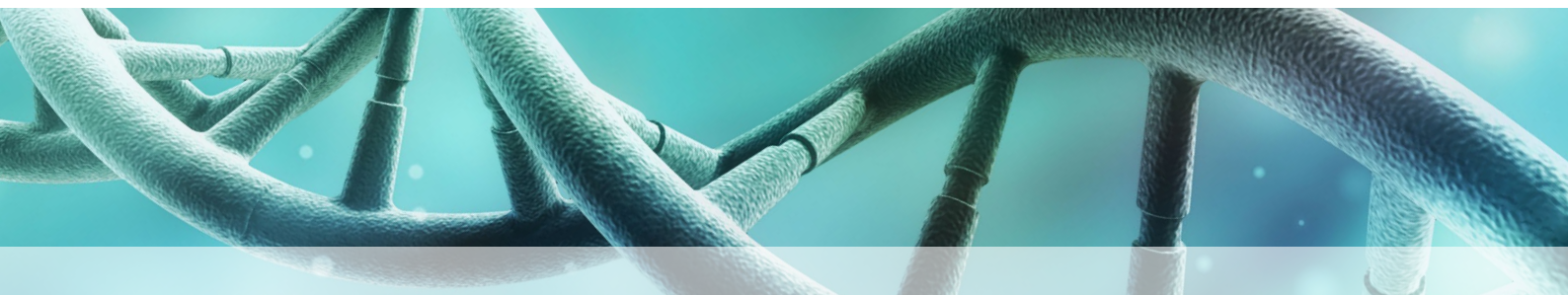


NucleoMag[®] cfDNA

Automated purification of cfDNA from plasma samples on the Hamilton NIMBUS[®] Presto workstation



Introduction

Liquid biopsies, specifically cell-free DNA (cfDNA) analysis, are minimally invasive techniques for genomic profiling with an enormous potential for clinical prognostic and diagnostic applications. Thereby, the techniques of cfDNA analysis are a tool for assessing biomarkers for cancer profiling, tumor burden monitoring, prenatal testing or quantification of minimal residual disease during patient follow-up.

The NucleoMag[®] cfDNA kit is designed for rapid and reliable isolation of circulating cell-free DNA from human plasma, derived from e.g. EDTA blood draw tubes or Cell-Free DNA BCT[®] Tubes. The kit enables the highly efficient recovery of fragmented DNA ≥ 50 bp. Elution can be performed with as little as 50–200 μ L, and purified DNA is ready for use in all common subsequent downstream applications like digital droplet PCR or NGS.

Together with Hamilton, MACHEREY-NAGEL has established its NucleoMag[®] cfDNA kit on the NIMBUS Presto workstation. In this Application Note, we demonstrate the reliable cfDNA purification from human plasma samples in a fully automated manner for your high-throughput nucleic acid extraction workflow.

NucleoMag[®] cfDNA

Technology	Magnetic beads
Sample material	Cell-free BCT (Streck), Plasma, Serum
Sample amount	≤ 2 mL in standard protocol (protocol adaptation is necessary for larger volumes)
Elution volume	50–200 μ L
Typical DNA yield	depending on sample type, storage and quality
Average fragment size	> 50 bp
Run time	with on-deck lysis: approx. 120 min* for 24 samples with external lysis : approx. 50 min* for 24 samples

*the user can decide whether lysis should take place externally or on-deck

NIMBUS Presto workstation

Technology	Automated liquid handling platform (Hamilton NIMBUS) with integrated magnetic rod processing unit (KingFisher [™] Presto)
Capacity	1–24 samples (≤ 2 mL sample volume)
Processable volume	50–5000 μ L
Footprint	L 1359 mm W 709 mm H 889 mm



