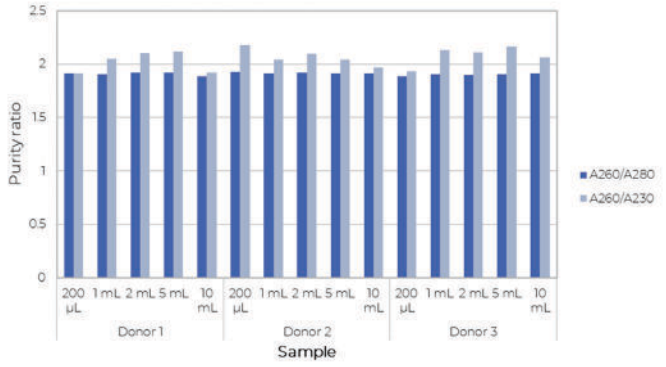

Figure 2. Purity ratios of DNA extracted from 200 µL, 1 mL, 2 mL, 5 mL and 10 mL whole blood of 3 donors obtained by UV-VIS with the NanoDrop One. Data presented are mean values (3 donors, n=3).

DNA integrity

Results from automated gel-electrophoresis are presented in Fig.3. All used sample volumes and processing methods result in high molecular weight DNA (peak size (bp): >60000)

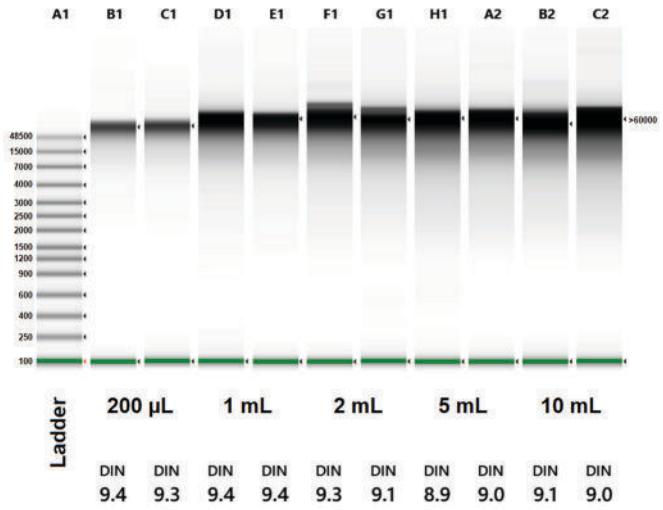

Figure 3. TapeStation gel image with DNA Integrity Numbers (DIN, 1 - 10) for gDNA extracted from 200 µL, 1 mL, 2 mL, 5 mL and 10 mL whole blood.

PCR compatibility

The Real-Time PCR results are presented in Fig. 4 below. The differences between reported Ct values for increasing sample volumes are consistent with the expected values considering the elution volumes used (150, 200, 400, 1000 and 2000 µL).

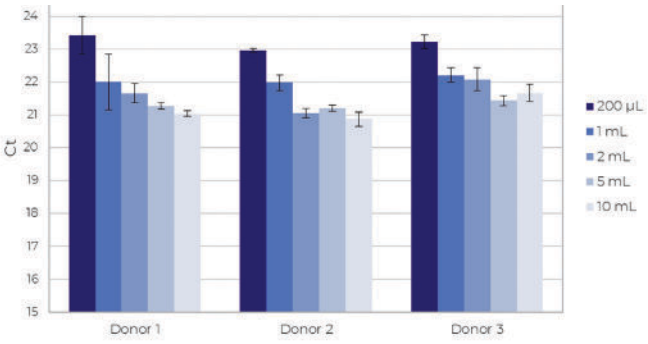

Figure 4. Ct values obtained after DNA extraction from different blood volumes. Ct values between 20.9 and 23.4 are reported by the AriaMx Real-Time PCR system. The data presented are mean values (n = 3, ±1 SD).

Automated DNA extraction from 200 µL whole blood

In Figure 5 and Table 3 below, nucleic acid concentration, intra-assay repeatability and extraction efficiency (compared to manual use) are presented for 3 different instruments suitable for use of 200 µL whole blood. On average, the automated methods recover 76% (PP32), 74% (PP96), 90% (KF96) of the DNA obtained with manual use, with a CV% of ≤9.6. All purity ratios indicate highly pure DNA (A260/A280>1.83, A260/A230>1.9, not shown).

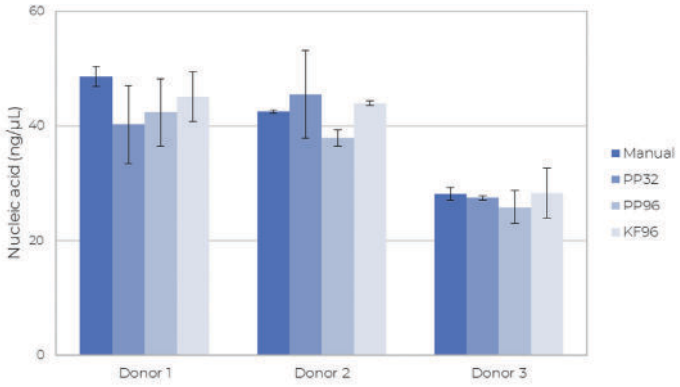

Figure 5. Nucleic acid concentrations measured after extractions from 200 whole blood of 3 donors using 3 different magnetic particle processors (PurePrep 32 Nucleic Acid Extraction System, PurePrep 96 Nucleic Acid Extraction System, KingFisher™ 96 Magnetic Particle Processor). The data presented are mean values (n = 3, ±1 SD).

