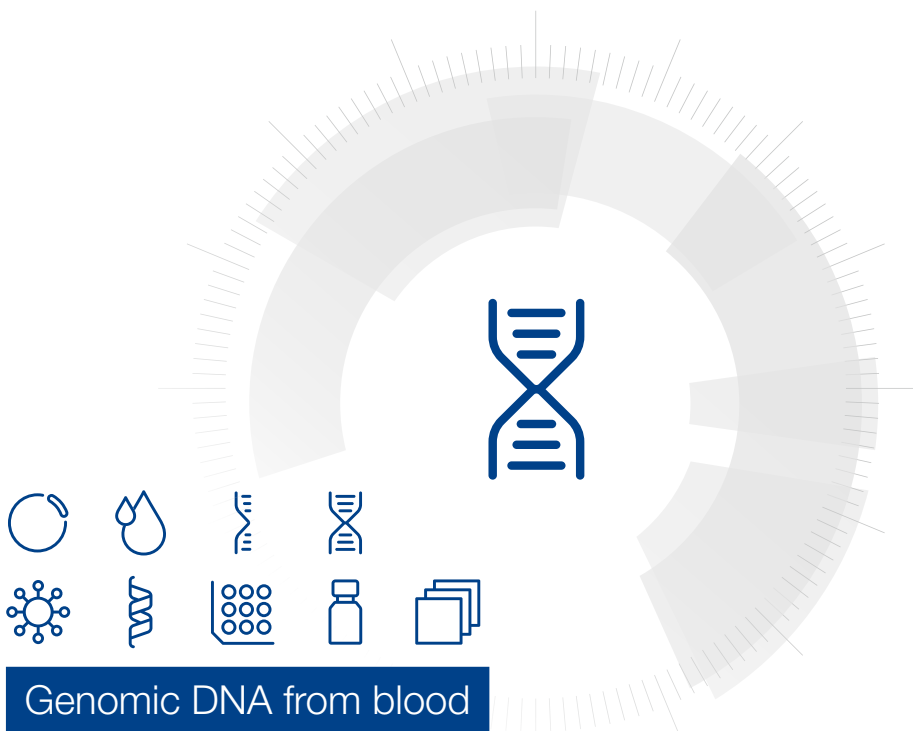


MACHEREY-NAGEL

# User manual



## Genomic DNA from blood

- NucleoMag® Blood 200  $\mu$ L
- NucleoMag® Blood 200  $\mu$ L Prefilled Plates

April 2025 / Rev. 08

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# 1 Components

## 1.1 Kit contents

NucleoMag® Blood 200 µL		
REF	1 × 96 preps 744501.1	4 × 96 preps 744501.4
NucleoMag® B-Beads	2 × 1.5 mL	12 mL
Lysis Buffer MBL1	13 mL	45 mL
Binding Buffer MBL2	40 mL	160 mL
Wash Buffer MBL3	300 mL	900 mL
Wash Buffer MBL4	125 mL	500 mL
Elution Buffer MBL5*	30 mL	125 mL
Proteinase K, lyophilized**	50 mg	4 × 50 mg
Proteinase Buffer PB	8 mL	15 mL
User manual	1	1

NucleoMag® Blood 200 µL Prefilled Plates	
REF	6 × 16 preps 744510
Lysis Buffer MBL1	13 mL
Prefilled 96-well NucleoMag® Blood 200 µL Reagent Plate	6 pieces
Liquid Proteinase K	2 mL
8-well Tip Combs	6 × 2 pieces
Leaflet	1

\* Elution Buffer MBL5: 5 mM Tris, pH 8.5

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

## 1.2 Material to be supplied by user

### Reagents

- 80 % ethanol (for the washing step)

### Equipment / Consumables

Equipment / Consumables	REF	Pack of
<ul style="list-style-type: none"> <li>• <b>Separation plate for magnetic beads separation, (manual use)</b> e.g., Square-well Block (96-well block with 2.1 mL square-wells)</li> </ul>	740481 740481.24	4 24
<ul style="list-style-type: none"> <li>• <b>Lysis tubes for incubation of samples and lysis,</b> e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)</li> </ul>	740477 740477.24	4 sets 24 sets
<ul style="list-style-type: none"> <li>• <b>Elution plate for collecting purified nucleic acids,</b> e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL U-bottom wells)</li> </ul>	740486.24	24
<ul style="list-style-type: none"> <li>• <b>For use of kit on KingFisher® instruments;</b> KingFisher® Accessory Kit B (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag® Blood 200 µL preps using KingFisher® Flex platform)</li> </ul>	744951	1 set
<ul style="list-style-type: none"> <li>• <b>For use of kit on MagnatePure32 Plus or IsoPure™ Mini:</b> 96 Deep-well plates for magnetic rod systems (already included in REF 744510 NucleoMag® Blood 200 µL Prefilled Plates)</li> </ul>	744955	25 pieces
<ul style="list-style-type: none"> <li>• <b>For use of kit on MagnatePure32 Plus or IsoPure™ Mini:</b> 8-well Tip Combs for magnetic rod systems (already included in REF 744510 NucleoMag® Blood 200 µL Prefilled Plates)</li> </ul>	744960	25 × 2 pieces

### 1.3 About this user manual

It is strongly recommended that first time users of the **NucleoMag® Blood 200 µL** kit read the detailed protocol sections of this user manual. 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](http://www.mn-net.com).

