Genomic DNA from Plasma

User manual

NucleoMag® DNA Plasma

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MACHEREY-NAGEL
www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoMag® DNA Plasma	
REF	**1 x 48 preps* 744550.1**	**4 x 48 preps* 744550.4**
Lysis Buffer MCF1	60 mL	2 x 125 mL
Binding Buffer MCF2	125 mL	4 x 125 mL
Wash Buffer MCF3	60 mL	250 mL
Wash Buffer MCF4**	25 mL	50 mL
Elution Buffer MCF5	13 mL	30 mL
Liquid Proteinase K	2 x 1.4 mL	2 x 6 mL
NucleoMag® P-Beads	2 x 1.4 mL	6 x 1.4 mL
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*The kits can be used for 1 x 48 preps or 4 x 48 preps calculated for a sample volume of 2 mL. Using a smaller or larger sample volume, the number of preps changes accordingly, see section 2.2.

** For preparation of working solutions and storage, see section 3.

1.2 Consumables and equipment to be supplied by user

Product	REF	Pack of
• **Magnetic separator**		
NucleoMag® SEP 24 (see section 2.7)	744903	1
NucleoMag® SEP Maxi (see section 2.7)	744902	1
• **Separation plate**		
24-Square-well Block U-bottom (24-well block with 10 mL square-wells)	740448.24	24
• **Lysis tubes for incubation of samples and lysis,**		
e.g., Snap Tubes (50 mL tubes)	740822.10	10
	740822.50	50
• **Lysis plate for incubation of samples and lysis**		
24-Square-well Block with silicone lid (24-well block with 10 mL flat bottom wells)	740679.4	4
• **Elution plate for collecting purified nucleic acids,**		
24-Square-well Block U-bottom (24-well block with 10 mL square-wells)	740448.24	24
24-Square-well Block with silicone lid (24-well block with 10 mL flat bottom wells)	740679.4	4
Elution Plate, U-bottom (96-well microplates with 300 µL U-bottom wells for the storage of eluates, including Self-adhering Foil)	740486.24	24
• **For automated use on KingFisher® instruments:**		
e.g., KingFisher® 24 Accessory Kit, (24 Deep-well Blocks, 24-tip combs, Plates for 5 x 24 NucleoMag® DNA Plasma preps using KingFisher® Flex platform)	744953	1 set
e.g., KingFisher® Duo Prime Accessory Kit B, (24 Deep-well Blocks, 6-tip combs, Plates for 8 x 6 NucleoMag® DNA Plasma preps using KingFisher® Duo platform)	744954	1 set

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoMag® 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 Product description

2.1 The basic principle

The **NucleoMag® DNA Plasma** kit is designed for the efficient isolation of circulating DNA from human blood plasma. Fragmented DNA as small as 50 bp and larger can be purified with high efficiency due to a reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions.

Sample lysis is achieved by incubation of a plasma sample with Liquid Proteinase K and Lysis Buffer MCF1 at 56 °C. For the adjustment of optimal binding conditions of nucleic acids and paramagnetic beads, Buffer MCF2 and the NucleoMag® P-Beads are added to the lysate. Three washing steps efficiently remove contaminating substances, such as PCR inhibitors, after magnetic separation. Subsequently residual ethanol from previous wash steps is removed by a drying step. Highly pure DNA is finally eluted from the paramagnetic beads within 50–200 µL of a slightly alkaline Elution Buffer MCF5 of low ionic strength (5 mM Tris/HCl, pH 8.5). Eluted DNA can be used directly for downstream applications. The **NucleoMag® DNA Plasma** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

- The **NucleoMag® DNA Plasma** kit is recommended for the rapid manual and automated isolation of circulating cell-free DNA from human EDTA plasma. The DNA yield strongly depends on the individual sample, but is typically in the range of 0.1 to 100 ng DNA per mL plasma. The **NucleoMag® DNA Plasma kit** enables the recovery of highly fragmented DNA ≥ 50 bp. Elution can be performed with as little as 50–200 µL elution buffer and the eluted DNA is ready to use for subsequent downstream applications like real-time PCR, next generation sequencing etc.

