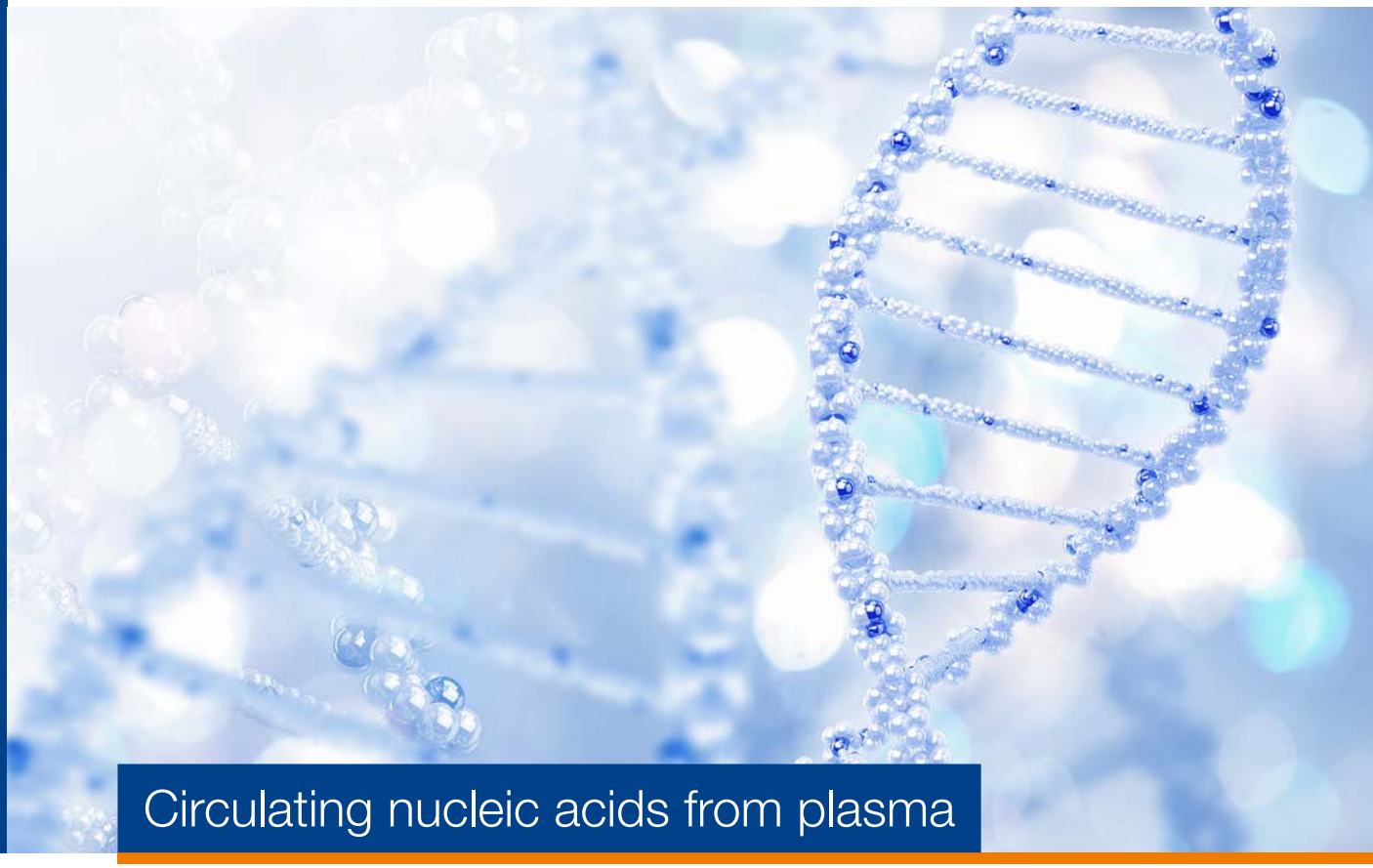
Bioanalysis

MACHEREY-NAGEL

Products for cfDNA and miRNA isolation



Circulating nucleic acids from plasma

- Flexible solutions for small and large blood plasma volumes
- Highly efficient recovery of nucleic acids from “Liquid Biopsies”
- Excellent sensitivities in downstream assays such as NGS, qPCR, ddPCR

