Steinbrenner Laborsysteme GmbH

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MagSi-DNA Body Fluid

Application Note | Scalable automated DNA extraction from human buffy coat

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. DNA extraction from buffy coat instead of whole blood offers an option for purifying high yields of DNA from relatively small sample volumes.

MagSi-DNA Body Fluid allows for a fast and cost-effective extraction of DNA from whole blood and saliva samples. This application note describes the use of MagSi-DNA Body Fluid for genomic DNA extraction from up to 200 µL buffy coat in 96 DeepWell plates on magnetic particle processing instruments, and provides an extensive quantity and quality analysis of the extracted DNA.

Volumes of 50 µL, 100 µL and 200 µL buffy coat were used for evaluation of efficiency and scalability of DNA extraction. Four different automated processing methods were evaluated; the 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) by Thermo Scientific™. DNA extraction performance was compared to the manual extraction (M) and a competitor method (C) also using the KingFisher™ 96 Magnetic Particle Processor. Extracted DNA samples were subjected to multiple analytical methods to evaluate suitability for typical applications involving nucleic acid amplification, including determination of total yield, concentration, purity, DNA integrity, and PCR compatibility.

Materials and methods

DNA extraction was performed with frozen buffy coat from K2EDTA blood (Cambridge Bioscience) which was stored at -20°C until use. Buffy coat samples were thawed by gently shaking at 37°C for 10 min.
First, 40 µL Proteinase K was aliquoted into the 96 DeepWell plates. Sample volumes of 50 µL, 100 µL and 200 µL were added for automated extraction on the PurePrep 32, PurePrep 96 and KingFisher™ 96 systems or in 2 mL microtubes for manual DNA extraction.

Sample volumes lower than 200 µL were added up to 200 µL with PBS buffer. Samples were vortexed briefly to initiate Proteinase K digestion, followed by addition of 200 µL Lysis Buffer U1 and incubation at 56 °C for 20 min on the extraction instruments, or on a Thermomixer C (Eppendorf) at 1200 rpm (for offline lysis). After lysis, the standard MagSi-DNA Body Fluid kit protocol was followed, with only minor adjustments in reagent volumes (25 µL MagSi-BF9 beads and 500 µL Binding Buffer U1). The DNA was eluted in 200 µL Elution Buffer.

For automated DNA extraction, 96 DeepWell 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 microtubes (MM-Separator M12 + 12, magtivio).

DNA concentration and purity of the eluates were measured with the Qubit™ dsDNA BR Assay Kit and 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 albumin (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, Germany).

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Results

Efficiency and scalability

Yield and purity

The DNA yield obtained from different sample volumes used is presented in Fig. 1. 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.

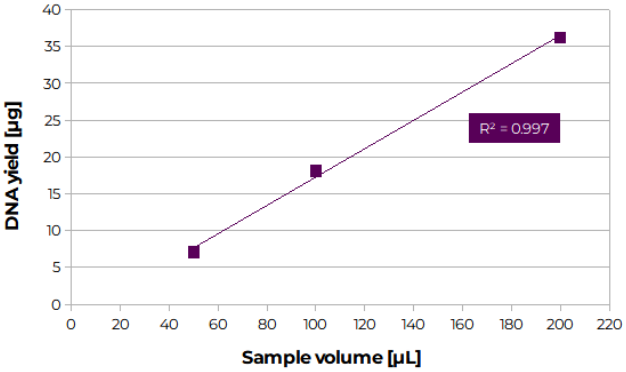

Figure 1. *DNA yields from 50 µL, 100 µL and 200 µL buffy coat obtained with manual extraction determined with the Qubit™ dsDNA BR Assay Kit and the obtained correlation efficients between sample volume and yields. The data presented are mean values (n = 3, n = 2 for 50 µL sample input).*

In Table 1 the DNA yield and purity obtained after manual extraction from different sample volumes are presented and compared to a competitor kit, used according to instructions from the manufacturer. For unknown reasons, extraction with the competitor kit shows no scalability, but a decreasing concentration with higher sample volumes.

Table 1. *Average DNA yields, according CV% and average purity ratios obtained from different sample volumes determined by UV-VIS with the NanoDrop One. Comparison of the manual processing and the competitor kit. Averages for n = 3, n = 2 for 50 µL sample input.*

	MagSi-DNA Body Fluid			Competitor		
Sample (µL)	**50**	**100**	**200**	**50**	**100**	**200**
DNA yield (µg)	9.7	20.7	45.0	11.5	7.4	1.5
CV% (yield)	6.4%	8.3%	6.4%	61.1%	98.5%	97.6%
A260/A280	1.82	1.83	1.87	1.94	1.96	2.18
A260/A230	2.08	2.18	2.21	1.99	1.85	1.01

Automation

Efficiency

In Table 2, nucleic acid concentration, intra-assay repeatability and extraction efficiency (compared to manual use) are presented for the different processing methods. An efficiency of up to 98% compared to manual use could be achieved with the Pureprep 96 System with online lysis.