	Manual	PP32	PP96	KF96
Nucleic Acid (ng/µL)	39.8	37.7	35.3	39.1
Intra-assay repeatability (CV%)	2.8%	11.6%	9.6%	8.6%
Efficiency	-	96%	89%	99%

Table 3. Mean nucleic acid concentrations, intra-assay repeatability and extraction efficiency (compared to manual use) of PurePrep Body Fluid Kit

Automated DNA extraction from 4 mL whole blood

In Fig.6, the DNA yield after extraction from 4 mL fresh and frozen whole blood of 6 donors on the PurePrep 24 Nucleic Acid Extraction System is presented. The average extraction efficiency compared to manual extraction from 200 µL of the same samples was 83% (fresh) and 93% (frozen), and the reported Ct values deviated only 0.2 Ct from the expected difference (ΔCt: 2.39 instead of 2.59, not shown).

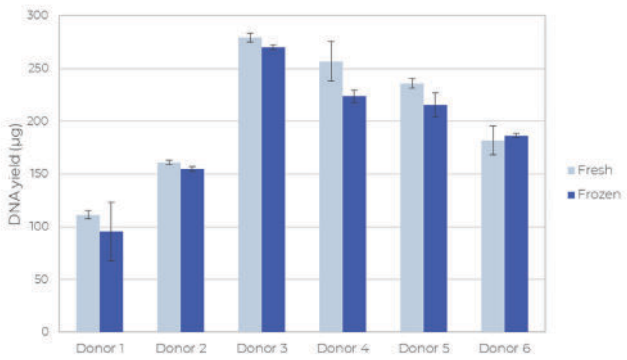

Figure 4. TapeStation gel snapshot with DNA Integrity Numbers (DIN) for gDNA extracted from 200 µL buffy coat obtained with different processing methods (manual (M), PurePrep 32 System (PP32), PurePrep 96 System with offline lysis (PP96 off), PurePrep 96 System with online lysis (PP96 on) and KingFisher™ 96 Magnetic Particle Processor (KF96)).

CONCLUSION

The data obtained shows that **The PurePrep Body Fluid Kit** can be successfully used for DNA extraction from samples volumes ranging from 200 µL to 10 mL without loss of efficiency. The kit is compatible with different magnetic particle processors for 200 µL with relatively small differences in DNA recovery and repeatability. For sample volumes ranging from 200 µL to 4 mL, the PurePrep 24 provides for a suitable solution with high DNA recovery. All DNA extractions performed for this study resulted in high concentrations of highly intact DNA with excellent purity values and demonstrated compatibility with applications involving nucleic acid amplification, e.g. PCR assays or DNA sequencing.

LITERATURE

- Product Manual PurePrep Body Fluid Kit, PM0020, Magtivio B.V.
- User Guide Qubit™ 1X dsDNA BR Assay, MAN0019617, ThermoFisher Scientific
- NanoDrop One UG, 269-309102, ThermoFisher Scientific
- DNA Integrity Number (DIN) For the Assessment of Genomic DNA Samples in Real-Time Quantitative PCR (qPCR) Experiments, Application Note 5991-6368EN, A. Padmanaban, Agilent Technologies, Inc.
- Pongers-Willemse et al., Real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia using junctional region specific TaqMan probes Leukemia (1998) 12, 2006–2014

PurePrep 32

PurePrep 24

PurePrep 96

ORDERING INFORMATION

ART. NO.	DESCRIPTION	AMOUNT
MG0000228	PurePrep Body Fluid Kit	96 preps
MG0000229	PurePrep Body Fluid Kit	10 x 96 preps
MGPP961001	PurePrep 96 Nucleic Acid Purification System	1 unit
MGPP321001	PurePrep 32 Nucleic Acid Purification System	1 unit
MGPP241001	PurePrep 24 Nucleic Acid Purification System	1 unit
MG96020050-01	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	50 pcs/pack
MG96010050-01	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	50 pcs/pack
MG96030050-03	96 well Tip-Comb for KingFisher™/PurePrep 96	50 pcs/pack
MG24040050-01	PurePrep 24 Tip-Comb + 24 DeepWell Plate	50 pcs/pack
MG24020050-01	PurePrep 24 DeepWell Plate	50 pcs/pack
MG32020050-01	PurePrep 16/32 DeepWell Plate	50 pcs/pack
MG32030200-01	PurePrep 16/32 Tip-Comb	200 pcs/pack

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