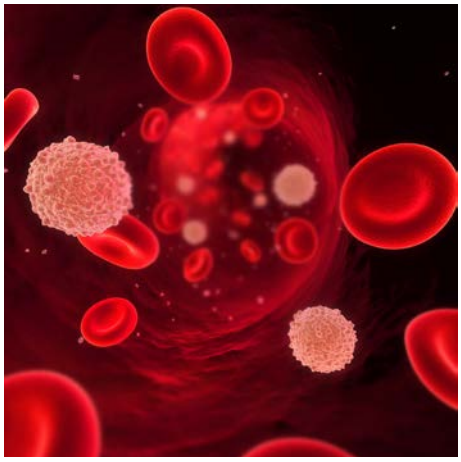
NEW
Kits for cfDNA isolation

Circulating nucleic acids in the bloodstream

Where do circulating nucleic acids derive from?

Cells in the human body occasionally undergo apoptosis. During this process, DNA is fragmented and secreted from the cells. Healthy cells, fetal cells, tumor cells, as well as transplanted cells can release DNA into the bloodstream. However, the amount of cell-free DNA (cfDNA) in the blood plasma is very low, usually below 10 ng/mL plasma.

Circulating miRNA in blood plasma is either present in exosomes that are secreted by the cells or is associated with RNA-binding proteins. In both cases, the miRNA is prevented from degrading.



Why analyze circulating nucleic acids?

The analysis of cell-free nucleic acids allows for non-invasive monitoring of 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 for monitoring disease progression, therapeutic effects of a treatment, or recurrence of cancer. In addition, the analysis of cell-free nucleic acids usually allows for an earlier diagnosis compared to invasive methods such as common tissue biopsies. Therefore, the analysis of circulating nucleic acids from body fluids is called a “Liquid Biopsy”.

How are circulating nucleic acids analyzed?

EDTA blood draw tubes or Cell-Free DNA BCT® (Streck, Inc.) are usually utilized in blood sampling for a “Liquid Biopsy“. After pelletting the cellular components of the collected blood, the supernatant is used for nucleic acid isolation. Next, quantification of cfDNA is ideally carried out by qPCR or capillary electrophoresis since common methods such as absorption measurement or fluorescent dye based quantification might lead to false results due to low DNA concentration. The cell-free nucleic acids are then analyzed by sensitive methods such as digital PCR, quantitative PCR, or NGS technologies to detect targets such as miRNA, single nucleotide variants, or chromosome mutations.

The new cfDNA products allow for processing of large plasma volumes, which is often required in order to increase the sensitivity of these downstream assays.

cfDNA isolation

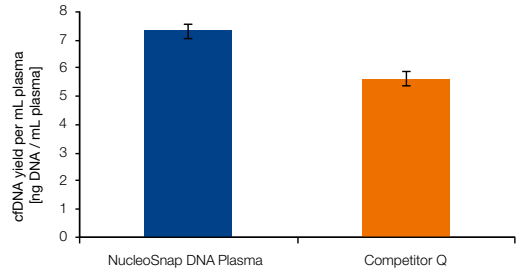
NucleoSnap DNA Plasma

Isolation of cfDNA from up to 10 mL blood plasma

Technology	Silica-membrane technology
Format	Snap-off column, vacuum processing
Sample material	1–10 mL plasma
Blood draw tubes	EDTA, Cell-Free DNA BCT® (Streck)
Fragment size	≥ 50 bp
Typical yield	Depending on sample source, storage and quality
Elution volume	20–100 µL
Preparation time	50 min/12 preps (EDTA plasma)

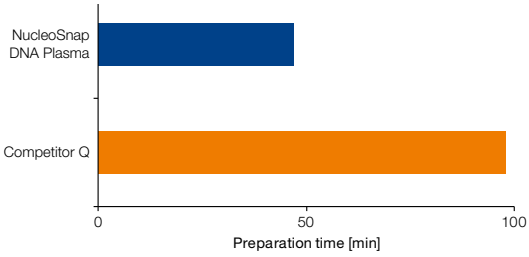
Snap-off column for processing large volumes

Application data



Efficient isolation of cfDNA from 5 mL human EDTA plasma

Isolation of cfDNA from EDTA plasma with the NucleoSnap DNA Plasma kit and a vacuum-based kit from a competitor (Competitor Q). DNA yields were quantified by qPCR (Quantifiler® Human DNA Quantification Kit, ThermoFisher Scientific on a Applied Biosystems 7500 Real-Time PCR Systems, ThermoFisher Scientific).