Table 2. *Nucleic acid concentrations, intra-assay repeatability and extraction efficiency (compared to manual use) of MagSi-DNA Body Fluid for 100 µL buffy coat. The data presented are mean values (n = 3, ±1 SD)*

	manual	PP32	PP96 off	PP96 on	KF96
Nucleic Acid (ng/µL)	115.0	67.9	95.9	113.3	83.6
Intra-assay CV	8.3%	5.8%	8.1%	21.4%	18.6%
Efficiency	-	59%	83%	98%	73%

Fig. 2 shows the nucleic acid concentrations of all methods used in a graphical way to easily compare their efficiency, including different sample input amounts.

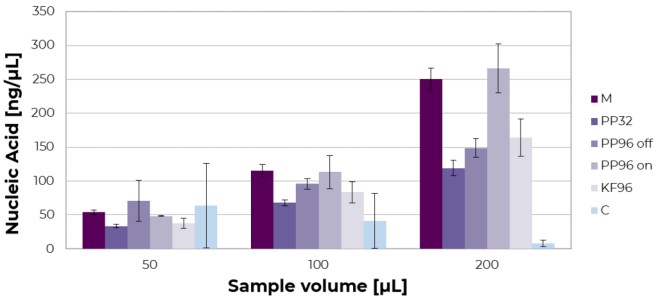

Figure. 2. *Nucleic acid concentrations measured after extractions from different buffy coat volumes obtained with different processing methods (manual (M), PurePrep 32 System (PP32), PurePrep 96 System with offline lysis (PP96 off), PurePrep 96 System with online lysis (PP96 on), KingFisher™ 96 Magnetic Particle Processor (KF96) and the competitor kit (C)). The data presented are mean values (n = 3, n = 2 for 50 µL sample input, ±1 SD).*

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Purity

The DNA purity obtained from different sample volumes used is presented in Fig. 3. All A260/A280 purity ratios are ≥1.80 and all A260/A230 ratios are ≥1.76, indicating highly pure DNA without protein or salt contamination.

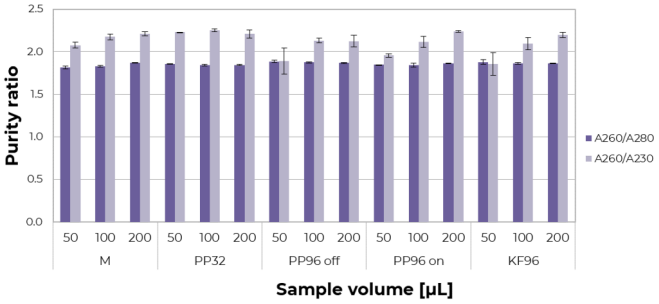

Figure 3. *Purity ratios of DNA extracted from 50 µL, 100 µL and 200 µL buffy coat obtained with different processing methods (manual (M), PurePrep 32 System (PP32), PurePrep 96 System with offline lysis (PP96 off), PurePrep 96 System with online lysis (PP96 on) and KingFisher™ 96 Magnetic Particle Processor (KF96)) determined by UV-VIS with the NanoDrop One. The data presented are mean values (n = 3, n = 2 for 50 µL sample input).*

DNA integrity

Results from automated gel electrophoresis are presented in Fig.4. All used processing methods result in high molecular weight DNA (peak size (bp): >60000).

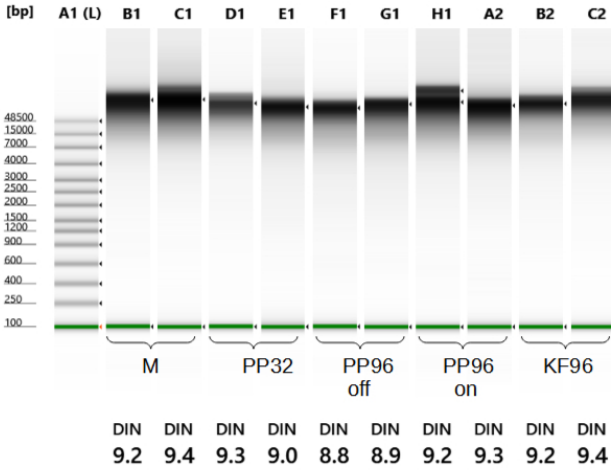

Figure 4. *TapeStation gel snapshot with DNA Integrity Numbers (DIN) for gDNA extracted from 200 µL buffy coat obtained with different processing methods (manual (M), PurePrep 32 System (PP32), PurePrep 96 System with offline lysis (PP96 off), PurePrep 96 System with online lysis (PP96 on) and KingFisher™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

PCR compatibility

Results from Real-Time PCR with the extracted DNA are presented in Fig. 5 below. The differences between reported Ct values for increasing sample volumes are consistent with the expected values (2x more: -1 Ct).

