Save time and improve quality with the Clean Blood & Tissue DNA kit

A comparison of hands-free magnetic bead vs. manual spin column extraction

Introduction

Efficient and high quality extraction of genomic DNA is critical for downstream applications in molecular biology. This study compares the performance of the magnetic bead-based Clean Blood & Tissue DNA kit (CleanNA) with that of a widely used spin column kit (Kit Q) for genomic DNA extraction from whole blood samples. The Clean Blood & Tissue DNA kit takes advantage of magnetic bead-based technology to enable an automated, centrifugation-free workflow – unlike the manual spin column protocol, which involves multiple centrifugation or vacuum steps that can be labor intensive and tedious. This not only simplifies the extraction process, but also enhances walk-away time by requiring minimal user intervention, without compromising DNA yield or quality. The liquid handling steps for the Clean Blood & Tissue DNA kit are integrated on the ASSIST PLUS pipetting robot (INTEGRA Biosciences), fully automating the magnetic separation step by controlling the up and down movement of the magnetic array on a HEATMAG module (INTEGRA Biosciences) during bead handling steps. An integrated heating step – often recommended to boost enzymatic performance (proteinase K) and maximize DNA yield – is also employed. The yield, purity and overall capabilities of each method are demonstrated, highlighting the automation potential of the Clean Blood & Tissue DNA kit on the ASSIST PLUS.

Quality checks for extracted DNA

DNA integrity is assessed using quality control techniques that include fluorescence-based concentration measurements, spectrophotometry measurements to screen for protein or organic contaminants, and quantitative PCR (qPCR) to confirm compatibility with downstream applications. Fluorescence-based DNA measurement uses specific DNA-binding dyes – like PicoGreen™ or SYBR™ Green – to enable precise, sensitive and reliable detection, making it ideal for extraction efficiency checks. Measurement of the optical density (OD) A_{260}/A_{280} ratio is a spectrophotometric method for the assessment of DNA purity; a reference value of ~1.8 indicates minimal protein contamination. The OD A_{260}/A_{230} ratio assesses DNA purity by detecting contaminants like phenol, guanidine and other salts, with a reference value between 2.0 and 2.2 indicating pure DNA. qPCR assesses the quality, integrity and concentration of extracted DNA, confirming its suitability for downstream applications by checking for amplifiability and the absence of potential inhibitors or contaminants.

Methods

Protocol. The extraction was carried out following the instructions provided by the kit suppliers, using the set-up outlined in **Table 1**.
Sample material. 12 EDTA whole blood samples were collected from individual donors, and stored at -80 °C for up to 6 weeks prior to extraction.
Quality control of extracted DNA. The DNA concentration (ng/µl) was measured using a DeNovix DS-11 FX spectrophotometer/fluorometer, with an input volume of 2 µl. Elution buffer was used to blank the device.
qPCR. The albumin gene was chosen as a target control for the qPCR assay, which was performed with the SensiFAST™ SYBR® Lo-ROX Kit (Meridian Bioscience) on the QuantStudio™ 5 (Thermo Fisher Scientific). The reaction volume (25 µl) included 12.5 µl SensiFAST SYBR Lo-ROX (2x), 1.5 µl of a mix with Alb-FW primer, Alb-RV primer and Alb-Probe (10 mM), 6.0 µl of nuclease-free water and 5 µl of template DNA. The cycling conditions included the following steps: 50 °C for 2 minutes, 95 °C for 10 minutes, 95 °C for 15 sec (40 cycles), 60 °C for 1 minute.[1,2]

Workflow set-up

	Automated set-up: magnetic beads	Manual set-up: spin columns
Sample input	200 µl EDTA blood*	200 µl EDTA blood
Kit chemistry	Magnetic beads Clean Blood & Tissue DNA Kit	Spin column Kit Q
Liquid handling	Automated on an ASSIST PLUS with a 6 channel VOYAGER pipette	Manually pipetted by the end user
Binding and washing	HEATMAG magnetic separation/heating module	Tabletop centrifuge Manual transfer in/out of centrifuge
Heating	HEATMAG magnetic separation/heating module, with integrated heating steps at 56 °C (lysis) and 65 °C (elution)	Thermal block, 56 °C Stand-alone
Consumables	GRIPTIPS® pipette tips, sterile, filter, low retention Deep well plate as reagent source Eppendorf Safe-Lock® Tubes, 1.5 ml	Standard tips Eppendorf Safe-Lock® Tubes, 1.5 ml
Sample number	12	12
Total time	~90 min	~60 min
Prep time	~20 min Reagent aliquoting	~10 min Tube labeling Sample transfer
Hands-on time	~5 min Tip box change (1x)	~40-50 min Continuous attention

**200 µl of EDTA blood was used with 50 µl 1x PBS to reach a total volume of 250 µl*

Table 1: Summary of the automated and manual set-ups.