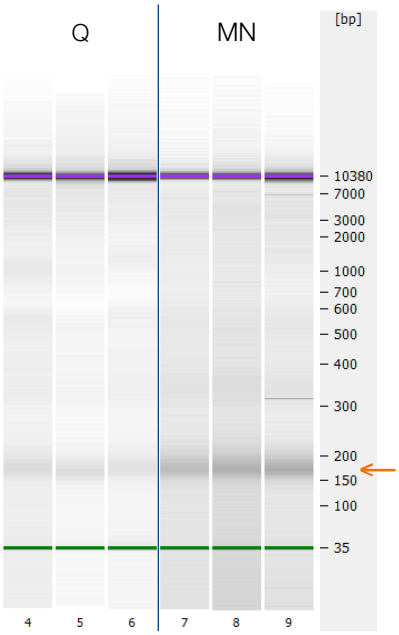


Time-saving procedure

cfDNA can be isolated from 12 EDTA preserved samples with the NucleoSnap DNA Plasma Kit in less than 50 min. whereas the procedure of competitor Q takes more than 80 min.

Convenient vacuum-processing

24 samples can be processed in parallel with our NucleoVac 24 Vacuum Manifold, and no centrifugation steps are required during binding and washing steps.



Optimized protocol for cfDNA isolation from Cell-Free DNA BCT® (Streck)

cfDNA was isolated from three independent Cell-free DNA BCT® preserved samples with the NucleoSnap DNA Plasma kit (MN) and a vacuum-processed kit from competitor (Q). The isolated cfDNA shows the typical peak at approx. 170 bp, indicated by an arrow (←). cfDNA was separated on a Bioanalyzer™ 2100 with a High Sensitivity DNA Kit (Agilent).

Ordering information

Product	Specifications	Preps	REF
NucleoSnap DNA Plasma	Manual vacuum processing, up to 10 mL plasma	10 / 50	740300.10 / .50

cfDNA isolation

NucleoSpin® DNA Plasma Midi

Isolation of cfDNA from up to 5 mL blood plasma

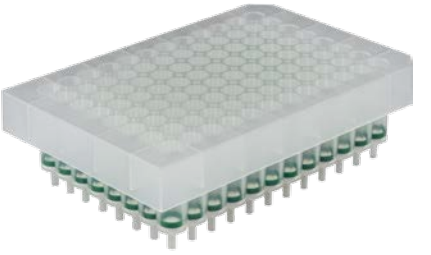
Technology	Silica-membrane technology
Format	Midi spin column
Sample material	1–5 mL plasma
Blood draw tubes	Human EDTA / Cell-Free DNA BCT® (Streck)
Fragment size	≥ 50 bp
Typical yield	Depending on sample source, storage and quality
Elution volume	200 µL (140 µL final eluate volume)
Preparation time	~ 90 min/24 preps

