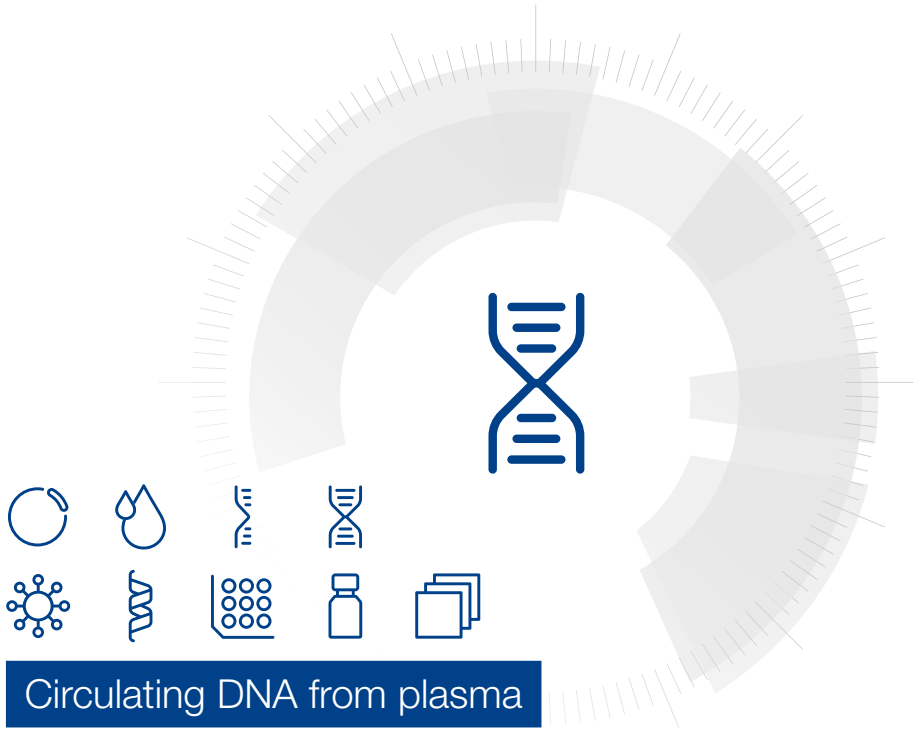
Bioanalysis

MACHEREY-NAGEL

User manual



Circulating DNA from plasma

■ NucleoSpin® cfDNA Midi

November 2023 / Rev. 04

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1 Components

1.1 Kit contents

	NucleoSpin® cfDNA Midi Kit
REF	**48 preps 740303.48**
Activation Buffer PMA	75 mL
Lysis Buffer PML	125 mL
Binding Buffer PMB	3 × 250 mL
Wash Buffer PMW1	2 × 125 mL
Wash Buffer PMW2 (Concentrate)*	50 mL
Elution Buffer PME	30 mL
Liquid Proteinase K	7 mL
NucleoSpin® cfDNA Midi Columns	48
Collection Tubes (1.5 mL)	48
24-Square-well Block 10 mL	4
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* For preparation of working solutions and storage, see section 3.

1.1 Kit contents *continued*

	NucleoSpin® cfDNA Midi Core Kit
REF	**48 preps 740302.48**
Activation Buffer PMA	75 mL
Lysis Buffer PML	125 mL
Binding Buffer PMB	3 × 250 mL
Wash Buffer PMW1	2 × 125 mL
Wash Buffer PMW2 (Concentrate)	50 mL
Elution Buffer PME	30 mL
Liquid Proteinase K	7 mL
NucleoSpin® cfDNA Midi Columns	48
Collection Tubes (1.5 mL)	48
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1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol

Consumables

- 50 mL tubes or large volume multiplate for plasma lysis
- Disposable pipette tips

Equipment

- NucleoVac 96 Vacuum Manifold (see ordering information section 6.2)
- NucleoVac Vacuum Regulator (see ordering information section 6.2)
- Starter Set Midi (see ordering information section 6.2)
- Vacuum pump
- Heater-shaker or water bath for lysis
- Multi channel pipettes or large volume pipettes with appropriate tips
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® cfDNA Midi** kit is used for the first time. All technical literature is available on the Internet at www.mn-net.com.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® cfDNA Midi** kit is designed for the efficient isolation of circulating DNA from human blood plasma. Fragmented DNA of 50 bp and larger can be purified with high efficiency. The **NucleoSpin® cfDNA Midi** kit can be used with standard manual and automated vacuum manifolds. The kit is fully automatable on many liquid handling robots.

The protocol follows state-of-the-art bind-wash-elute procedures: lysis is performed for 30 minutes with Proteinase K and lysis buffer. Afterwards, a binding buffer is added and the solution is applied onto the columns in several steps and DNA is bound to the silica membrane. Three washing steps efficiently remove contaminating substances, such as PCR inhibitors. Drying of silica is achieved by applying vacuum and pure DNA is finally eluted.

