MACHEREY-NAGEL

NucleoSpin® Blood L Vacuum

Purification of genomic DNA from 2 mL blood samples using vacuum filtration

Introduction

Genetic analysis and related molecular assays require the use of high quality DNA as a template. Starting with DNA of high purity, concentration, and integrity is a critical step towards reaching successful results at each point in the workflow. For long-term storage, purified DNA samples are more stable and more compact than whole blood samples.

This application note describes the use of MACHEREY-NAGEL's NucleoSpin® Blood L Vacuum kit. This vacuum filtration based kit is designed for simultaneous purification of up to 24 whole blood samples with starting volumes that range from 1 mL to 2 ml. Vacuum filtration technology eliminates tedious centrifugation steps as well as the need to handle columns manually during the process (i.e., to discard flow through). NucleoSpin® Blood L Vacuum kit uses SBS format-based column holders to ensure compatibility with both manual manifolds (such as NucleoVac 96 Vacuum Manifold) as well as automated liquid handling platforms.

Blood samples are lysed using a Proteinase K incubation in the presence of a chaotropic salt-based buffer. Following the initial lysis step, a binding buffer is added to the samples and the lysate is loaded in two subsequent steps onto the binding columns. DNA is bound to the silica membrane as vacuum is applied to the column and buffers are filtered through. Impurities are removed from the bound DNA during several washing steps. DNA is eluted after a final drying step. 24 samples can typically be processed in approximately 90 min.

NucleoSpin® Blood L Vacuum kit can be used for fresh or frozen human blood samples stored in EDTA, citrate, or other common anticoagulants. Depending on the leukocyte count, typical yields up to 78 ug can be achieved. DNA purity ($A_{260}/_{280}$) is typically in the range of 1.7–1.9.

Material and Methods

The NucleoSpin® Blood L Vacuum kit on the NucleoVac 96 Vacuum Manifold was used according to the standard protocol. The complete user manual can be found on the MACHEREY-NAGEL website (www.mn-net.com/Bioanalysis). For vacuum processing, the NucleoVac 96 Vacuum Manifold, and Starter Set Midi are needed. The Starter Set Midi contains the necessary Column Holder Midi, Wash Plate Midi, Elution Tube Holder Midi, and Dummy Columns for processing NucleoSpin® L (Midi) Columns on the NucleoVac 96 Vacuum Manifold.

Procedure at a glance

NucleoSpin® Blood L Vacuum	
Lyse	50 µL Proteinase K 2 mL blood Mix 3 times (by pipetting up and down) 750 µL Buffer BLV1 Mix 3 times (by pipetting up and down)
	Shake at 1000 rpm at 56 °C, 30 min
Bind	4 mL BLV2 Mix 5 times (by pipetting up and down)
	Load 3.5 mL lysate + 300 µL Buffer BLV4 overlay -0.2–0.4 bar*, 3–5 min Load 3.5 mL lysate + 300 µL Buffer BLV4 overlay -0.2–0.4 bar*, 3–5 min
Wash	4 mL Buffer BLV3 RT, 5 min -0.2–0.6 bar*, 2 min
	2 mL Buffer BLV4 -0.2–0.6 bar*, 2 min
	2 mL Buffer BLV4 -0.2–0.6 bar*, 2 min
Dry	-0.6 bar, 10 min
Elute	300 µL Buffer BLV5 RT, 2 min -0.4 bar*, 30 s -0.6 bar*, 30 s
	300 µL Buffer BLV5 RT, 2 min -0.4 bar*, 30 s -0.6 bar*, 30 s

* Reduction of atmospheric pressure

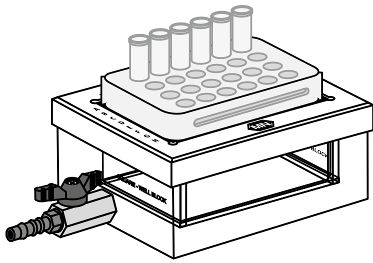

Figure 1: Setup of the NucleoVac 96 Vacuum Manifold

A maximum of 24 samples can be processed in parallel. Unused positions in the Column Holder Midi are closed with Dummy Columns.

