

NucleoSpin® 96 cfDNA

Automated purification of cell-free DNA from 1 mL plasma samples on the epMotion® 5075vt



Introduction

The analysis of cfDNA in plasma or serum allows a non-invasive monitoring of, e.g., a disease, the detection of aneuploidy of an unborn child, or the rejection of a transplant. Cell-free nucleic acids are especially promising targets in the field of cancer diagnostics. Tumor cells release DNA in the blood flow by a combination of apoptosis, necrosis and active secretion (1). The circulating-free DNA can be used for monitoring disease progression, therapeutic effects of a treatment, or recurrence of cancer (2, 3).

There are various challenges related to the isolation procedure of cfDNA from plasma or serum samples such as the removal of potential inhibitors, the low concentration of cfDNA in comparison to the genomic DNA content and the inherent sample variability. A main obstacle is the low abundance and the small fragment size of cfDNA (< 200 bp).

In order to recover sufficient amount of pure cfDNA for subsequent biomolecular applications MACHEREY-NAGEL developed the NucleoSpin® 96 cfDNA kit designed for isolation of cell-free DNA from plasma and serum in a silica membrane 96-well plate format.

This application note describes the purification process on the automated liquid handling platform epMotion® from Eppendorf® using the NucleoSpin® 96 cfDNA kit from MACHEREY-NAGEL. The novel optimized protocol allows the processing of 8 to 96 samples per run (variable sample number in multiples of 8). The processing time for 48 samples (2 mL) is 100 minutes and for 96 samples (1 mL) is 104 minutes.

Products at a glance

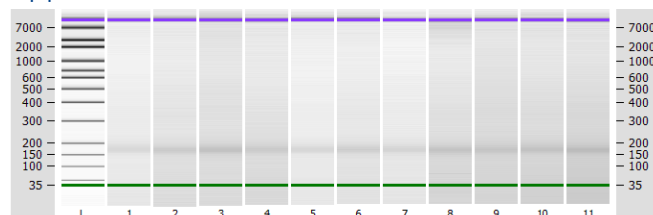
| NucleoSpin® 96 cfDNA | |
|----------------------|---|
| Technology | Silica membrane technology |
| Sample material | 0.5–2 mL plasma or serum |
| Preparation time | Approx. 104 min for 96 samples (1 mL) and 100 min for 48 samples (2 mL) on epMotion® 5075vt (excluding lysis) |
| Blood draw tubes | Human EDTA/ Cell-Free DNA BCT® (Streck) |
| Elution volume | 100 µL (70 µL final eluate volume) |
| Fragment size | ≥ 50 bp |

Material and methods



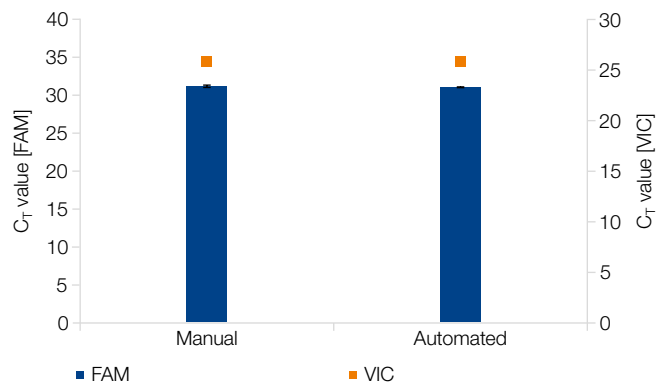
This protocol is programmed to process up to 96 samples (1 mL) or 48 samples (2 mL) in parallel and developed for the epMotion® 5075vt platform. Sample lysis is performed externally according to the user manual. Following lysis all subsequent steps are performed on the epMotion® 5075vt. The protocol starts with the adjustment of binding condition in a unique manner in order to allow a consecutive binding of nucleic acids to the silica membrane. Contaminants such as proteins or lipids are then removed from the silica membrane by three washing steps, while nucleic acids are reversibly bound to the silica membrane. Highly pure cfDNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer (PME).