Medium to high throughput solutions

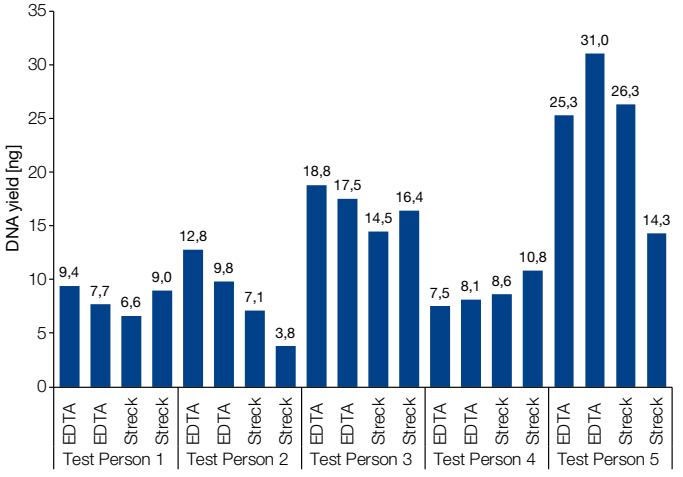
NucleoSpin® 96 DNA Plasma

Isolation of cfDNA from up to 2 mL blood plasma

Technology	Silica-membrane technology
Format	96-well plates
Sample material	0.5–2 mL plasma
Blood draw tubes	Human EDTA / Cell-Free DNA BCT® (Streck)
Fragment size	≥ 50 bp
Typical yield	Depending on sample source, storage and quality
Elution volume	100 µL (70 µL final eluate volume)
Preparation time	~ 90 min/96 preps



Application data



Efficient isolation of cfDNA from 2 mL plasma in 96-well format

Comparison of the cfDNA yield from 2 mL plasma samples from different individuals and different blood draw tubes. The results show that the NucleoSpin® 96 DNA Plasma kit enables the successful isolation of cfDNA from commonly used blood collection tubes. DNA was quantified by qPCR (Quantifiler® Human DNA Quantification Kit, Thermo Fisher Scientific).

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® DNA Plasma Midi	Medium-throughput isolation of cfDNA	48	740303.48
NucleoSpin® DNA Plasma Midi Core Kit	Medium-throughput isolation of cfDNA	48	740302.48
NucleoSpin® 96 DNA Plasma	High-throughput isolation of cfDNA	1 x 96 / 4 x 96	740873.1 / .4
NucleoSpin® 96 DNA Plasma Core Kit	High-throughput isolation of cfDNA	1 x 96 / 4 x 96	740874.1 / .4

miRNA isolation from plasma and exosomes

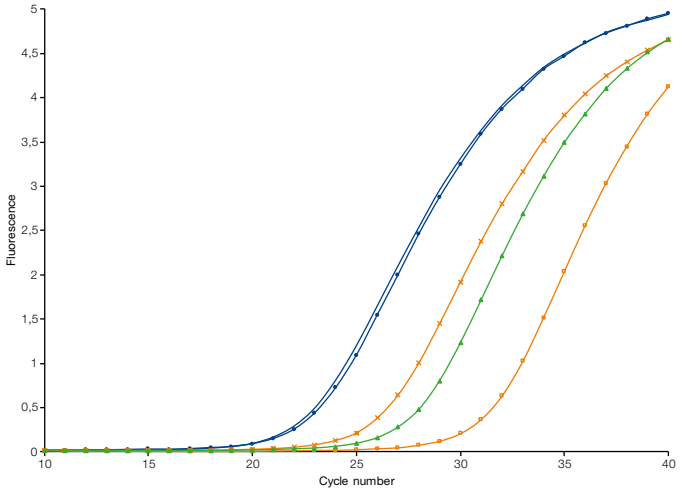
NucleoSpin® miRNA Plasma

Efficient isolation of miRNA from plasma or serum without phenol/chloroform

Technology	Silica-membrane technology
Format	Mini spin columns
Sample material	300 µL plasma or serum (900 µL with multiple loading steps)
Blood draw tubes	EDTA
Fragment size	> 15 nt
Typical yield	Depending on sample source, storage and quality
Elution volume	20–50 µL
Preparation time	40 min/10 preps (without rDNase digestion) 70 min/10 preps (with rDNase digestion)
Binding capacity	200 µg

Simple procedure without phenol / chloroform

Application data



- NucleoSpin® miRNA Plasma (without rDNase)
- NucleoSpin® miRNA Plasma (with rDNase)
- Competitor Q (CNA Kit)
- Competitor Q (miRNA Kit)
- Competitor A

Higher miRNA yields

Purified miRNA (2 µL of each eluate) was used as a template in quantitative realtime RT-PCR for miR-16 miRNA (Applied Biosystems, TaqMan® MicroRNA RT Kit, hsa-miR-16 MicroRNA Assay). The results show that C_T values are lowest for NucleoSpin® miRNA Plasma, indicating highest miRNA yields. As a result, NucleoSpin® miRNA Plasma shows superior performance with or without optional DNase digestion.