Kit specifications at a glance

Parameter	NucleoMag® DNA Plasma
Technology	Magnetic bead technology
Format	Highly reactive superparamagnetic beads
Sample material	Human EDTA/Cell-Free DNA BCT® plasma
Sample amount	1–10 mL per preparation
Typical yield	Depending on sample source, storage, and quality
Elution volume	50–200 µL

- The **NucleoMag® DNA Plasma** is designed for extractions of 2 mL plasma samples, but it can also be scaled individually from 1 mL sample volume up to 10 mL. The table below describes the estimated number of extractions depending on different sample volumes:

Table 1: Amount of estimated number of extractions per sample volume

Sample volume	Number of extractions (1 x 48 preps REF 744550.1)	Number of extractions (4 x 48 preps REF 744550.4)
1 mL	96	384
2 mL	48	192
4 mL	24	96
6 mL	16	64
8 mL	12	48
10 mL	10	40

For a detailed adaption of sample volume for each buffer, NucleoMag® P-Beads, and Liquid Proteinase K please refer to section 5.1 and 5.2

2.3 Size and yield of DNA from plasma

Usually, DNA concentrations in plasma samples are in a range of 0.1 ng to several 100 ng DNA per mL. 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.

2.4 Handling of sample material

The yield and quality of circulating DNA 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.

2.5 Elution procedures

The recommended standard elution volume is 100 µL. A reduction of the elution volume to 50 µL will increase DNA concentration. It is essential to cover the NucleoMag® P-Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation pate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, higher elution volumes might be necessary to cover the whole pellet.

Note: Elution is possible at room temperature. Yield can be increased by 15–20 % if elution is performed at 56 °C.

2.6 Stability of isolated DNA

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

2.7 Magnetic separation systems

For handling of the **NucleoMag® DNA Plasma kit**, the use of the magnetic separator NucleoMag® SEP 24 is recommended. Separation is carried out in a 24-Square-well Block U-bottom (see ordering information). The magnetic separator NucleoMag® SEP Maxi is recommend for separation in 50 mL tubes. The kit can also be used with other common separators.

Static magnetic separators
Separators with static magnets, such as the NucleoMag® SEP 24 or other common magnetic separators are suitable for manual and automated liquid handling workstations. The NucleoMag® SEP 24 Separator is recommended to be used in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required; the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for subsequent resuspension of the beads.

Movable magnetic systems
Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators
Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution, the beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.8 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker, to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and washing steps:

- Load 5 mL and 1 mL of dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue to increase the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100–200 µL dyed water to the wells of the collection plate and proceed as described above.

Note: Shaker settings have to be adjusted using the respective volumes for processing larger sample volumes than 2 mL.

2.9 Handling of beads

Distribution of beads
A homogeneous distribution of the NucleoMag® P-Beads to the individual wells of the separation plate, is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogeneous distribution of the beads to the individual wells of the separation plate. During automation it is recommended to keep the beads resuspended before aspirating them to the samples. Make sure to implement an additional step to premix the beads / binding buffer mixture within the reservoir.

Magnetic separation time
Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads
Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips, needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Small elution volume possible	Number of tips needed
Magnetic mix	+	++	+	Low
Shaker	++	++	+++	Low
Pipetting	+++	+*	++	High

+: acceptable, ++: good, +++: excellent
* depending on the multichannel system of the used automation platform.

3 Storage conditions and preparation of working solutions

Attention: *Buffers MCF1, MCF2, and MCF3 contain chaotropic salt! Wear gloves and goggles!*

CAUTION: Buffer MCF1, MCF2, and MCF3 contain guanidine hydrochloride / guanidinium thiocyanate 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.
- The waste generated with the **NucleoMag® DNA Plasma** 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 to local safety regulations.

Before starting the **NucleoMag® DNA Plasma** protocol, prepare the following:

- Wash Buffer MCF4: Add the indicated volume of ethanol (96–100 %) to Buffer MCF4 Concentrate before use. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer MCF4 at room temperature (18–25 °C) for up to one year.

	NucleoMag® DNA Plasma	
REF	**1 x 48 preps 744550.1**	**4 x 48 preps 744550.4**
Wash Buffer MCF4 (Concentrate)	25 mL Add 100 mL ethanol	50 mL Add 200 mL ethanol

- **Liquid Proteinase K** is ready to use. After first use, **store Liquid Proteinase K at 4 °C or -20 °C**.
- **NucleoMag® P-Beads** are ready to use. After first use, store NucleoMag® P-Beads at 4 °C. **Do not store the NucleoMag® P-Beads at -20 °C.**

4 Safety instructions

The following components of the **NucleoMag® 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.