2.2 Kit specifications

- The **NucleoSpin® cfDNA Midi** kit is recommended for the isolation of circulating cell-free DNA from human EDTA plasma.
- The **NucleoSpin® cfDNA Midi** kit is designed for high recovery of fragmented DNA ≥ 50 bp.
- Up to 5 mL plasma can be used as sample material with a single column.
- DNA yield strongly depends on the individual sample, but is typically in the range of 0.1 to 100 ng DNA per mL plasma.
- DNA is ready to use for downstream applications like real-time PCR or NGS.
- The preparation time is approximately 90 min for up to 24 plasma samples.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® cfDNA Midi
Technology	Silica membrane technology
Format	NucleoSpin® Midi Column
Sample material	Human EDTA / Cell-Free DNA BCT® plasma
Sample amount	1 – 5 mL per preparation
Typical yield	Sample dependent
Elution volume	200 µL
Preparation time	Approx. 90 min/24 preps
Use	For research use only

2.3 Required hardware

Vacuum processing

The **NucleoSpin® cfDNA Midi** kit is used with the NucleoVac 96 Vacuum Manifold (see ordering information, section 6.2). Additional to the vacuum manifold, special adapter frames (included in the Starter Set Midi) are needed for processing up to 24 NucleoSpin® cfDNA Midi Columns on the NucleoVac 96 Vacuum Manifold. The Starter Set Midi (see ordering information, section 6.2) contains a Column Holder Midi for holding up to 24 NucleoSpin® cfDNA Midi Columns, a Wash Plate Midi, for preventing cross-contamination, and the Elution Tube Holder Midi for holding the Elution Tubes inside the vacuum manifold. For the use of less than 24 columns, Dummy Columns are included.

The manifold may be used with a vacuum pump, house vacuum, or water respirator. We recommend a vacuum of -0.2 to -0.6 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information, section 6.2) is recommended.

2.4 Size and yield of DNA from plasma

Usually, DNA concentrations in plasma are in a range of 0.1 ng up to several 100 ng DNA per mL of plasma. The amount of circulating DNA in plasma depends on health condition of the donor, sampling and handling of the blood, plasma preparation, DNA isolation method, and others.

A significant portion of the cell-free DNA in plasma originates from apoptotic cells. Therefore, a considerable percentage of this circulating DNA is known to be highly fragmented. However, the degree of fragmentation and the ratio of fragmented DNA to high molecular weight DNA depends on several parameters like origin of the DNA (e.g., fetal, tumor, microbial DNA), health condition of the blood donor, blood sampling procedure, and handling of the sample.

2.5 Handling of sample material

Circulating DNA yield and quality is largely influenced by blood sampling technique, handling, storage, and plasma preparation. It is highly recommended to perform these steps as uniform as possible in order to achieve highest reproducibility.

Plasma can be isolated according to the following recommendation:

Preparation of plasma from human EDTA blood or Streck Cell-Free DNA BCT®

1 Centrifuge samples for 10 min at 2,000 x *g*.

2 Remove the plasma without disturbing sedimented cells and particles.

3 Clear plasma of residual cellular debris by means of centrifugation (10 min at 5,000 x *g*).

4 If necessary, freeze plasma samples in fresh tubes. Upon thawing, check for precipitates and remove them with a final centrifugation step.

2.6 Elution procedures

The recommended standard elution procedure comprises two steps of 100 µL. This will result in about 140 µL eluate. The retained volume will contain very little amounts of DNA because the majority will be present in the eluted fraction.

2.7 Stability of isolated DNA

Due to the low DNA content in plasma and the resulting low total amount of isolated DNA, its fragmentation, and the absence of DNase inhibitors (the elution buffer does NOT contain EDTA) the eluates should be kept on ice for short term storage and frozen at -20 °C for long term storage.

3 Storage conditions and preparation of working solutions

Attention: *Buffers PML, PMB, and PMW1 contain guanidinium hydrochloride (chaotropic salt) which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste. Wear gloves and goggles!*

Storage conditions:

- All kit components can be stored at room temperature (18 – 25 °C) and are stable for at least one year.
- If there is any precipitate present in the buffers, warm the buffer up to 25 – 37 °C to dissolve the precipitate before use.

Before starting any **NucleoSpin® cfDNA Midi** protocol prepare the following:

- Ethanol has to be added to Wash Buffer PMW2 according to the instructions on the label and in this user manual. All other kit components are ready to use.
- Prepare plasma sample according to section 2.5.
- Set heating block or water bath to 56 °C for lysis.
- Set up the **NucleoVac 96** Vacuum Manifold.
- **Liquid Proteinase K** is ready to use. After first opening, store Liquid Proteinase K at -20 °C.
- When using multi-well plates, samples have to be split into suitable aliquots.