References

Stückrath *et al.*, 2015
Oncotarget. 2015 May 30; 6(15): 13387–13401
Aberrant plasma levels of circulating *miR-16*, *miR-107*, *miR-130a* and *miR-146a* are associated with lymph node metastasis and receptor status of breast cancer patients
In this study, miRNA was isolated from 300–600 µL plasma and analyzed by microarray profiling as well as qRT-PCR.

Vigneron *et al.*, 2016
Molecular Oncology August 2016, 10 (7): 981–992
Towards a new standardized method for circulating miRNAs profiling in clinical studies: Interest of the exogenous normalization to improve miRNA signature accuracy
This paper includes a product comparison with competitors A and Q: "First, to maximize profiling signals and to reduce miRNA expression variability, three isolation kits were compared and the NucleoSpin® kit provided higher miRNA concentrations than the other widely used kits."

Combine with Exosome Precipitation Solution*

Ordering information

Product	Specifications	Preps/Pack of	REF
NucleoSpin® miRNA Plasma	miRNA isolation from plasma and serum	10 / 50 / 250	740981.10 / .50 / .250
Exosome Precipitation Solution* (Serum/ Plasma)	Enrichment of exosomes for miRNA isolation	2 / 12 / 60 mL	740398.2 / .12 / .60
Exosome Precipitation Solution* (Urine)	Enrichment of exosomes for miRNA isolation	12 / 50 / 250 mL	740399.12 / .50 / .250

* not available in the USA

Kits for cfDNA and miRNA isolation

Ordering information

cfDNA from plasma	Preps/Pack of	REF
NucleoSnap DNA Plasma	10 / 50	740300.10 / .50
NucleoSpin® DNA Plasma Midi	48	740303.48
NucleoSpin® DNA Plasma Midi Core Kit	48	740302.48
NucleoSpin® 96 DNA Plasma	1 x 96 / 4 x 96	740873.1 / .4
NucleoSpin® 96 DNA Plasma	1 x 96 / 4 x 96	740874.1 / .4
NucleoSpin® Plasma XS	10 / 50 / 250	740900.10 / .50 / .250
miRNA from plasma and exosomes		
NucleoSpin® miRNA Plasma	10 / 50 / 250	740981.10 / .50 / .250
Exosome Precipitation Solution (Serum/Plasma)*	2 / 12 / 60 mL	740398.2 / .12 / .60
Exosome Precipitation Solution (Urine)*	12 / 50 / 250 mL	740399.12 / .50 / .250
Accessories		
NucleoVac 24 Vacuum Manifold	1	740299
NucleoVac Mini Adapter	100	740297.100
NucleoVac Valves	24	740298.24
NucleoVac 96 Vacuum Manifold	1	740681
NucleoVac Vacuum Regulator	1	740641
Starter Set Midi	1	740744
Related products		
NucleoSpin® miRNA *Small and large RNA isolation from various sample types*	10 / 50 / 250	740971.10 / .50 / .250
NucleoZOL *Universal RNA isolation reagent for small and large RNA*	200 mL	740404.200
NucleoSpin® RNA Set for NucleoZOL *Mini spin kit for processing NucleoZOL lysates*	10 / 50	740406.10 / .50
NucleoSpin® Virus *Isolation of viral RNA and DNA from serum and plasma*	10 / 50 / 250	740983.10 / .50 / .250
NucleoSpin® 96 Virus *High throughput viral RNA and DNA isolation from serum and plasma*	2 x 96 / 4 x 96	740691.2 / .4
NucleoMag® Virus *Magentic bead-based isolation of viral RNA and DNA*	1 x 96 / 4 x 96	744800.1 / .4
NucleoMag® VET *Pathogen nucleic acid isolation from various veterinary samples*	1 x 96 / 4 x 96	744200.1 / .4

* not available in the USA

Your distributor

www.mn-net.com

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