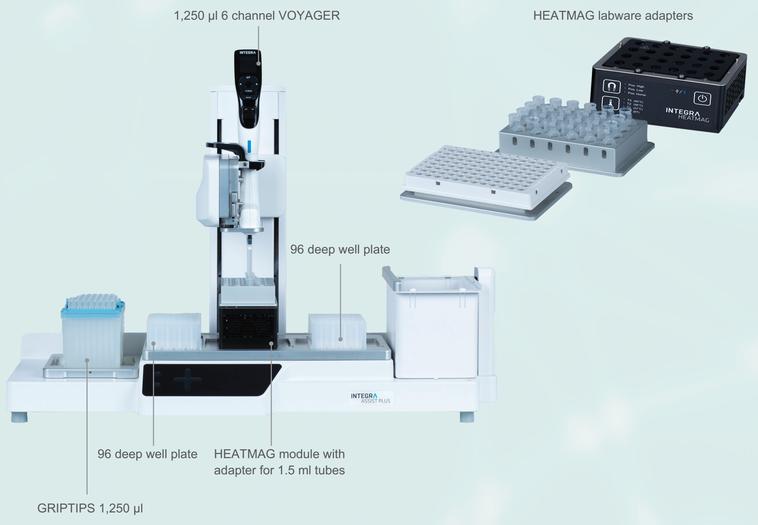
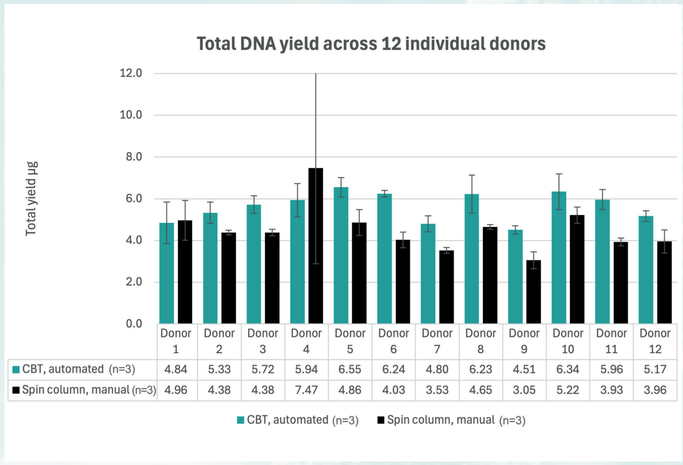


Figure 1: All-in-one INTEGRA automated set-up for extraction of 12 samples using the Clean Blood & Tissue DNA kit together with the HEATMAG module and available labware adapters for 96 well PCR plates and Eppendorf Safe-Lock Tubes.

Results

Total yield of DNA

The total DNA yield after extraction, measured by fluorescence, was higher across individual donors for the magnetic bead approach compared to the spin column method. Variations in the yield observed between donors are expected, as blood composition – including the number of white blood cells with nuclei, the primary source of nucleic acids – is an inherent characteristic of each individual (**Figure 2**).



	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 8	Donor 9	Donor 10	Donor 11	Donor 12
CBT, automated (n=3)	4.84	5.33	5.72	5.94	6.55	6.24	4.80	6.23	4.51	6.34	5.96	5.17
Spin column, manual (n=3)	4.96	4.38	4.38	7.47	4.86	4.03	3.53	4.65	3.05	5.22	3.93	3.96

Figure 2: The total DNA yield was determined by multiplying the concentration by the elution volume.

qPCR assay DNA compatibility

Lower Ct values were observed for all samples purified using the Clean Blood & Tissue DNA Kit, indicating that this method produced a higher concentration of amplifiable DNA (**Figure 4**). The qPCR signal obtained from eluates after spin column extraction was likely inhibited by the presence of contaminants, as indicated by A_{260}/A_{280} and A_{260}/A_{230} ratio measurements that deviated above or below the reference values, respectively (**Figures 3 and 5**).