Results

The DNA purification results reproducibility was measured using pooled blood samples as well as individual blood samples. DNA yield and purity were determined by UV spectroscopy. Highly reproducible results were obtained when using pooled blood samples, which demonstrates the robustness of the purification method (figure 2). DNA was purified from identical aliquots of 2 mL fresh or frozen human blood. An average DNA yield of 68 µg with a CV of 9.5 % was obtained from fresh blood samples and an average yield of 33.9 µg with a CV of 12 % from frozen samples.

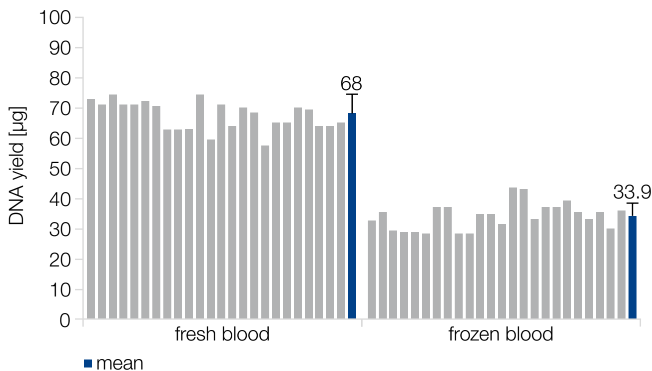

Figure 2: DNA yield from 2 mL fresh or frozen pooled blood samples purified by NucleoSpin® Blood L Vacuum.

For further investigation, individual blood samples were purified. Results are shown in figure 3. DNA yields from 2 mL fresh blood samples were in the range of 33–78 ug, while DNA yields from 2 mL frozen blood samples ranged between 27–55 ug. DNA yield from individual blood samples strongly depends on the leukocyte count of the donor.

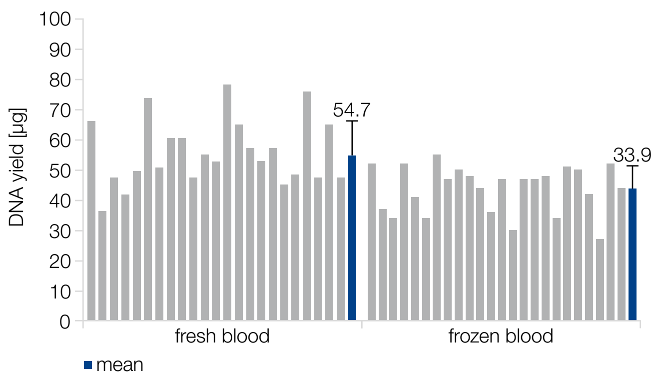

Figure 3: Yield of the purified DNA from individual blood samples.

Excellent purity ratios were obtained from all individual blood samples, A_{260}/A_{280} between 1.7–1.9, with an average A_{260}/A_{280} of 1.9 (data not shown). Ratios in this range indicate the absence of protein carry over in the purified DNA samples.

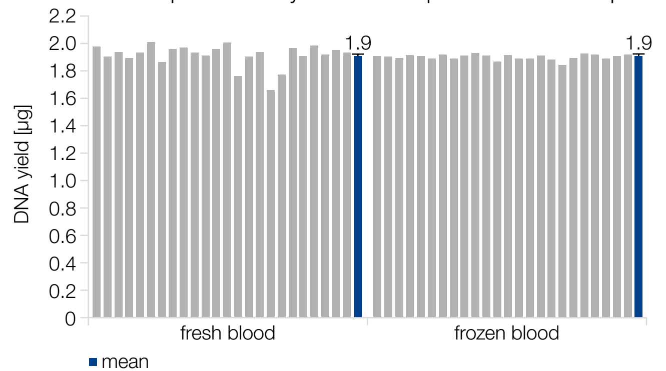

Figure 4: Purity of the purified DNA from individual blood samples.