Component ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**GHS symbol** ***GHS-Symbol***	**Hazard phrases** ***H-Sätze***	**Precaution phrases** ***P-Sätze***
MCF1	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %* CAS 50-01-1	 WARNING *ACHTUNG*	302, 315, 319	264W, 280sh, 301+312, 330
MCF2	Guanidinium thiocyanate 30–45 % + ethanol 35–55 % *Guanidiniumthiocyanat 30–45 % + Ethanol 35–55 %* CAS 593-84-0, 64-17-5	 WARNING ACHTUNG	226, 302, 412, EUH031	210, 260D, 264W, 273, 301+312, 330
MCF3	Guanidine hydrochloride 24–36 % + ethanol 35–55 % *Guanidinhydrochlorid 24–36 % + Ethanol 35–55 %* CAS 50-01-1, 64-17-5	 WARNING ACHTUNG	226, 302	210, 264W, 301+312, 330
Liquid Proteinase K	Proteinase K liquid 90–100 % *Proteinase K flüssig 90–100 %* CAS 39450-01-6	 WARNING ACHTUNG	317	261sh, 280sh,

 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. Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Hazard phrases

H226 Flammable liquid and vapor.
Flüssigkeit und Dampf entzündbar.

H302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

H315 Causes skin irritation.
Verursacht Hautreizungen.

H317 May cause an allergic skin reaction.
Kann allergische Hautreaktionen verursachen.

H319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase*

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.*
P260D	Do not breathe vapours *Dampf nicht einatmen.*
P261	Avoid breathing dust/fume/gas/mist/vapours/spray. *Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden.*
P264	Wash thoroughly after handling. *Nach Handhabung … gründlich waschen.*
P273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P280	Wear protective gloves/protective clothing/eye protection/face protection. *Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz 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.*

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

5.1 Protocol at a glance

The procedure below describes the isolation of cell-free DNA from 2 mL human EDTA plasma. A range of 1–10 mL plasma can be processed by corresponding adjustment of the Proteinase K, magnetic beads, and buffer volumes:

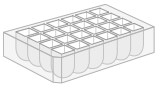
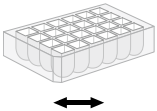
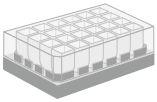
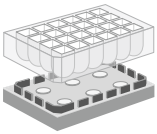
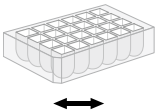
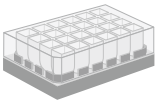
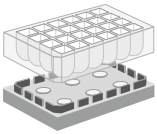
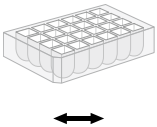
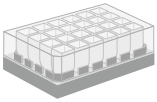
Plasma volume [mL]	Proteinase K [µL]	Buffer MCF1 [mL]	Buffer MCF2 [mL]	NucleoMag® P-Beads [µL]	Wash Buffer volume [mL]
1	25	0.45	1	15	0.5
2	50	0.9	2	30	1
4	100	1.8	4	60	1
6	150	2.7	6	60	2
8	200	3.6	8	60	2
10	250	4.5	10	90	2

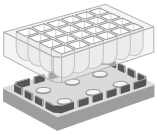
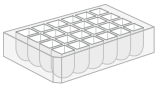
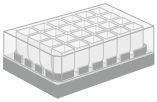
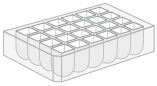
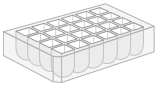
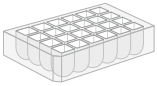
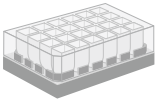
Before starting the preparation:

- Prepare plasma sample according to section 2.4
- Check if Wash Buffer MCF4 was prepared according to section 3.
- Set water bath or heating block to 56 °C (for lysate incubation).

