Application note

Performance of the sbeadex Blood DNA Purification Kit and its compatibility with anticoagulant preservatives

Introduction

The sbeadex™ Blood DNA Purification Kit from LGC Biosearch Technologies™ is designed for purification of nucleic acids from whole blood samples using magnetic separation. The binding mechanism consists of superparamagnetic particles coated with sbeadex surface chemistry with a nucleic acid specific adapter, which work in a two-step binding process allowing water-based wash steps to remove impurities present in the sample matrix before final elution. The absence of organic solvents in the final washing steps reduces inhibitory influence in subsequent analysis. The sbeadex Blood DNA Purification Kit is capable of purifying high-quality DNA from a range of storage media, including EDTA, heparin and citrate anticoagulants with no consequences of inhibition in further downstream processing. The sbeadex Blood DNA Purification Kit has shown to be compatible with manual as well as automated handling in a 96-well format, where results for both are reproducible.

Here we demonstrate the application of the sbeadex Blood DNA Purification Kit for purification of DNA from whole blood samples against a market-leading competitor (Competitor A) on the KingFisher Flex 96 (Thermo Fisher Scientific). These experiments assessed the effects of blood preservation anticoagulants used in common storage vacutainers in relation to the quality of purified DNA and their influence on further downstream processes. Kit performance was verified through qPCR and enzymatic analysis.

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Methods

Blood samples

Validation blood samples were drawn from twenty healthy individuals in 6 mL vacutainers with either EDTA (K2), heparin (sodium heparin - 15 USP activity units) or citrate ACD (solution B) as the anticoagulant. An additional 1588 blood samples stored in EDTA (K2) vacutainers were sourced from our in-house production laboratory to test the reproducibility of the sbeadex Blood DNA Purification Kit.

DNA purification

DNA was purified using the sbeadex Blood DNA Purification Kit and Competitor A chemistry, while utilising an optimised one-hour KingFisher Flex protocol and 200 µL of whole blood. The one-hour KingFisher Flex protocol for both 100 µL and 200 µL are readily available for customer use. (For any further enquiries, please contact the Technical and Application support team at Biosearch Technologies at techsupport@lgcgroup.com). Purified DNA mass estimations and quality assessments were made using the NanoQuant Plate and Infinite 200 PRO plate reader (Tecan, Switzerland). All purified DNA samples were normalised to 12 ng (approximately 2900 genome copies) using TE (10 mM Tris, 1 mM EDTA). DNA normalisation was performed using a Freedom EVO 200 (Tecan, Switzerland) to minimise the introduction of variation.

Real-time PCR

To determine downstream performance of sbeadex-purified DNA in real-time PCR (qPCR), 1 µL of purified DNA was used as template in a 10 µL reaction to target Ribonuclease P (RNaseP) using Biosearch Technologies' BHQ probes, primers and BHQ Probe Master Mix according to manufacturer's protocols. This was performed using the Bio-Rad CFX 384.

Enzymatic analysis

A small subset of samples from the validation experiment was tested using 1 µg of purified DNA, digested by 10 U of EcoRI in buffer for 2 hours and analysed through gel electrophoresis. For this gel electrophoresis test, control samples were also used consisting of 1 µg of purified BamHI digested lambda DNA that was subsequently religated using T4 DNA ligase. Control samples were run alongside the sample DNA that had been purified using the sbeadex Blood DNA Purification Kit. Ligation was performed directly in digestion cutsmart buffer with no clean up in between. Enzymatic fragmentation of genomic DNA was performed using dsDNA fragmentase. All enzymes were supplied by New England BioLabs, UK.

Validation experiment

A small number of blood samples from twenty participants were used for a validation experiment to assess the overall performance of the sbeadex Blood DNA Purification Kit in comparison to Competitor A in a series of tests. The tests covered the quantity and quality of DNA purified and its suitability for further downstream processing (qPCR and enzymatic analysis). Details of the methods for these experiments are as described in relevant sections above.

