MACHEREY-NAGEL

NucleoSpin® Blood L Vacuum

Automated purification of genomic DNA from 1 mL blood samples on the automation platform ep*Motion*® 5075vt

Introduction

The automated routinely performed isolation of genomic DNA from whole blood samples is an initial and crucial step for various diagnostic workflows. Clinical applications such as genotyping, HLA typing, biomarker discovery, newborn screening, and pharmacogenetics are widely performed in laboratories worldwide. Subsequent biomolecular detection methods like qPCRs, next-generation sequencing, and microarray analysis, are constantly improved leading to a better sensitivity and molecular diagnostic performance.

A main aspect to face these workflow requirements, is to facilitate the extraction of highly pure DNA in substantial amounts. To provide a fast and consistent sample processing, MACHEREY-NAGEL designed the NucleoSpin® Blood L Vacuum kit, specialized for automated, vacuum based purification of genomic DNA from medium volume whole blood samples (fresh or frozen, EDTA or citrate treated) in a 24-well format.

This application note describes the purification process on the automated liquid handling platform ep*Motion*® from Eppendorf® using the NucleoSpin® Blood L Vacuum kit from MACHEREY-NAGEL. The novel optimized protocol allows the processing of 24 samples each with a volume of 1 mL within 110 minutes including a sample lysis on the ep*Motion*®.

Products at a glance

NucleoSpin® Blood L Vacuum	
Technology	Silica membrane technology
Sample material	≤ 1 mL whole blood (fresh or frozen, EDTA or citrate treated)
Preparation time	Approx. 110 min on ep*Motion*® 5075vt for 24 samples including samples lysis
Typical yield	50–80 µg (2 mL blood; depending on sample quality)
Elution volume	2 x 300 µL
Binding capacity	250 µg

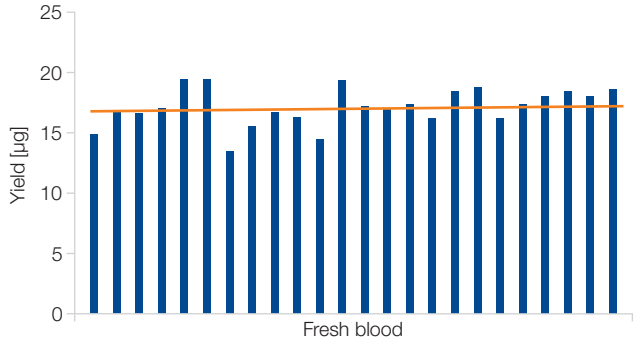
Material and methods

This protocol is programmed to process up to 24 samples in parallel and developed for the ep*Motion*® 5075vt platform.

Fresh or frozen blood samples, treated with EDTA as anticoagulant, and may be derived from humans or animals (e.g., pig or cow).

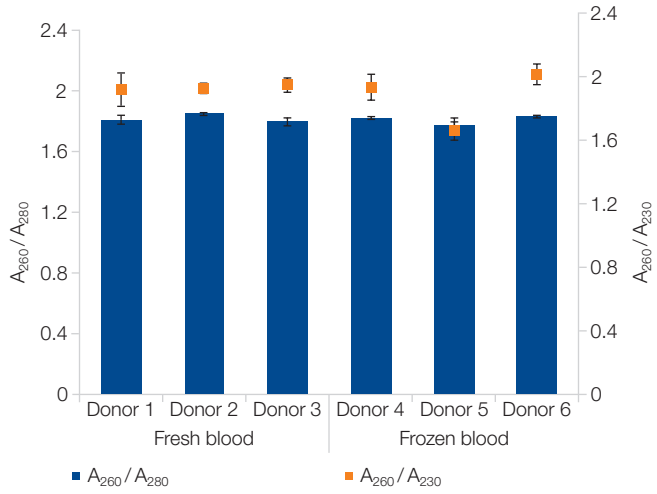
Up to 1 mL of each blood sample needs to be filled into individual wells of the lysis plate. All subsequent steps are performed automatically. Blood samples are lysed by Proteinase K and Lysis Buffer BLV1. This step is followed by the addition of Binding Buffer BLV2, which allows binding of the DNA to the silica membrane as vacuum is applied and the lysate is filtered through. Subsequent washing steps remove impurities and pure DNA is finally eluted after a final drying step.