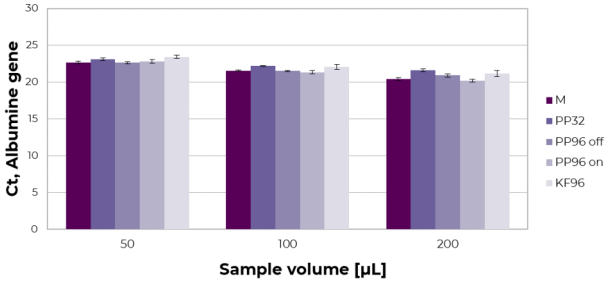

Figure 5. *Ct values measured after DNA extraction from different buffy coat volumes obtained with different processing methods (manual (M), PurePrep 32 System (PP32), PurePrep 96 System with offline lysis (PP96 off), PurePrep 96 System with online lysis (PP96 on) and KingFisher™ 96 Magnetic Particle Processor (KF96)). Ct values between 20.18 and 23.43 are reported by the AriaMx Real-Time PCR system. Data presented are mean values (n = 3, n = 2 for 50 µL sample input, ±1 SD).*

Conclusion

The data obtained show that MagSi-DNA Body Fluid can be successfully used for DNA extraction from sample volumes ranging from 50 µL to 200 µL. The kit is compatible with different automated magnetic particle processors using a sample volume of up to 200 µL buffy coat. Comparison to a competitor kit highlights the scalability with different sample volumes, which could not be achieved with the competitor kit. All DNA extractions performed for this study resulted in high concentrations of highly intact DNA with excellent purity values, demonstrating compatibility with applications involving nucleic acid amplification, e.g. PCR assays or DNA sequencing.

Literature

- *Product Manual MagSi-DNA Body Fluid, PM0020, magtivio B.V.*
- *User Guide Qubit™ 1X dsDNA BR Assay, MAN0019617, ThermoFisher Scientific*
- *NanoDrop One UG, 269-309102, ThermoFisher Scient.*
- *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 acute lymphoblastic leukemia using junctional region specific TaqMan probes Leukemia (1998) 12, 2006–2014*

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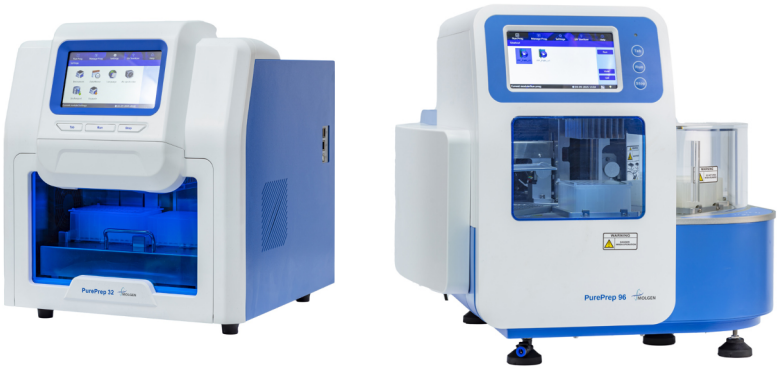
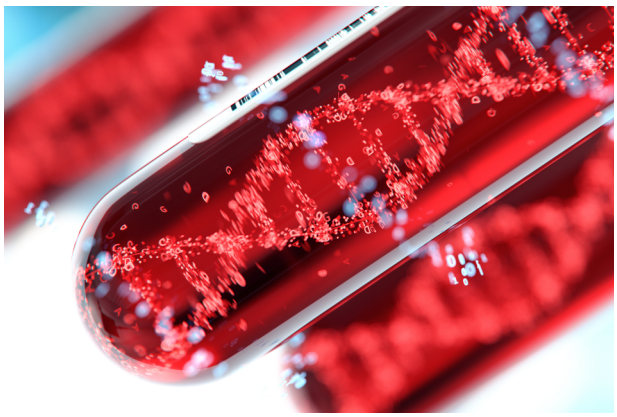


PurePrep 32 and 96 Nucleic Acid Purification Systems

Ordering information

Art. No.	Description	Amount
MDKT00140096	MagSi-DNA Body Fluid	96 preps
MDKT00140960	MagSi-DNA Body Fluid	10 x 96 preps
AS00001	PurePrep 96 Nucleic Acid Purification System	1 unit
AS00002	PurePrep 32 Nucleic Acid Purification System	1 unit
MDPL00200050	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	50 pcs/pack
MDPL00190060	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	60 pcs/pack
MDPL00210060	96 well Tip-Comb for KingFisher™/PurePrep 96	60 pcs/pack
MDPL00300050	PurePrep 16/32 DeepWell Plate	50 pcs/pack
MDPL00310200	PurePrep 16/32 Tip-Comb	200 pcs/pack



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