Protocol for 2 mL

1	**Lyse sample**	**Add 50 µL Liquid Proteinase K to 2 mL plasma** **Mix** **RT, 15 min**
		Add 900 µL MCF1 **Mix** **56 °C, 30 min**

2	**Bind** DNA to NucleoMag® P-Beads	**Add 30 µL NucleoMag® P-Beads** **Add 2 mL Buffer MCF2**	
		Mix by shaking for 10 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 5 min separation using the NucleoMag® SEP 24	
3	**Wash** with MCF3	**Remove Square-well Block from NucleoMag® SEP 24** **Add 1 mL MCF3**	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation using the NucleoMag® SEP 24	
4	**Wash** with MCF4 (1st)	**Remove Square-well Block from NucleoMag® SEP 24** **Add 1 mL MCF4**	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation using the NucleoMag® SEP 24	

5	**Wash** with MCF4 (2nd)	**Remove Square-well Block from NucleoMag® SEP 24** **Add 1 mL MCF4**	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation using the NucleoMag® SEP 24	
6	**Dry** the magnetic beads	**15 min at RT** *(Optional: Air-dry at 56 °C)*	
7	**Elute** DNA	**Add 50–200 µL MCF5** *(Optional: Elute at 56 °C)*	
		Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Separate 2 min using the NucleoMag® SEP 24 and transfer DNA from supernatant into the elution plate	

5.2 Detailed protocol

The procedure below describes the isolation of cell-free DNA from 2 mL human EDTA plasma. A range of 1–10 mL plasma can be processed by corresponding adjustment of the Proteinase K, magnetic beads and buffer volumes:

Plasma volume [mL]	Proteinase K [µL]	Buffer MCF1 [mL]	Buffer MCF2 [mL]	NucleoMag® P-Beads [µL]	Wash Buffer volume [mL]
1	25	0.45	1	15	0.5
2	50	0.9	2	30	1
4	100	1.8	4	60	1
5	125	2.25	5	60	2
6	150	2.7	6	60	2
8	200	3.6	8	60	2
10	250	4.5	10	90	2

Before starting the preparation:

- Prepare plasma sample according to section 2.4
- Check if Wash Buffer MCF4 was prepared according to section 3.
- Set water bath or heating block to 56 °C (for lysate incubation).

Protocol for 2 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 further processing. However, the supernatant may still contain suspended matter (e.g., lipids). This floating material does not interfere with further processing.

2 Lyse sample

Pre-dispense **50 µL Proteinase K** to a suitable reaction tube or plate and add **2 mL plasma** sample. Carefully **mix** the contents.

Incubate **15 min** at **room temperature** (18–25 °C). Add **900 µL Buffer MCF1** to the tube. **Mix** well by vortexing, shaking or repeated pipetting up and down and incubate at 56 °C for 30 min ideally with shaking.

Allow the samples to cool down for **5 min** and transfer the lysate to a 24-Square-well Block (U-bottom) or any other reaction tube or plate suitable for magnetic separation.

Note: Prolong the incubation time at 56 °C to 60 min for plasma samples from Streck Cell-Free DNA BCT®.

3 Bind DNA

Resuspend and add **30 µL P-Beads** and **2 mL Buffer MCF2** to the lysed sample **(NucleoMag® P-Beads and Buffer MCF2 can be pre-mixed).**

Mix by pipetting up and down 6 times and shake for **5–10 min** at **room temperature**. Alternatively, when processing the kit without a shaker, vortex or pipette up and down 10 times and incubate for 5–10 min at room temperature.

Note: Be sure to resuspend the NucleoMag® P-Beads before removing them from the storage tube or bottle. Vortex storage tube or bottle briefly until a homogeneous suspension has been formed.

Separate the magnetic beads against the side of the wells by placing the 24-Square-well Block (U-bottom) on the NucleoMag® SEP 24 magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might be not visible in this step. Remove supernatant from the opposite side of the well.

4 Wash with MCF3

Remove the 24-Square-well Block from the NucleoMag® SEP 24 Magnetic Separator.

Add **1 mL Buffer MCF3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**2–3 min**). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the the magnetic beads against the side of the wells by placing the 24-Square-well Block (U-bottom) on the NucleoMag® SEP 24 magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

5 Wash with MCF4 (1st)

Remove the 24-Square-well Block from the NucleoMag® SEP 24 Magnetic Separator.

Add **1 mL Buffer MCF4** to each well and resuspend the beads by shaking until the beads are resuspended completely (**2–3 min**). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the the magnetic beads against the side of the wells by placing the 24-Square-well Block (U-bottom) on the NucleoMag® SEP 24 magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

6 Wash with MCF4 (2nd)

Remove the 24-Square-well Block from the NucleoMag® SEP 24 Magnetic Separator.