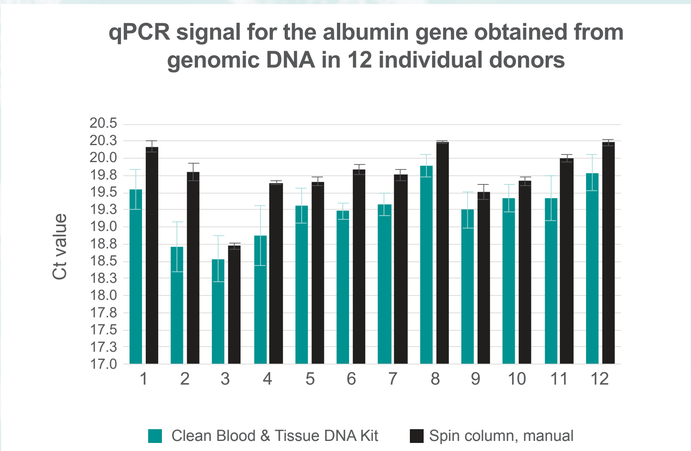


Figure 4: Ct values obtained for the albumin gene from genomic DNA in eluates purified using the Clean Blood & Tissue DNA Kit and spin column methods (error bars represent standard deviation from n=3 replicates).

DNA purity

OD A_{260}/A_{280} values for the automated Clean Blood & Tissue DNA Kit set-up were closer to the reference value of 1.8 compared to the results for the spin column extraction method indicating higher purity and less protein contamination (**Figure 3**).

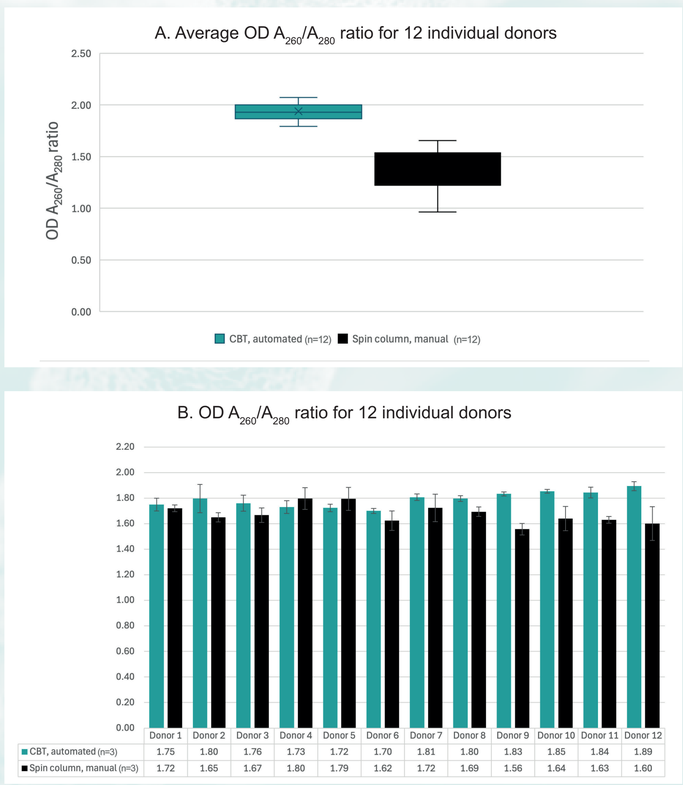


Figure 3: OD A_{260}/A_{280} values: A. Average values for 12 samples; B. Individual values for each donor.

OD A_{260}/A_{230} values for the automated set-up with the Clean Blood & Tissue DNA Kit were closer to the reference range of 2.0-2.2 indicating no contaminants. Significantly lower values were obtained for the spin column extraction method (**Figure 5**).

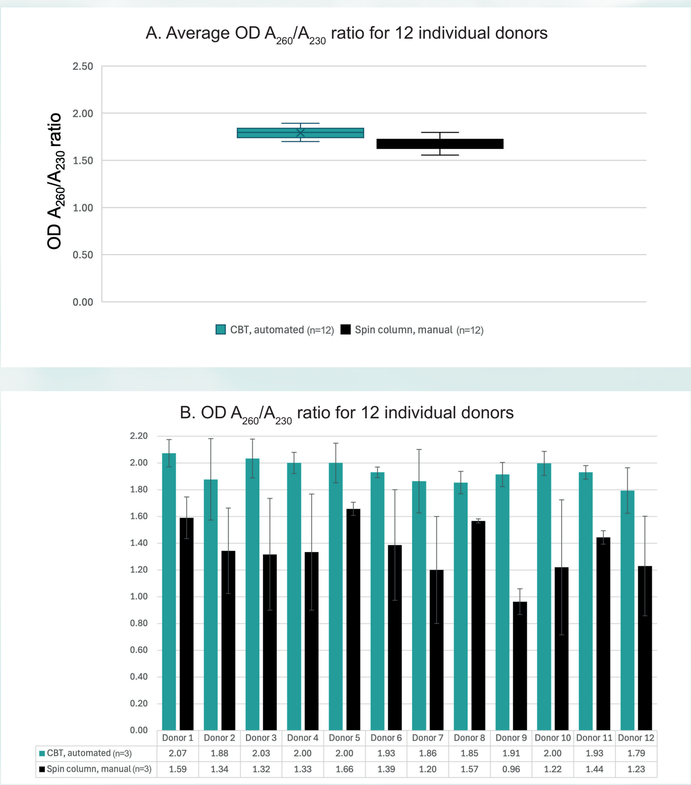


Figure 5: OD A_{260}/A_{230} values: A. Average values for 12 samples; B. Individual values for each donor.

