Circulating DNA from plasma

User manual

NucleoSnap® DNA Plasma

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MACHEREY-NAGEL
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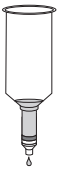
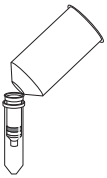
Circulating DNA from plasma

Protocol at a glance (Rev. 02)

		NucleoSnap® DNA Plasma
1 Prepare sample	 	4,500 x *g*, 10 min
2 Lyse sample		45 µL Proteinase K 3 mL plasma Mix RT, 5 min 3 mL VL Mix 56 °C, 5 min
3 Prepare Binding conditions		3 mL ethanol (96–100%) Mix
4 Bind DNA	 Vacuum	500 µL CC 0.4–0.6 bar*, 30 s Load lysate 0.4 bar*, 5–15 min
5 Wash silica membrane	 Vacuum	1 mL VW1 0.2–0.4 bar*, 1 min 500 µL WB 0.2–0.4 bar*, 30 s
6 Dry silica membrane	 	Remove upper part and discard 11,000–20,000 x *g*, 3 min
7 Elute DNA	 	50 µL Elution Buffer RT, 3 min 11,000 x *g*, 1 min

* Reduction of atmospheric pressure

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1 Components

1.1 Kit contents

	NucleoSnap® DNA Plasma	
REF	**10 preps 740300.10**	**50 preps 740300.50**
Column Conditioner CC	13 mL	30 mL
Lysis Buffer VL	60 mL	275 mL
Wash Buffer VW1	13 mL	75 mL
Wash Buffer WB	10 mL	50 mL
Elution Buffer*	13 mL	13 mL
Liquid Proteinase K	800 µL	4 mL
NucleoSnap® DNA Plasma Columns	10	50
Collection Tubes (2 mL)	10	50
Collection Tubes (1.5 mL)	10	50
NucleoVac Mini Adapter	10	50
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* Composition of Elution Buffer: 5 mM Tris/HCL, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents:

- 96–100 % ethanol

Consumables:

- 50 mL tubes for plasma lysis
- Disposable pipette tips

Equipment:

- Vacuum manifold
- Centrifuge for microcentrifuge tubes
- Valves (recommended, e.g., 740298.24, see ordering information, section 7.2)
- Heating-block or water bath for 56 °C incubation of 50 mL tubes
- Manual pipettors
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)
- Vacuum regulator (recommended, e.g., 740641, see ordering information, section 7.2)

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSnap® DNA Plasma** kit before using this product. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available online at ***www.mn-net.com***.

2 Kit specifications

- The **NucleoSnap® DNA Plasma** kit is recommended for the isolation of circulating cell-free DNA from human EDTA plasma and Cell-Free DNA BCT® plasma tubes.
- The **NucleoSnap® DNA Plasma** 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 loading step.
- DNA yield strongly depends on the individual sample, but is typically in the range of 0.1 to 100 ng DNA per mL plasma.
- Elution can be performed with as little as 20–50 µL elution buffer. DNA is ready to use for downstream applications like real-time PCR or others.
- The preparation time is approximately 50 min for 6 plasma samples.

Table 1: Kit specifications at a glance

Parameter	**NucleoSnap® DNA Plasma**
Technology	Silica membrane technology
Format	NucleoSnap® Column
Sample material	Human EDTA/Cell-Free DNA BCT® plasma 1–10 mL*
Sample amount	1–5 mL per preparation
Typical yield	Sample dependent
Elution volume	20–50 µL
Preparation time	50 min/6 preps

* For processing larger volumes than 5 mL, Lysis Buffer VL and Proteinase K have to be purchased separately (see ordering information, section 7.2).

3 Product description

3.1 The basic principle

The **NucleoSnap® DNA Plasma** kit is designed for the efficient isolation of circulating DNA from human blood plasma. Fragmented DNA as small as 50–1000 bp can be purified with high efficiency. Due to a special snap-off design, the **NucleoSnap® DNA Plasma Columns** allow processing of up to 5 mL plasma (or 10 mL plasma, which requires extra buffer; see ordering information) along with very small elution volumes (20–50 µL), resulting in highly concentrated DNA.
The protocol follows state-of-the-art bind-wash-elute procedures: After mixing a plasma sample with Liquid Proteinase K, lysis buffer and binding buffer, the mixture is applied to the **NucleoSnap® DNA Plasma Column**. Upon loading of the mixture onto the column and applying the vacuum, DNA binds to the silica membrane.
Two washing steps efficiently remove contaminating substances, such as PCR inhibitors. Subsequently, the upper part of the snap-off column is removed, and highly pure DNA is finally eluted with 20–50 µL of a slightly alkaline elution buffer of low ionic strength (5 mM Tris/HCl, pH 8.5) from the lower part of the snap-off column.