DNA integrity for fresh and frozen blood samples was investigated by agarose gel electrophoresis (figure 5 and 6). 10 µL of the DNA purified from 2 mL pooled fresh and frozen blood samples was loaded on a 1 % TAE agarose gel (ethidium bromide stain). High molecular weight DNA combined with the absence of low molecular weight smear or degradation products demonstrate the integrity and quality of the purified gDNA.

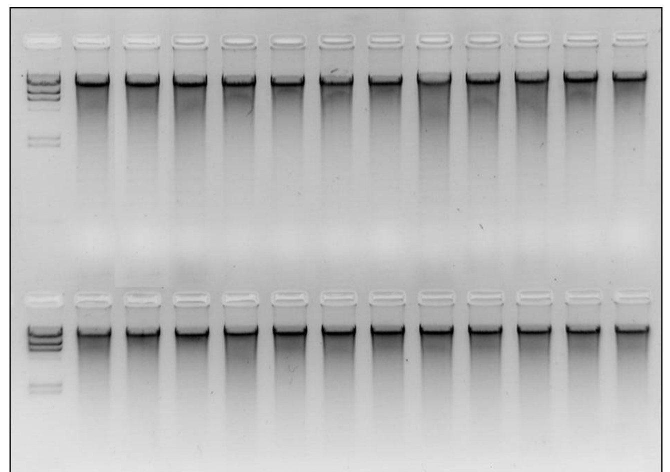

Figure 5: Agarose gel analysis of the purified DNA from pooled fresh blood samples.

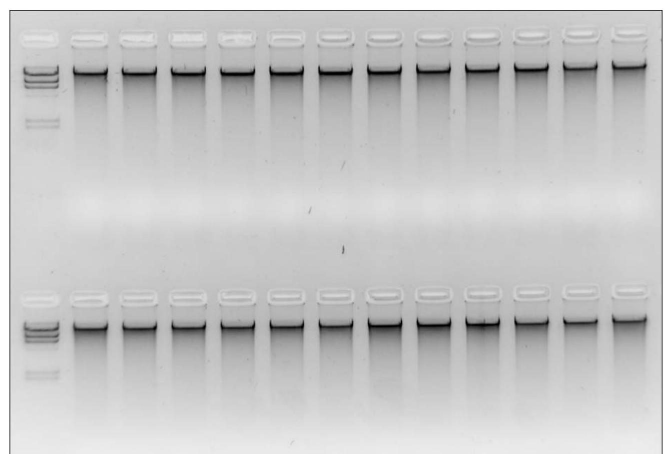

Figure 6: Agarose gel analysis of the purified DNA from pooled frozen blood samples.

Purified DNA was analyzed by qPCR to measure performance and presence of inhibitors in a real-time PCR assay targeting the beta-actin gene. 2.5 µL of the purified DNA was used as template in a 25 µL qPCR reaction. All purified samples were amplified with homogenous results. PCR amplification plots indicate the absence of inhibition (Figure 7).

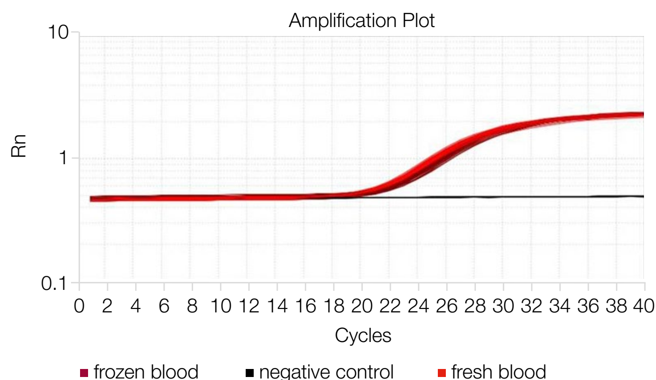

Figure 7: Real-Time PCR analysis of DNA purified from human whole blood (amplification plots).