Add **1 mL Buffer MCF4** to each well and resuspend the beads by shaking until the beads are resuspended completely (**2–3 min**). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the the magnetic beads against the side of the wells by placing the 24-Square-well Block (U-bottom) on the NucleoMag® SEP 24 magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

7 Dry NucleoMag® P-Beads

Remove the 24-Square-well Block (U-bottom) from the NucleoMag® SEP 24 Magnetic Separator and air-dry the magnetic bead pellet for **10–15 min** at **room temperature**. Alternatively air-dry the magnetic bead pellet at **56 °C** with moderate shaking.

Note: Be sure to remove all of the residual wash buffers from the final wash in order to dry the bead-pellet.

8 Elute DNA

Add **desired volume of Elution Buffer MCF5 (50–200 µL)** to each well of the 24-Square-well Block (U-bottom) and resuspend the beads by shaking **5 min** at **room temperature.** Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5–10 min** at **room temperature** or **56 °C**.

Separate the magnetic beads against the side of the wells by placing the 24-Square-well Block (U-bottom) on the NucleoMag® SEP Magnetic Separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified DNA to either elution plates or other reaction tubes (see ordering informations).

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

Note: For alternative elution procedures see section 2.5.

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 from approximately 0.1–1000 ng DNA per mL of plasma have been reported (see remarks in section 2.3). *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. *Incomplete sample lysis* • Sample was not thoroughly homogenized and mixed with Lysis buffer and Proteinase K. The mixture has to be shaken continuously. Alternatively, prolong incubation time with Proteinase K. *Reagents not prepared properly* • Prepare Buffer MCF4 according to the instructions (section 3). *Insufficient Elution buffer volume* • Bead pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation was too short or aspiration speed was too high.

Problem	Possible cause and suggestions
Turbed eluates / Low eluates	*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 2.3). Alternatively increase the volume of Binding Buffer MCF2. *Insufficient air-drying of magnetic beads.* • Be sure to remove all of the residual wash buffers from the final wash in order to dry the bead-pellet. Alternatively prolong the air-drying step or air-dry the bead pellet at 56 °C.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* • Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs. *Insufficient air-drying of magnetic beads.* • Be sure to remove all of the residual wash buffers from the final wash in order to dry the bead pellet. Alternatively prolong the air-drying step or air-dry the bead pellet at 56 °C.
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the 24-Square-well Block when transferring the lysate. If the rim of the wells is contaminated, seal the Square-well Block with Self-adhering PE Foil (see ordering information) before starting the shaker.
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
NucleoMag® DNA Plasma	744550.1 744550.4	1 x 48 preps 4 x 48 preps
NucleoMag® SEP 24	744903	1
NucleoMag® SEP Maxi	744902	1
24-Square-well Block U-bottom (24-well block with 10 mL square-wells for magnetic separation)	740448.24	24
24-Square-well Block with silicone lid (24-well block with 10 mL flat bottom wells)	740679.4	4
Elution Plate, U-bottom (96-well microplates with 300 µL U-bottom wells for the storage of eluates, including Self-adhering Foil)	740486.24	24 sets
Self-adhering PE Foil	740676	50 sheets
Snap Tubes (50 mL)	740822.10 740822.50	10 50
KingFisher® 24 Accessory Kit (24 Deep-well Blocks, 24-tip combs, Plates for 5 x 24 NucleoMag® DNA Plasma preps using KingFisher® Flex platform)	744953	1
KingFisher® Duo Prime Accessory Kit B (24 Deep-well Blocks, 6-tip combs, Plates for 8 x 6 NucleoMag® DNA Plasma preps using KingFisher® Duo platform)	744954	1
Liquid Proteinase K	740396	5 mL

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

6.3 Product use restriction / warranty

NucleoMag® 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.

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).

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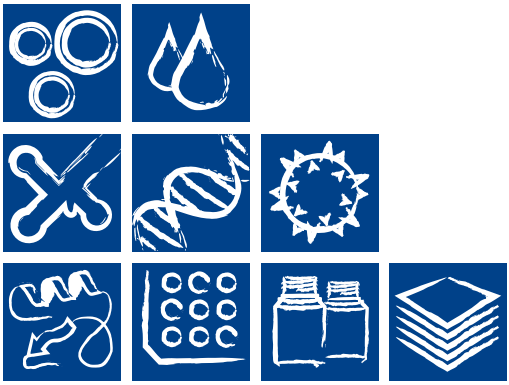

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