Application data



Consistent cfDNA recovery

The isolation of cfDNA from 1 mL human EDTA plasma using the NucleoSpin® 96 cfDNA kit on the epMotion® 5075vt shows the characteristic peak at approx. 170 bp after measurement by capillary gel electrophoresis using the Agilent Bioanalyzer™ 2100 system with the High Sensitivity DNA kit.



Comparison of automated and manual processing of 1 mL plasma sample

DNA was isolated from human plasma (n = 8; 1 mL each) using the NucleoSpin® 96 cfDNA kit automated on the epMotion® 5075vt platform or via manual purification using the NucleoVac 96 vacuum manifold (MN). The final cfDNA recovery was determined by quantitative real time PCR, using the Quantifiler® Human DNA Quantification kit. The TaqMan® probe for detecting the target region (human telomerase reverse transcriptase gene) of interest is labeled with a FAM reporter dye (blue bars). VIC dye was used for detecting the amplified Internal PCR control DNA (orange squares), enabling verification that the polymerase, the assay, and the detection instrumentation are working correctly.

| Number | Concentration [pg/μL] | total yield [ng] |
|-----------|-----------------------|------------------|
| Sample 1 | 22.96 | 2.76 |
| Sample 2 | 21.21 | 2.55 |
| Sample 3 | 21.06 | 2.53 |
| Sample 4 | 23.14 | 2.78 |
| Sample 5 | 28.22 | 3.39 |
| Sample 6 | 27.46 | 3.3 |
| Sample 7 | 24.69 | 2.96 |
| Sample 8 | 22.89 | 2.75 |
| Sample 9 | 25.67 | 3.08 |
| Sample 10 | 34.53 | 4.14 |

Reliable purification of cfDNA from plasma

Human cfDNA was isolated from plasma samples (n = 10) using the NucleoSpin® 96 cfDNA kit on the epMotion® 5075vt. The total yield was determined using the Agilent Bioanalyzer™ 2100 system.

Automate your DNA extraction from plasma samples

MACHERY-NAGEL and Eppendorf® deliver a sophisticated solution for your high throughput DNA extraction from plasma and serum samples. We adapted the NucleoSpin® 96 cfDNA kit on the epMotion® 5075vt to speed up your nucleic acid purification workflow.

- Reliable performance and excellent yields from plasma sample material
- Variable sample number in multiples of 8 (8–96 samples)
- Fast processing of 96 samples (1 mL) volume within 104 min and 48 samples (2 mL) within 100 min

References

1. Stroun et al., 2001 "About the possible origin and mechanism of circulating DNA apoptosis and active DNA release". Clinica Chimica Acta
2. Crowley et al., 2013 "Liquid biopsy: monitoring cancer-genetics in the blood" Nature Reviews Clinical Oncology
3. Alix-Panabières and Pantel 2016 "Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy" Cancer Discovery

Ordering information

| Product | Specifications | Pack of | REF |
|-------------------------------|--|-----------|---------------------------------|
| NucleoSpin® 96 cfDNA | Kit based on silica membrane technology for the isolation of cfDNA from plasma samples | 1 x 96 | 740873.1 |
| | | 4 x 96 | 740873.4 |
| NucleoSpin® 96 cfDNA Core Kit | Kit based on silica membrane technology for the isolation of cfDNA from plasma samples | 1 x 96 | 740874.1 |
| | | 4 x 96 | 740874.4 |
| Square-well Block | Square-well Block with U-bottom suitable for sample lysis of 1 mL samples | 4 pieces | 740481 |
| | | 24 pieces | 740481.24 |
| 24-Square-well Block 10 mL | 24-Square-well Block 10 mL suitable for sample lysis of 2 mL samples | 4 pieces | 740679.4 |
| epMotion® 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 | 1 | 5075000304 www.eppendorf.com |

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