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Results and discussion

DNA quantity and quality

Photometric measurements of DNA purified from whole blood using the sbeadex Blood DNA Purification Kit and Competitor A chemistry demonstrates that they are comparable (table 1). Samples purified using sbeadex Blood DNA Purification Kit were of the same standard as those extracted with the Competitor A kit.

The average DNA concentration obtained using sbeadex was 7.7 ng/µL and samples demonstrated excellent 260/230 and 260/280 values within desired optimum ranges. The performance of the sbeadex Blood DNA Purification Kit is confirmed to be compatible with a range of commonly used anticoagulants in blood vacutainers, with no observed hinderance to the DNA yield or quality.

Variable	Purification kit	n	Mean (ng/µL)	Standard deviation
260/280	Competitor A	185	1.9	0.07
	sbeadex	372	1.9	0.02
260/230	Competitor A	185	2.2	0.4
	sbeadex	372	1.9	0.2
DNA concentration	Competitor A	185	7.0	2.6
	sbeadex	372	7.7	4.2

Table 1. Summarised data for DNA concentration, 260/280 and 260/230 from purification of whole blood samples using the sbeadex Blood DNA Purification Kit and Competitor A chemistry.

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Enzymatic analysis

The enzymatic analysis was performed to prove the DNA purification capabilities of the sbeadex Blood DNA Purification Kit alongside commonly used storage methods, testing their suitability for downstream processing. The EcoRI columns in figure 1 illustrate successful digestion of sbeadex Blood DNA with visible smear and banding for treated samples. Untreated genomic samples (lanes 4, 6, 8, 10) remain as an intact single band. Ligated sample (lane 12) shows a successful ligation, with all samples that were successfully digested with EcoRI (lanes 5, 7, 9, 11) merging into a single band following the T7 ligation. This enzymatic analysis confirms that storage of whole blood in vacutainers containing anticoagulants prior to DNA purification with sbeadex does not affect DNA digestion using a commonly available restriction endonuclease.