All samples were purified with high consistency. An average C_T value of 23.1 with a standard deviation of 0.3 was obtained from fresh blood samples and an average of C_T of 23.9 with a standard deviation of 0.2 from frozen blood samples (figure 8).

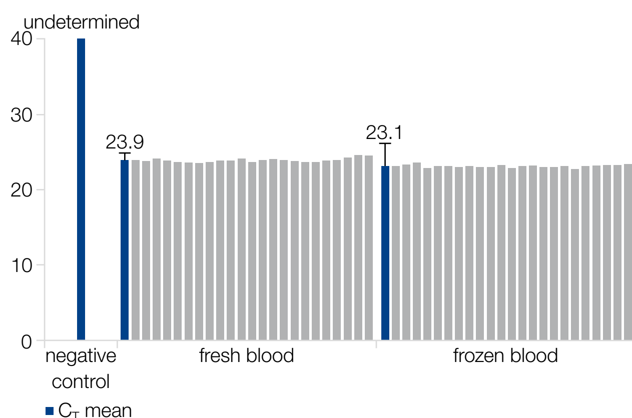

Figure 8: Real-Time PCR analysis of DNA purified from human whole blood (C_T values).

To confirm the absence of cross contamination, a sample plate with checkerboard pattern was analyzed (figure 9). The negative PBS samples were placed next to the blood samples in a checkerboard pattern and monitored for cross contamination. The PBS samples were tested for contaminating DNA using a 40 cycle qPCR targeting the beta-actin gene. Figure 9 B shows that PCR amplification was observed only in the wells that contained preloaded blood samples. No amplification was observed in the wells with PBS buffer. These results demonstrate that no cross contamination occurred during processing.

A)

	1	2	3	4	5	6
A	sample	no sample	sample	no sample	sample	no sample
B	no sample	sample	no sample	sample	no sample	sample
C	sample	no sample	sample	no sample	sample	no sample
D	no sample	sample	no sample	sample	no sample	sample

B)

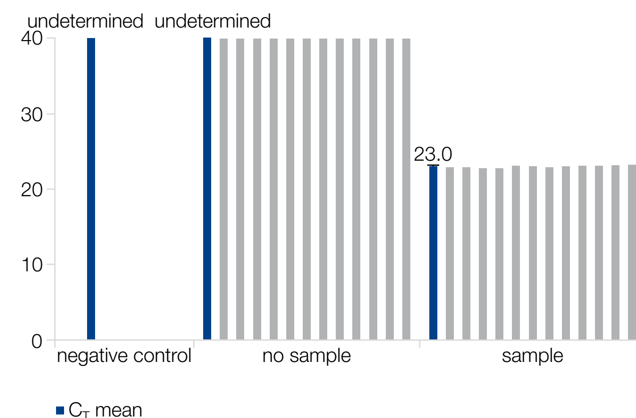

Figure 9: Cross-contamination analysis

A) Pipetting pattern

B) Amplification results

Conclusion

The NucleoSpin® Blood L Vacuum kit provides an optimal solution for manual, vacuum based DNA purification for 2 mL fresh and frozen blood samples. Convenient processing of up to 24 samples is possible using the Starter Set Midi in combination with the NucleoVac 96 Vacuum Manifold. The results demonstrate that both fresh and frozen human blood samples are suitable. The purified DNA is of high quality and suitable for qPCR analysis without any inhibition. The use of SBS format based column holders make it possible to integrate NucleoSpin® Blood L Vacuum into suitable liquid handling platforms.

Ordering information

Product	Preps	REF
NucleoSpin® Blood L Vacuum	24	740954.24
Product accessories	**Pack of**	**REF**
NucleoVac 96 Vacuum Manifold	1	740681
NucleoVac 96 Vacuum Regulator		740641
Starter Set Midi		740744

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