Discussion

The total DNA yield after the extraction of blood samples, as measured by fluorescence, was higher across individual donors for the magnetic bead-based Clean Blood & Tissue DNA kit compared to the spin column method **(Figure 2)**. The spectrophotometric values met the reference standards for DNA isolated using magnetic beads, confirming that the nucleic acid was of high purity and free from protein contamination **(Figures 3 and 5)**. In contrast, DNA obtained through the spin column extraction method exhibited a low OD A_{260}/A_{230} ratio, suggesting the presence of organic contaminants such as phenol, guanidine, salts or solvents, likely due to incomplete washing of the silica column. These compounds are also known to interfere with applications such as qPCR or sequencing, reducing the efficiency and accuracy of downstream analyses. The study confirmed the correlation between a low OD A_{260}/A_{230} ratio and lower qPCR efficiency, indicating that inhibiting residuals are still present after column purification[3] **(Figures 4 and 5).** This could indicate that column-extracted nucleic acids may not be suitable for sensitive applications, and additional purification steps may be necessary to remove contaminants before use.

Summary

The Clean Blood & Tissue DNA kit provides consistent yields of high quality genomic DNA from whole blood samples. The magnetic bead-based kit offered higher yields with increased purity when compared to the traditional spin column method. The Clean Blood & Tissue DNA Kit purified nucleic acids also provided more robust and consistent results in qPCR. This shows that the quality of DNA obtained with the Clean Blood & Tissue DNA Kit matched or exceeded that of samples extracted using the spin column method across all parameters tested.

The automated INTEGRA workflow with the Clean Blood & Tissue DNA Kit offers several advantages over the manual spin column workflow, including:

- Minimal hands-on time, fully automated and walk-away workflow for 12 samples, freeing up valuable lab time for scientists while ensuring reliable and reproducible results
- Integrated heating step using a HEATMAG module to maximize yield, especially when working with small sample volumes or samples with reduced white blood cells
- High quality, reproducible DNA yields for all samples
- Streamlined and gentle nucleic acid isolation, eliminating the mechanical shearing stresses associated with spin columns
- Low reagent dead volumes
- User-friendly software with an integrated magnetic module for automated magnetic bead handling (**Figure 6**)

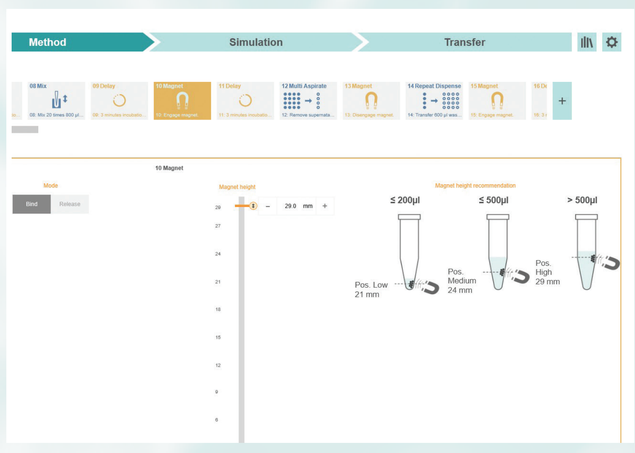


Figure 6: User-friendly commands to integrate the automated magnetic bead handling steps with the MAG/HEATMAG module on the ASSIST PLUS pipetting robot.

References

1. Laurendeau I, Bahuau M, Vodovar N, Larramendy C, Olivi M, Bieche I, Vidaud M, Vidaud D. TaqMan PCR-based gene dosage assay for predictive testing in individuals from a cancer family with INK4 locus haploinsufficiency. *Clin Chem.* 1999 Jul;45(7):982-6. PMID: 10388473.
2. Yang Q, Li X, Ali HA, Yu S, Zhang Y, Wu M, Gao S, Zhao G, Du Z, Zhang G. Evaluation of suitable control genes for quantitative polymerase chain reaction analysis of maternal plasma cell-free DNA. *Mol Med Rep*. 2015 Sept;12(5):7728-7734.

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