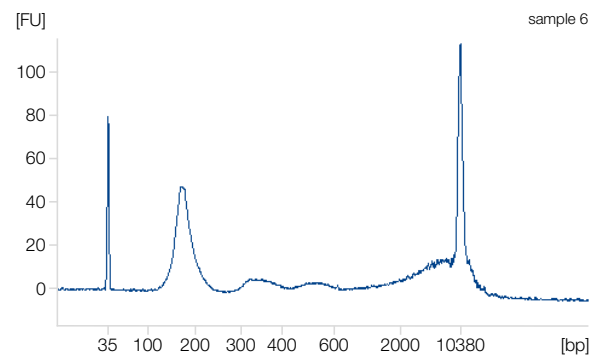
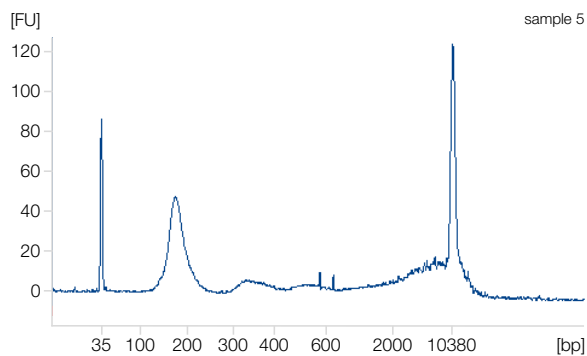
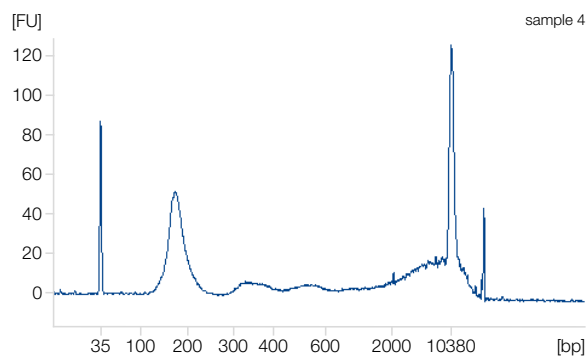
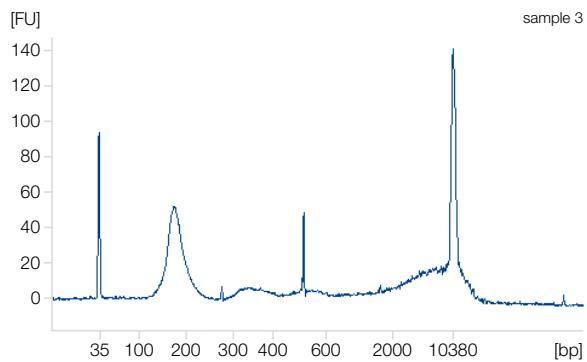
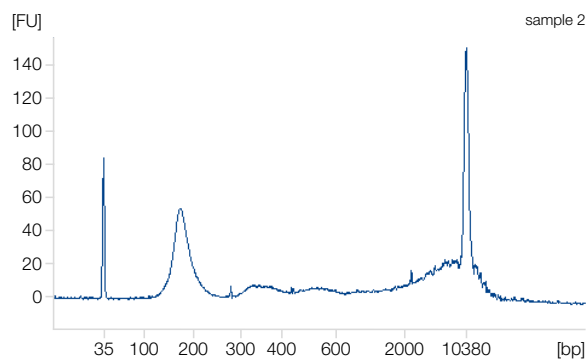
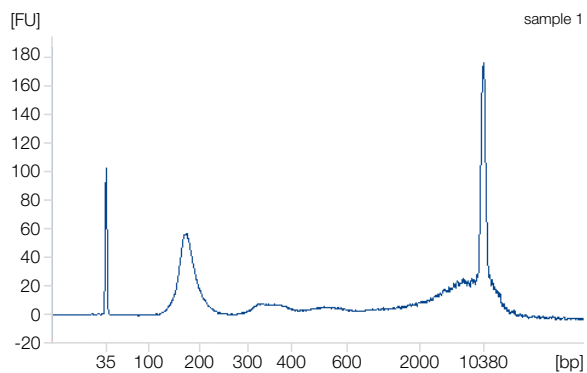
The NIMBUS Presto workstation combines liquid handling and magnetic rod processing for fully automated, high-throughput nucleic acid extractions.

Material and Methods

In this Application Note, we demonstrate the automated purification workflow exemplarily for human plasma samples using the MACHEREY-NAGEL NucleoMag[®] cfDNA and the Hamilton NIMBUS Presto System.