3.2 Size and yield of DNA from plasma

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

The **NucleoSnap® DNA Plasma** purification system is designed for the efficient isolation of highly fragmented DNA in a range of 50–1000 bp. Within this range, both small and large fragments are recovered with high efficiency.

3.3 Handling of sample material

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

Plasma can be isolated according to the following recommendation:

Preparation of plasma from human EDTA blood

1 Centrifuge fresh blood sample for 10 min at 2,000 x *g*.

2 Transfer the plasma without disturbing sedimented cells and particles into a fresh tube.

3 Freeze plasma at -20 °C for storage until DNA isolation.

4 Thaw frozen plasma samples prior to DNA isolation and centrifuge for 3 min at ≥ 11,000 x *g* in a mini centrifuge for small plasma volumes or 10 min at 4,500 x *g* in a tabletop centrifuge for larger volumes of plasma in order to remove residual cells, cell debris, and particulate matter. Use the supernatant for DNA isolation.

Preparation of plasma from Cell-Free DNA BCT®
Please follow the procedures recommended in the Cell-Free DNA BCT® user manual.

3.4 Elution procedures

The recommended standard elution volume is 50 µL. A reduction of the elution volume to 20 µL will increase DNA concentration, but the total DNA yield will decrease by this elution procedure. An increase of the elution volume to 100 µL or more will only slightly increase total DNA yield, but reduces DNA concentration.

3.5 Stability of isolated DNA

Due to the low DNA content in plasma, the resulting low total amount of isolated DNA, 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.

4 Storage conditions and preparation of working solutions

Attention: *Buffers VL and VW1 contain chaotropic salt! Wear gloves and goggles!*

CAUTION: Buffer VL and VW1 contain guanidinium hydrochloride 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.

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 the **NucleoSnap® DNA Plasma** protocol:

- No buffers or enzymes have to be prepared – all kit components are ready to use.
- Set up the vacuum manifold (please follow the instructions provided by the manufacturer).
- **Liquid Proteinase** is ready to use. After first use, store Liquid Proteinase K at 4 °C or -20 °C.

5 Safety instructions

The following components of the **NucleoSnap® DNA Plasma** contain hazardous contents.

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

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***
VL	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %* CAS 50-01-1	 WARNING *ACHTUNG*	302, 315, 319	264W, 280sh, 301+312, 330
VW1	Guanidine hydrochloride 36–50 % + 2–propanol 20–35 % *Guanidinhydrochlorid 36–50 % + 2–Propanol 20–35 %* CAS 50-01-1	 WARNING *ACHTUNG*	226, 302, 319, 336	210, 260D, 264W, 280sh, 301+312, 330
WB	Ethanol 55–75 % *Ethanol 55–75 %* CAS 64-17-5	 DANGER *GEFAHR*	225	210, 233
Column Conditioner CC	Potassium hydroxide solution 0.50–1.00 % *Kaliumhydroxid–Lösung 0,50–1,00 %* CAS 1310-58-3	 WARNING *ACHTUNG*	290, 315, 319	280s, 390

Hazard phrases

H225	Highly flammable liquid and vapor. *Flüssigkeit und Dampf leicht entzündbar.*
H226	Flammable liquid and vapor. *Flüssigkeit und Dampf entzündbar.*
H290	May be corrosive to metals. *Kann gegenüber Metallen korrosiv sein.*
H302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H315	Causes skin irritation. *Verursacht Hautreizungen.*
H319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*

H336	May cause drowsiness or dizziness. *Kann Schläfrigkeit und Benommenheit verursachen.*

Precaution phrases

P210	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.*
P233	Keep container tightly closed. *Bei Hautreizung oder –ausschlag:*
P261D	Do not breathe vapors. *Dampf nicht einatmen.*
P264W	Wash with water thoroughly after handling *Nach Gebrauch mit Wasser gründlich waschen.*
P280sh	Wear protective gloves/eye protection. *Schutzhandschuhe/Augenschutz tragen.*
P301+312	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.*
P330	Rinse mouth. *Mund ausspülen.*
P390	Absorb spillage to prevent material damage. *Verschüttete Mengen aufnehmen, um Materialschäden zu vermeide*