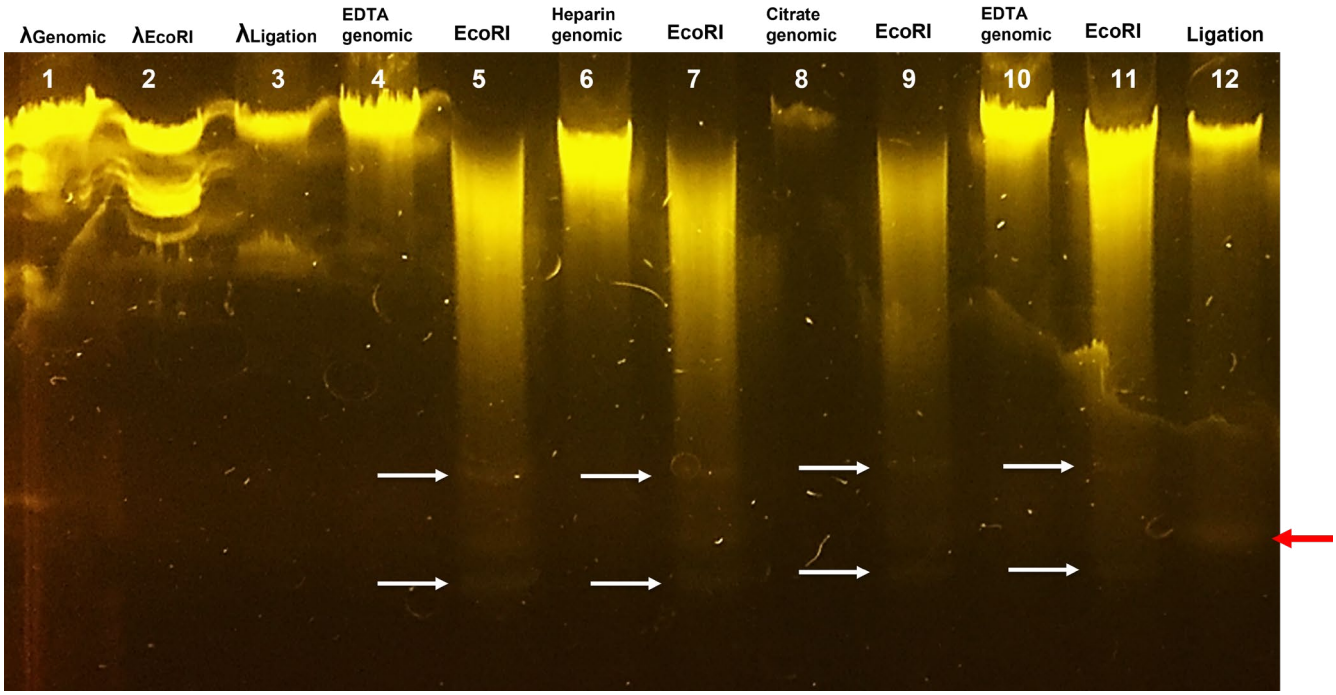

Figure 1. DNA digestion (using EcoRI) and ligation test performed on 0.8% agarose gel. Digested samples (lanes 5, 7, 9, 11) show two faint bands which are no longer present following the ligation test (lane 12).

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Heparin samples

Purification of whole blood samples utilising a heparin vacutainer has shown to be compatible with the sbeadex Blood DNA Purification Kit. Figure 2 shows electrophoresis analysis of heparin-containing samples purified using the sbeadex Blood DNA Purification Kit (lanes 9, 11, 13) and digested with EcoR1; these samples are of high molecular weight and hence will be suitable for many downstream applications.

The quality of DNA from heparin samples purified with Competitor A chemistry is lower, illustrating reduced DNA integrity which is likely to affect suitability for downstream applications (lanes 8, 10, 12). Following EcoR1 digestion, Competitor A-purified samples demonstrated greater degradation than sbeadex-purified samples; the absence of banding indicates over-fragmentation and poor DNA distribution, likely due to low dsDNA compliment.