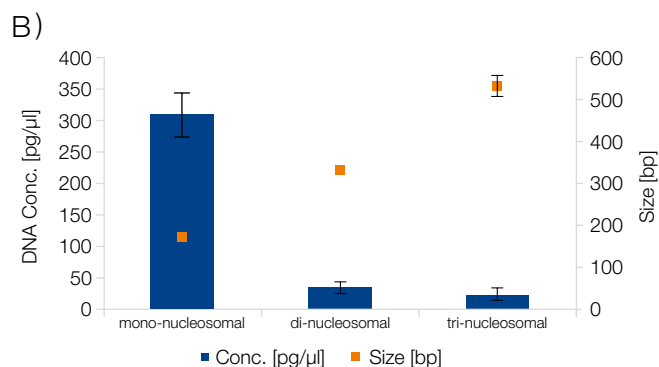
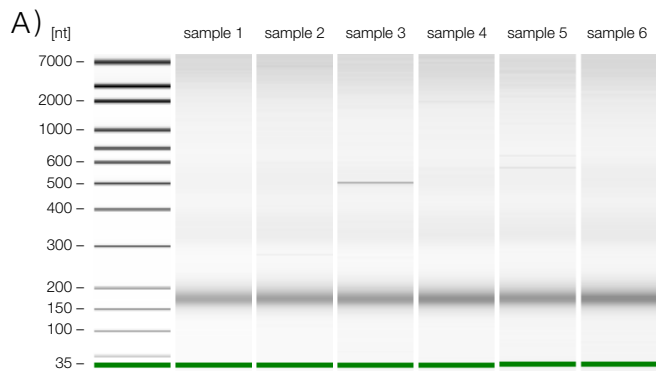
Total cfDNA from 2 mL human EDTA plasma derived from six different donor samples was purified. Plasma samples are mixed with liquid Proteinase K and lysis buffer prior to lysis incubation. The subsequent isolation is based on reversible adsorption of nucleic acids to magnetic beads (NucleoMag[®] P-Beads). Nucleic acid binding is enabled by the addition of Binding Buffer MCF2. After magnetic separation and removal of the supernatant, contaminants and salts are removed by three subsequent washing steps. The NucleoMag[®] P-Beads are air-dried before highly pure nucleic acids are finally eluted under low ionic strength conditions in Elution Buffer MCF5.

Application data



Reliable purification of cfDNA from various plasma samples

The NucleoMag® cfDNA kit was used to isolate cfDNA from human EDTA plasma samples. Efficient purification of cfDNA is demonstrated by the characteristic peak at approx. 150–170 bp using the Bioanalyzer™ 2100 system and the High Sensitivity DNA Kit from Agilent. First and last peaks correspond to internal markers that were run with each of the samples.



Consistent cfDNA recovery, regardless of plasma sample

A) The isolation of cfDNA from human EDTA plasma using the NucleoMag[®] cfDNA kit shows clear bands at the expected size of approx. 170 bp in the virtual gel image using the Agilent Bioanalyzer[™] 2100 system with the High Sensitivity DNA kit.

B) Concentration of the respective nucleosomal units of the above-mentioned samples were determined by integrating the peak areas from the bioanalyzer analysis. The fragment length distributions of mono-nucleosomal, di-nucleosomal and tri-nucleosomal DNA fractions were analyzed and indicate the typical gradual decrease in concentration with increasing nucleosome number.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag [®] cfDNA	Magnetic bead-based kit for the isolation of circulating DNA from plasma samples; including NucleoMag [®] P-Beads, buffers, Liquid Proteinase K	1 x 48 preps	744550.1
		4 x 48 preps	744550.4
NIMBUS Presto	Automated liquid handling platform with 4 pipetting channels, a CO-RE Gripper, barcode scanner, and many additional features		Hamilton*

NucleoMag[®] is a registered trademark of MACHEREY-NAGEL; Hamilton[®] and NIMBUS[®] are registered trademarks of Hamilton Company; KingFisher[™] is a trademark of Thermo Fisher Scientific.

* For more detailed information, please visit www.hamiltoncompany.com/robotics. To find a Hamilton subsidiary or distributor in your area, please visit <http://www.hamiltoncompany.com/contacts>.

A rapid, fully automated solution for cfDNA isolation from plasma samples

MACHEREY-NAGEL and Hamilton deliver a tailored solution for your high-throughput cfDNA purification from human plasma samples. We have adapted the NucleoMag[®] cfDNA kit procedure on the NIMBUS Presto workstation to meet the expectations of cfDNA analysis and profiling.

Here, we demonstrate the successful use of the NucleoMag[®] cfDNA kit for nucleic acid isolation from human plasma and subsequent fragment size analysis.

The powerful combination of the NucleoMag[®] technology and the NIMBUS Presto workstation has several advantages over standard nucleic acid purification procedures:

- Save hands-on time by using automated plate-prefilling and plate-handling performed by the NIMBUS workstation
- Benefit from the high-speed extraction procedure of the integrated KingFisher[™] Presto unit
- Efficient purification of fragmented DNA as small as 50 bp
- No PCR inhibition, regardless of your preferred sample volume