6 Protocols for the isolation of cell-free DNA from plasma

6.1 Protocol for the isolation of DNA from 1–5 mL EDTA plasma

The procedure below describes the isolation of cell-free DNA from 3 mL human EDTA plasma. A range of 1–5 mL plasma can be processed by corresponding adjustment of the Proteinase K, lysis buffer, and ethanol volumes:

Plasma volume [mL]	Proteinase K [µL]	Buffer VL [mL]	Ethanol [mL]
1	15	1	1
2	30	2	2
3	45	3	3
4	60	4	4
5	75	5	5

Before starting the preparation:

- Prepare plasma sample according to section 3.3.
- Set water bath or heating block for 50 mL tubes to 56 °C (for lysate incubation).
- Set up vacuum manifold (please follow the instructions provided by the manufacturer).

Protocol for 3 mL

1 **Prepare sample**

Remove residual blood cells: Centrifuge plasma for at least **10 min** at **4,500 x *g*** in order to remove residual cells and cell debris. Use supernatant, discard sediment.

Note: Sediment should not be used for futher processing. However, the supernatant may still contain suspended matter (e.g., lipids). This floating material does not interfere with further processing.

4,500 x *g*, 10 min

2 **Lyse sample**

Add **45 µL Proteinase K** to a 50 mL tube. Add **3 mL plasma** to the tube. Carefully **mix** the contents of the tube without moistening the tube lid.

Incubate **5 min** at **room temperature** (18–25 °C). Add **3 mL Buffer VL** to the tube. **Mix** the tube contents by vortexing for approximately 20 s in order to obtain a homogenous lysate.

Incubate tube at **56 °C** for **5 min** (e.g., in a water bath).

+ 45 µL Proteinase K

+ 3 mL plasma

Mix

RT, 5 min

+ 3 mL VL

Mix

56 °C, 5 min

3 **Prepare binding conditions**

Carefully open the tube lid and add **3 mL ethanol** (96–100 %).

Mix the tube contents by vortexing for approximately 10 s in order to obtain a homogenous lysate.

+ 3 mL ethanol (96–100 %)

Mix

4 **Bind DNA**

+ 500 µL CC

0.4–0.6 bar*, 30 s

Load lysate

0.4 bar*, 5–15 min

Place the NucleoSnap® DNA Plasma Column onto a vacuum manifold (do not apply vacuum yet).

Note: The use of reusable NucleoVac Stop-cocks is recommended (not provided, see ordering information, section 7.2)

Add **500 µL Column Conditioner CC** to the column.

Apply vacuum (**approximately 0.4–0.6 bar*** differential pressure) until the solution has passed the column (usually several seconds). Release vacuum.

Note: Perform adding Column Conditioner CC and applying vacuum 1–5 min before adding the lysate to the NucleoSnap® Column.

Apply the **lysate from step 3** to the NucleoSnap® Column.

Apply vacuum (**approximately 0.4 bar*** differential pressure). Wait until the lysate has passed through the column. Approximate duration:

for 3 mL lysate (corresponding to 1 mL plasma): up to 5 min;

for 9 mL lysate (corresponding to 3 mL plasma): up to 10 min;

for 15 mL lysate (corresponding to 5 mL plasma): up to 15 min.

The vacuum can be adjusted to ≥ 0.6 bar* at the end of this step in case the lysate has not yet completely passed the column.

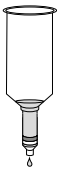
Close stopcocks for each column as soon as the lysate has passed the column!

* Reduction of atmospheric pressure

5 **Wash silica membrane**

Add **1 mL Buffer VW1** to the column.
Apply vacuum (**approximately 0.2–0.4 bar*** differential pressure) until the wash buffer has completely passed through the column. Approximate duration is up to one minute. Release vacuum.

Add **500 µL Buffer WB** to the column.
Apply vacuum (**approximately 0.2–0.4 bar*** differential pressure) until the wash buffer has passed through the column. Approximate duration is 30 s. Release vacuum.