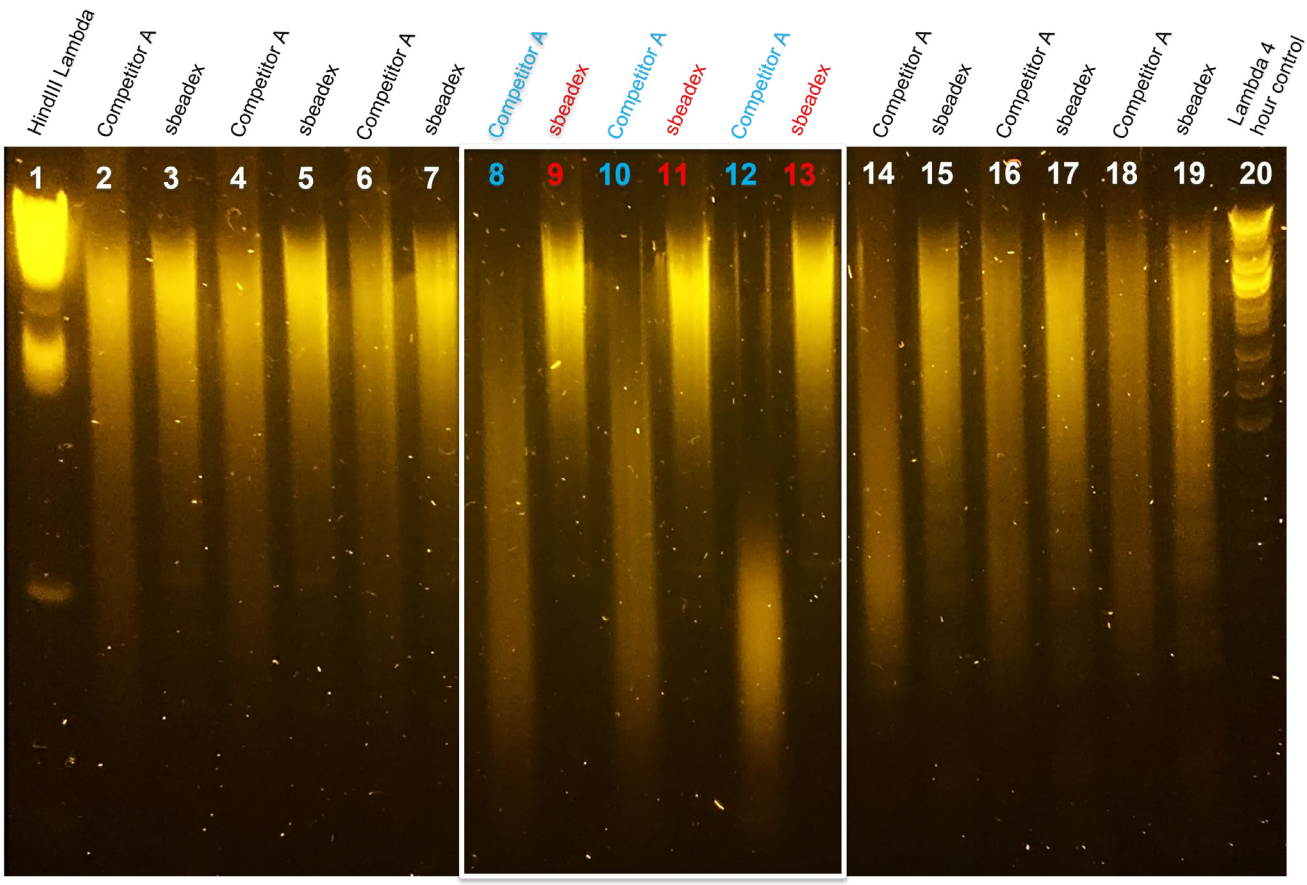

Figure 2. 0.5 µg genomic DNA, purified with both sbeadex Blood DNA Purification Kit and Competitor A chemistry, digested with 20 U/µL EcoRI for 2, 3 and 4 hours. Lanes 8 to 13: Purified DNA from heparin samples. Lanes 2 to 7: Purified DNA from EDTA samples. Lanes 14 to 19: Purified DNA from citrate samples.

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Reproducibility

Figure 3 illustrates DNA quantity and quality for over 1600 samples purified using the sbeadex Blood DNA Purification Kit for the two test laboratories. DNA quality and quantity are comparable across these parameters, with just a slight shift in the overall mean for the 260/230 values. Results from the production laboratory used a larger volume of samples, capturing a wider range of possible variabilities, but still produced data that was consistent with those samples in the validation experiment. The consistency of this data in spite of the variability across laboratories, inherent sample variability and number of samples processed demonstrates the reliability of the sbeadex Blood DNA Purification Kit and its ability to perform consistently.