	NucleoSpin® cfDNA Midi / NucleoSpin® cfDNA Midi Core Kit
REF	**48 preps 740303.48 / 740302.48**
Wash Buffer PMW2 (Concentrate)	50 mL Add 200 mL ethanol

4 Safety instructions

When working with the NucleoSpin® cfDNA Midi kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at http://***www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer PML, PMB and PMW1 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the NucleoSpin® cfDNA Midi kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

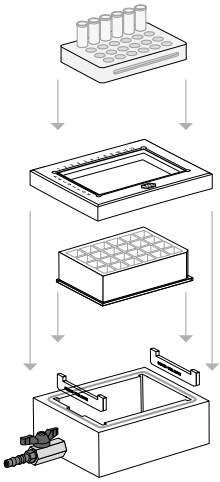
4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocol for the isolation of DNA from plasma

Setup of vacuum manifold:

Binding and washing step

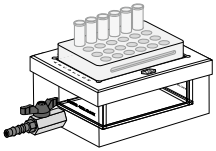


Step 4:
Place the NucleoSpin® Midi Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.

Step 3:
Place the manifold lid on top of the manifold base.

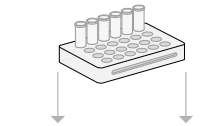
Step 2:
Place the Sample Waste Block in the manifold.

Step 1:
Insert spacers 'SQUARE-WELL BLOCK' in the manifold.

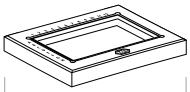


Final setup

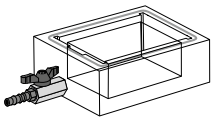
Drying step



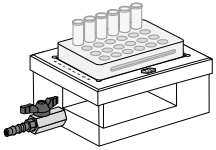
Step 3:
Place the NucleoSpin® Midi Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.



Step 2:
Place the manifold lid on top of the manifold base.

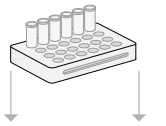


Step 1:
Insert the equiped waste container in the manifold base.

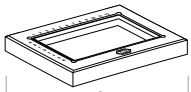


Final setup

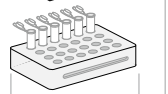
Elution step



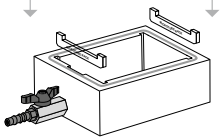
Step 4:
Place the NucleoSpin® Midi Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.



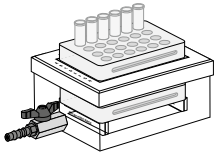
Step 3:
Place the manifold lid on top of the manifold base.



Step 2:
Place the 1.5 mL Collection Tubes inserted the Elution Tube Holder Midi in the manifold.



Step 1:
Insert spacers 'MICROTUBE RACK' in the manifold base.



Final setup

The procedure below describes the isolation of cell-free DNA from **5 mL human plasma**. Adjusting reagent volumes according to the table below allows for processing of plasma volumes from 1 – 5 mL.

Plasma volume [mL]	Liquid Proteinase K [µL]	Lysis Buffer PML [µL]	Binding Buffer PMB [mL]
1	25	400	2
2	50	800	4
3	75	1200	6
4	100	1600	8
5	125	2000	10

Before starting the preparation:

For hardware requirements, refer to section 2.3.

- For detailed information regarding vacuum manifold, see page 12.
- Check if Buffer PMW2 was prepared according to section 3.
- Set a thermal heating shaker to 56 °C.

1 Lyse sample

Add **125 µL Liquid Proteinase K** to a 50 mL tube.

125 µL Liquid Proteinase K

Add **5 mL plasma** to the tube.

+ 5 mL plasma

Vortex briefly.

+ 2 mL PML

Add **2 mL Buffer PML** to the tube.

Mix

Mix the tube contents by briefly vortexing the tube.

56 °C, 30 min

Incubate at **56 °C** for **30 min** (for Streck Cell-Free DNA BCT®, incubate 60 min; ideally with shaking).

Insert spacers 'MTP/MULTI-96 PLATE', the Waste Container and the Wash Plate Midi (with the mark in the upper left hand corner) into the manifold base. Place the manifold lid on top and then the Column Holder Midi (with the mark in the upper left hand corner) equipped with binding and dummy columns as needed.

While incubating the lysis, apply **1 mL Buffer PMA** to the columns. Incubate one minute, then apply vacuum of **-0.4 bar*** for **1 min**.