+ 1 mL VW1

0.2–0.4 bar*, 1 min

+ 500 µL WB

0.2–0.4 bar*, 30 s

6 **Dry silica membrane**

Remove the NucleoSnap® Column from the vacuum manifold and insert it into a Collection Tube (2 mL, provided). **Remove the upper part of the column and discard** it.

Note: Tightly hold the upper part of the column in one hand and the labeled Collection Tube (2 mL) holding the NucleoSnap® Column in the other hand and carefully snap off the upper part of the column. Do not label the lower (spin) part of the NucleoSnap® Column because the ink might be washed off.

Centrifuge the lower part of the column in the Collection Tube for **3 min** at **full speed** (> 11,000 x *g* up to 20,000 x *g*). Discard the Collection Tube with residual flow through and insert the column into a fresh Collection Tube (1.5 mL, provided).

Remove upper part and discard

11,000–20,000 x *g*, 3 min

7 **Elute DNA**

Add **50 µL Elution Buffer** to the column. Incubate **3 min** at **room temperature**. Centrifuge for **1 min** at **11,000 x *g***.

Store eluted DNA at 4 °C for short-term and at -20 °C for long-term storage.

Note: For alternative elution procedures see section 3.4.

50 µL Elution Buffer

RT, 3 min

11,000 x *g*, 1 min

* Reduction of atmospheric pressure

6.2 Protocol for the isolaton of DNA from 6–10 mL EDTA plasma

A range of 6–10 mL plasma can be processed by corresponding adjustment of the Proteinase K, lysis buffer, and ethanol volumes:

Plasma volume [mL]	Proteinase K [µL]	Buffer VL [mL]	Ethanol [mL]
6	90	6	6
7	105	7	7
8	120	8	8
9	135	9	9
10	150	10	10

Note: For processing larger volumes than 5 mL, Lysis Buffer VL and Proteinase K have to be purchased separately (see ordering information, section 7.2).

Continue with step 4 of protocol 6.1.

6.3 Protocol for the isolaton of DNA from 1–5 mL plasma from Cell-Free DNA BCT® (Streck, Inc.)

Step 2 (lyse sample) of the standard protocol has to be modified as follows:

Add **45 µL Proteinase K** to a 50 mL tube. Add **3 mL plasma** to the tube. Carefully mix the content of the tube without moistening the tube lid. Incubate **15 min** at **room temperature** (18–25 °C). Add **3 mL Buffer VL** to the tube. **Mix** the tube content by vortexing for approximately 20 s in order to obtain a homogenous lysate.

Incubate tube at **56 °C** for **60 min** (e.g., in a water bath).

Continue with step 3 of protocol 6.1.

7 Appendix

7.1 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 from approximately 0.1–1000 ng DNA per mL of plasma have been reported (see remarks in section 3.2). *Inaccurate yield determination* • If the DNA concentration is measured with double strand specific dyes, e.g., PicoGreen®, make sure not to heat the eluted 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 might 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 plasma samples that have been centrifuged in order to remove cells and cell debris (see remarks in section 3.3).
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.

7.2 Ordering information

Product	REF	Pack of
NucleoSnap® DNA Plasma	740300.10 / 50	10 / 50
NucleoVac 24 Vacuum Manifold	740299	1
NucleoVac Mini Adapter	740297.100	100
NucleoVac Valves	740298.24	24
NucleoSpin® Plasma XS	740900.10 / 50 / 250	10 / 50 / 250
Collection Tubes (2 mL)	740600	1000
Lysis Buffer VL	740833.200	200 mL
Liquid Proteinase K	740396	5 mL

Visit ***www.mn-net.com*** for more detailed product information.

7.3 Product use restriction / warranty

NucleoSnap® DNA Plasma 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.

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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.

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mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

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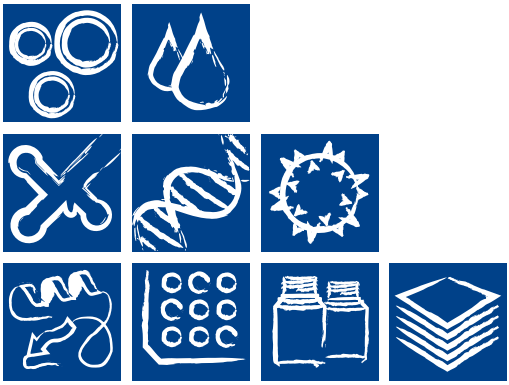

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