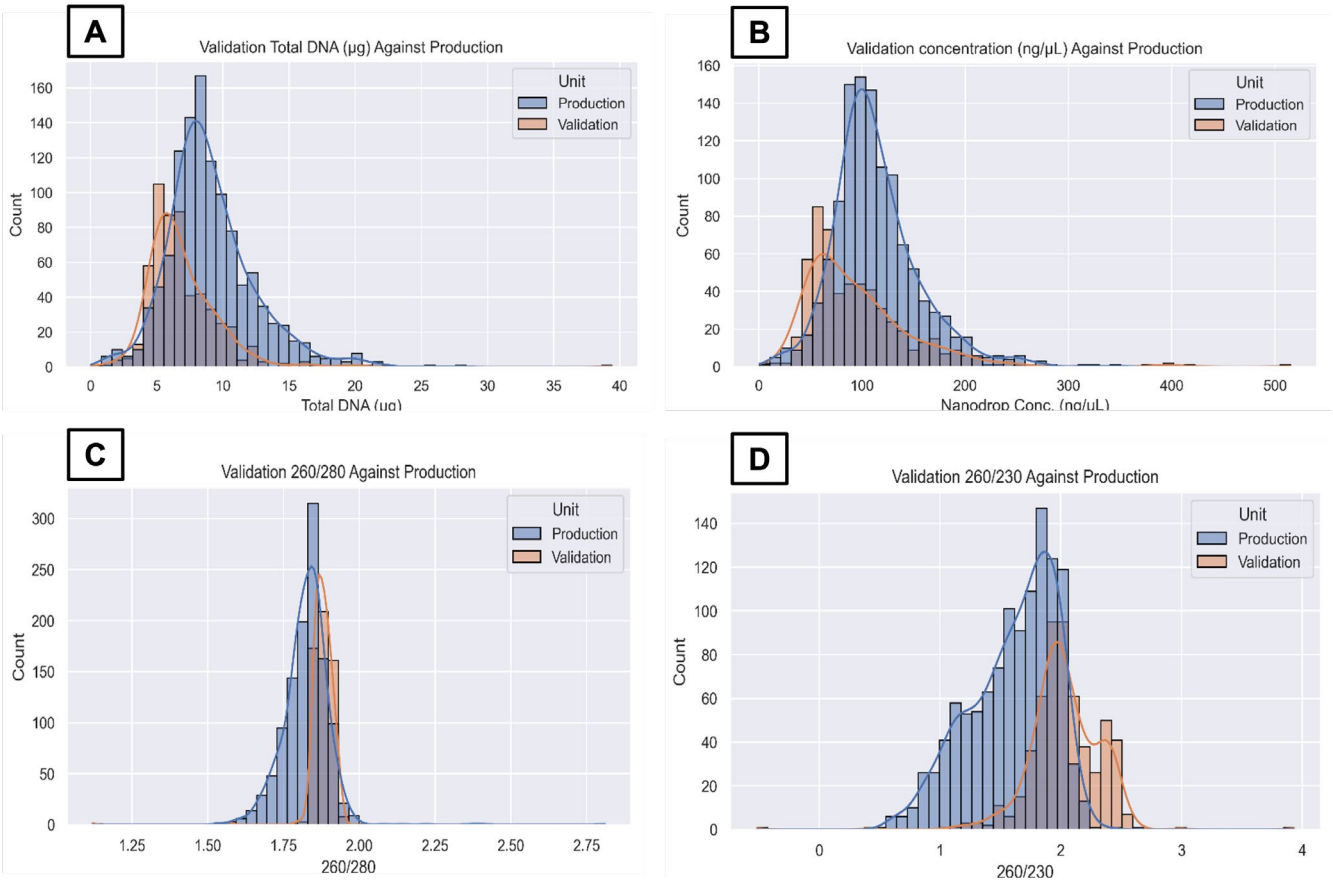

Figure 3. Histograms showing total DNA yield (A), DNA concentration (B), 260/280 (C) and 260/230 (D) for DNA purified from whole blood samples in two different laboratories using the same sbeadex chemistry.

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Real-time PC

Figure 4 illustrates qPCR results for DNA purified using both the sbeadex Blood DNA Purification Kit and Competitor A chemistry. Results demonstrate equivalent performance between the two chemistries, both obtaining comparable relative C_q values. This confirms that there was no significant inhibitory influence of the tested anticoagulants (including heparin) when using purified DNA in downstream qPCR. A small inhibitory influence of approximately 0.2 C_q was observed for the heparin-containing samples for both purification chemistries, indicating that heparin is unlikely to affect downstream processes such as Sanger sequencing and copy number variant analysis.