+ 1 mL PMA

-0.4 bar*, 1 min

* Reduction of atmospheric pressure

2	**Adjust binding conditions** Carefully open the tube and add **10 mL Buffer PMB**. **Mix** the tube contents by vortexing.	**+ 10 mL PMB** **Mix**
3	**Bind DNA** Apply prepared lysates to the **NucleoSpin® cfDNA Midi Column** in aliquots of **3.5 mL**. Apply vacuum of **-0.4 bar*** for **5 min** for each load. Remove, empty and replace Waste Container after the second loading step as well as after column loading is completed.	**3.5 mL lysate** **-0.4 bar*, 5 min**
4	**Wash membrane** **1st wash** Once all lysates have passed the membrane, add **4 mL Buffer PMW1** to each column. Incubate for **1 min**, and then apply vacuum of **-0.4 bar*** for **5 min**. **2nd wash** Add **2 mL Buffer PMW2**, and then apply vacuum of **-0.4 bar*** for **2 min**. **3rd wash** Repeat 2nd wash. Remove and empty Waste Container. Remove Wash Plate.	**+ 4 mL PMW1** **-0.4 bar*, 5 min** **+ 2 mL PMW2** **-0.4 bar*, 2 min** **+ 2 mL PMW2** **-0.4 bar*, 2 min**
5	**Dry silica membrane** Apply strongest possible vacuum of at least **-0.6 bar*** for **10 min** to dry the silica membrane. After drying, blot column outlets on tissue paper to remove residual ethanol. Insert spacers 'MICROTUBE RACK' and the **Elution Tube Holder** equipped with elution tubes without caps into the vacuum manifold base.	**-0.6 bar*, 10 min**

* Reduction of atmospheric pressure

6	**Elute highly pure DNA**	
	Add **100 µL Buffer PME** (first elution step) to the membrane. Incubate **1 min**.	**+ 100 µL PME** **RT, 1 min**
	Apply vacuum of **-0.4 bar*** for **30 s**.	**-0.4 bar*, 30 s**
	Add **100 µL Buffer PME** (second elution step) to the membrane.	**+ 100 µL PME**
	Apply vacuum of **-0.6 bar*** for **30 s**.	**-0.6 bar*, 30 s**
	Cap elution tubes and store at 4 °C for short term storage and at -20 °C for long term storage.	

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA yield	*Low DNA content of the sample* • The content of cell-free DNA in human plasma may vary over several orders of magnitude. DNA contents in the range of 0.1 – 1000 ng DNA per mL of plasma have been reported (see remarks in section 2.4). • If the DNA concentration is measured with double strand specific dyes, e.g., PicoGreen®, make sure not to heat the DNA before measurement. Due to denaturation of DNA during the heat incubation step and the double strand specificity of certain DNA dyes, e.g., PicoGreen®, results may be inaccurate.
Column clogging	*Sample contains residual cell debris or cells* • The plasma sample may have contained residual cells or cell debris. Make sure to use only clear plasma samples (see remarks in section 2.5).
Discrepancy between A_{260} quantification values and PCR quantification values	*Silica abrasion from the membrane* • Due to the typically low DNA content in plasma and the resulting low total amount of isolated DNA, a DNA quantification via A_{260} absorption measurement is often hampered due to the low sensitivity of the absorption measurement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect A_{260} quantification of small DNA amounts, centrifuge the eluate for 30 s at > 11.000 x *g* and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen® fluorecent dye).
Unexpected A_{260} / A_{280} ratio	*Measurement not in the range of photometer detection limit* • In order to obtain a significant A_{260} / A_{280} ratio, it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpected A_{260} / A_{280} ratios.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® cfDNA Midi	740303.48	48
NucleoSpin® cfDNA Midi Core Kit	740302.48	48
24-Square-well Block, 10 mL	740679.4	4
Lysis Buffer PML	740835.125	125 mL
Binding Buffer PMB	740836.250	250 mL
Liquid Proteinase K	740396	5 mL
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Starter Set Midi	740744	1

Note: The product has been formerly distributed under the name NucleoSpin® DNA Plasma Midi. The product code (REF) and kit content have not been changed.

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

Last updated: 08/2022, Rev. 04

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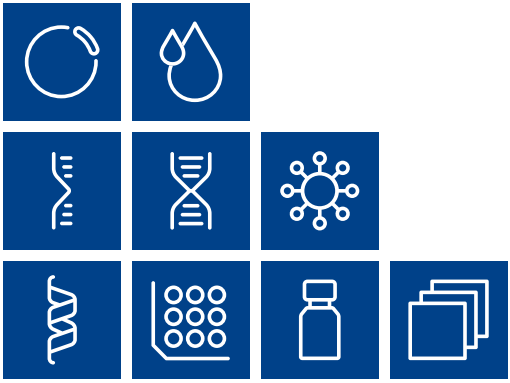

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