Application data



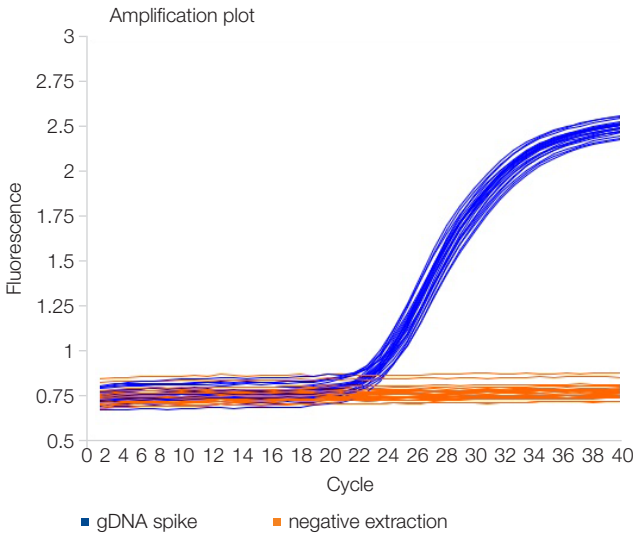
Reproducibility of nucleic acid purification

DNA was isolated from a fresh human blood sample pool (1 mL; n = 24) using the NucleoSpin® Blood L Vacuum kit on an ep*Motion*® 5075vt platform. The total yield was determined by UV spectrometry (blue bars), resulting in an average yield of 17.14 µg ± 1.56 (orange line).



Purity of isolated nucleic acids

DNA was isolated from 1 mL fresh and frozen human blood samples (n = 4) using the NucleoSpin® Blood L Vacuum kit on an ep*Motion*® 5075vt workstation. The total purity was determined by UV spectroscopy. The ratios of A_{260}/A_{280} value is shown in dark blue bars and ratios of A_{260}/A_{230} value in orange squares.



Determination of cross-contamination

One cause of false positive results can be the accidental contamination of samples or reagents with positive samples (cross-contamination) during vacuum processing, such as aerosol formation or spraying. The tailored protocol to isolate 1 mL blood with the NucleoSpin® Blood L Vacuum kit on an ep*Motion*® 5075vt was verified regarding the cross-contamination using 12 positive samples, spiked with genomic DNA (blue lanes) and 12 negative samples (orange lanes, containing water) in a chessboard pattern. The samples were subsequently analyzed by qPCR using a ß-actin amplicon of 250 bp.

Automate your DNA extraction from 1 mL blood samples

MACHEREY-NAGEL and Eppendorf® deliver a sophisticated solution for your automated DNA extraction from various medium volume blood samples, such as fresh, frozen, treated with EDTA or citrate. We adapted the NucleoSpin® Blood L Vacuum kit on the ep*Motion*® 5075vt to speed up your nucleic acid purification workflow.

- Reliable performance and excellent yields from plasma and serum sample material
- High reproducibility without cross contamination
- Fast processing of 24 samples (1 mL) volume within 110 min.

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® Blood L Vacuum	Kit based on silica membrane technology for the isolation of genomic DNA from 2 mL blood samples. Containing NucleoSpin® Blood L Columns with Collection Tubes, Collection Tubes (1.5 mL), buffers, Proteinase K	1 x 24	740954.24
Starter Set Midi	Column Holder for processing NucleoSpin® Blood L vacuum on a vacuum manifold	1 set	740744
24-Square-well Block 10 mL	24-Square-well Block 10 mL suitable for sample lysis	4 pieces	740679.4
ep*Motion*® 5075vt	Basic device incl. vacuum system, gripper, vac frame 2, vac frame holder, Eppendorf ThermoMixer®, epBlue™ software, mouse, waste box, 100–240 V ±10 % / 50–60 Hz ±5 %, 0.2 µL–1 mL		5075000304