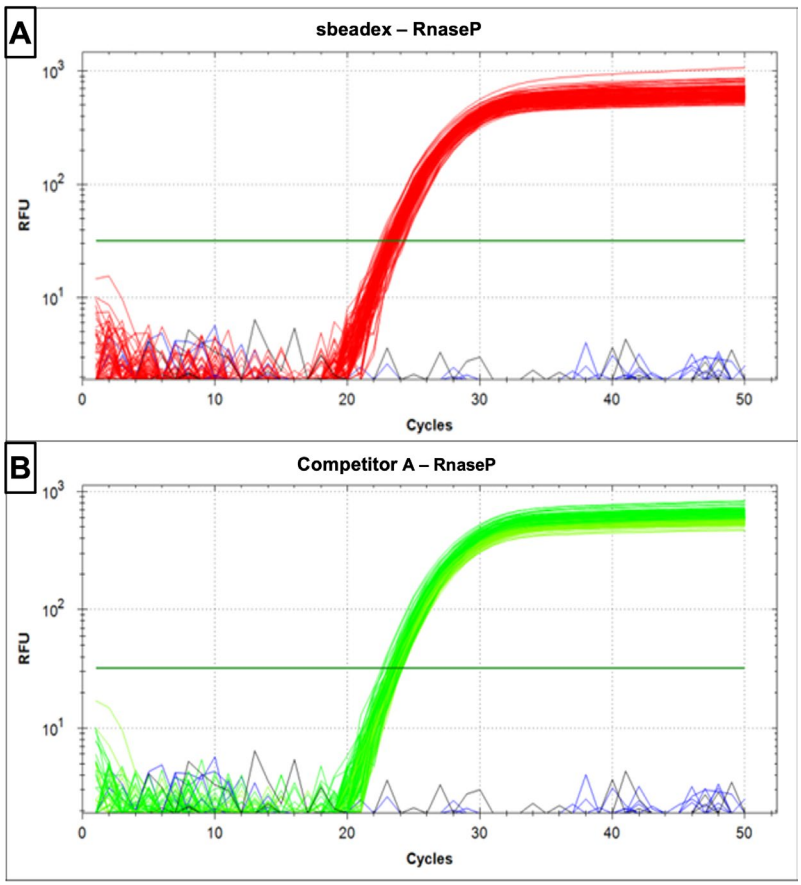

Figure 4. Amplification of the RNaseP gene using DNA purified with the sbeadex Blood DNA Purification Kit (Graph A) and Competitor A chemistry (Graph B). sbeadex Blood DNA Purification Kit chemistry (A): mean C_q value 23.5 (N = 120, standard deviation 0.36). Competitor A (B): mean C_q value 23.3 (N = 112, standard deviation 0.35).

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Summary

Here we demonstrate the success of the sbeadex Blood DNA Purification Kit for purification of blood samples from storage vacutainers containing common anticoagulants (citrate, EDTA and heparin). Purified DNA was of high quality (table 1) and demonstrated equivalent yield and purity ratios to DNA purified using a market-leading competitor chemistry. DNA quality obtained using the sbeadex Blood DNA Purification Kit was reproducible as illustrated in the through the production laboratory comparison (figure 3).

Performance of purified blood was also tested in downstream applications. DNA purified from samples stored with common anticoagulants using the sbeadex Blood DNA Purification Kit was successfully digested and ligated, indicating suitability in downstream applications including sequencing. For heparin-stabilised samples, samples purified with sbeadex performed better than those purified with Competitor A chemistry as they did not show degradation. Purified DNA was also used as template in qPCR and no significant inhibitory effects of the anti-coagulants were observed.

The sbeadex Blood DNA Purification Kit is compatible with samples stored in vacutainers containing common anticoagulants and purifies DNA suitable for use in enzymatic downstream applications including qPCR. Purification with this kit can be automated, making it an excellent choice for higher throughput processing of stabilised blood samples in your laboratory.

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