Circulating DNA from plasma

User manual

NucleoSpin® cfDNA Midi

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MACHEREY-NAGEL
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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 x 250 mL
Wash Buffer PMW1	2 x 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
User manual	1

* 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 x 250 mL
Wash Buffer PMW1	2 x 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
User manual	1

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

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

The following components of the **NucleoSpin® cfDNA Midi** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS-Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
Activation Buffer PMA	Sodium hydroxide solution 0.5–1.0 % *Natriumhydroxid-Lösung 0,5–1,0 %* CAS 1310-73-2d	 WARNING *ACHTUNG*	315, 319	280sh
Lysis Buffer PML	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %* CAS 50-01-1	 WARNING *ACHTUNG*	302, 315, 319	264W, 280sh, 301+312, 330
Binding Buffer PMB	Guanidine hydrochloride 24–36 % and ethanol 35–55 % *Guanidinhydrochlorid 24–36 % und Ethanol 35–55 %* CAS 50-01-1, 64-17-5	 WARNING *ACHTUNG*	226, 302	210, 264W, 301+312, 330
Wash Buffer PMW1	Guanidine hydrochloride 36–50 % and 2-propanol 20–35 % *Guanidinhydrochlorid 36–50 % und 2-Propanol 20–35 %* CAS 50-01-1, 67-63-0	 WARNING *ACHTUNG*	226, 302, 319, 336	210, 260D, 264W, 280sh, 301+312, 330

Hazard phrases

H 226	Flammable liquid and vapor *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 315	Causes skin irritation. *Verursacht Hautreizungen.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung*

H 336	May cause drowsiness or dizziness. *Kann Schläfrigkeit und Benommenheit verursachen.*

Precaution phrases

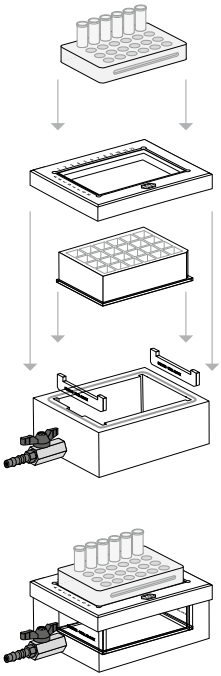
P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. *Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen*
P 260D	Do not breathe vapors. *Dampf nicht einatmen.*
P 264W	Wash with water thoroughly after handling. *Nach Gebrauch mit Wasser gründlich waschen.*
P 280sh	Wear protective gloves/eye protection. *Schutzhandschuhe/Augenschutz tragen*
P 301+312	IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt anrufen.*
P330	Rinse mouth. *Mund ausspülen.*

 The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

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.

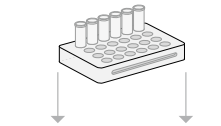
Step 3:
Place the manifold lid on top of the manifold base.

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

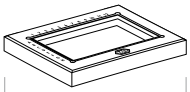
Step 1:
Insert spacers 'SQUARE-WELL BLOCK' in the manifold.

Final setup

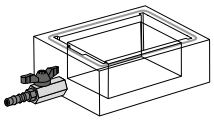
Drying step



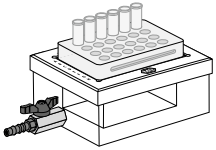
Step 3:
Place the NucleoSpin® Midi Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.



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

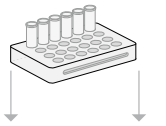


Step 1:
Insert the equiped waste container in the manifold base.

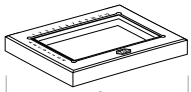


Final setup

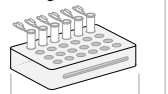
Elution step



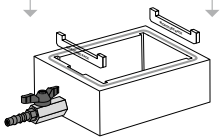
Step 4:
Place the NucleoSpin® Midi Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.



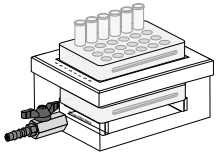
Step 3:
Place the manifold lid on top of the manifold base.



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 13.
- 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

NucleoSpin® cfDNA Midi kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or

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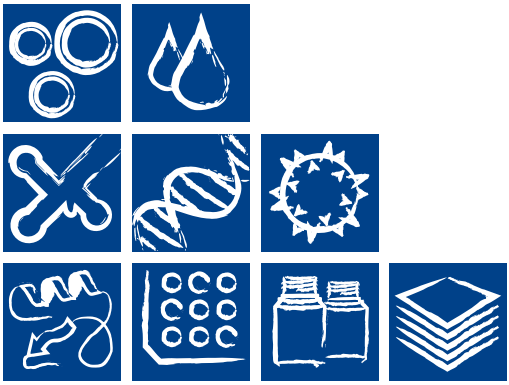

www.mn-net.com

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