### 1.4 Automation support

MN extraction kits are designed for streamlined automation, offering compatibility with a range of leading open robotic systems. Whether you're using magnetic rod systems or liquid handlers like Hamilton, Tecan, Eppendorf, or other platforms, our kits ensure efficient and reliable extraction processes. Reach out to us for comprehensive support and tailored automation solutions, making your extraction experience seamless and effortless.

Questions about MACHEREY-NAGEL's scripting support or automation service?

Please contact us for personal assistance:

Phone: +49 2421 969-333

E-mail: [support@mn-net.com](mailto:support@mn-net.com)

## 2 Product description

### 2.1 The basic principle

The **NucleoMag® Blood 200 µL** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Whole blood is lysed with Lysis Buffer MBL1 and Proteinase K. Following lysis incubation, magnetic beads are added and binding conditions under which the DNA binds to the magnetic beads are adjusted by addition of Binding Buffer MBL2. After magnetic separation and removal of the supernatant, the paramagnetic beads are washed three times to remove contaminants and salt. There is no need for a drying step as ethanol from previous wash steps is removed by Wash Buffer MBL4. Finally, highly purified DNA is eluted with low-salt Elution Buffer MBL5 and can directly be used for downstream applications. The **NucleoMag® Blood 200 µL** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit [www.mn-net.com/automation](http://www.mn-net.com/automation)

### 2.2 Kit specifications

**NucleoMag® Blood 200 µL** is designed for rapid manual and automated small-scale preparation of highly pure genomic DNA from 200 µL whole blood using the NucleoMag® 96 SEP (see ordering information) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The obtained DNA can be used directly as template for PCR, blotting, or any kind of enzymatic reactions.

**NucleoMag® Blood 200 µL Prefilled Plates** (REF 744510) are prefilled 96 deep-well plates specifically for the use on MagnetaPure 32 Plus or IsoPure™ Mini systems. Binding Buffer MBL2, NucleoMag® B-Beads, Wash Buffer MBL3, 80 % ethanol as well as elution buffer MBL5 are prefilled into their respective wells. Sample preparation/lysis is performed according to the standard procedure of the NucleoMag® Blood 200 µL kit. Subsequently, lysed samples are transferred to the reagent wells containing binding buffer MBL2 and NucleoMag® B-Beads for further processing on the MagnetaPure 32 Plus or IsoPure™ Mini instruments.

**NucleoMag® Blood 200 µL** allows easy automation on common liquid handling instruments or automated magnetic separators, for example Thermo Scientific's KingFisher® instruments. The actual processing time depends on the configuration of your instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on the automation platform.

The kit provides reagents for the purification of 2–8 µg of pure genomic DNA from 200 µL whole blood with an  $A_{260}/A_{280}$  ratio  $\geq 1.6$ –1.9 and typical concentration of 20–40 ng/µL. Depending on the health status of the blood donor and the elution volume used, concentrations of 10–160 ng/µL can be obtained.

Fresh or frozen blood treated either with EDTA or citrate can be used. The procedure is optimized for a sample volume of 200  $\mu\text{L}$ .

For research use only.

**NucleoMag® Blood 200  $\mu\text{L}$**  can be processed completely at room temperature, however, elution at 55 °C or 72 °C will increase the yield by about 15–20 %.

**NucleoMag® Blood Beads** are highly reactive, superparamagnetic beads. The binding capacity is approximately 0.4  $\mu\text{g}$  of gDNA per 1  $\mu\text{L}$  of NucleoMag® Blood Bead Suspension, 1  $\mu\text{L}$  of suspension contains 140  $\mu\text{g}$  of beads.

## 2.3 Magnetic separation systems

For use of **NucleoMag® Blood 200  $\mu\text{L}$** , the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481 / .24)
NucleoMag® SEP Mini (MN REF 744901)	1.5 mL or 2 mL reaction tubes (Sarstedt)
NucleoMag® SEP Maxi (MN REF 744902)	50 mL tubes (Falcon)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

### Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended 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 resuspension of the beads.

### Movable magnetic systems

Separators with moving magnetic pins, for example Te-MagS™ (for automated use only): 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 the movement.

### **Automated separators (e.g., King Fisher® instruments)**

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 beads are collected again with the rod-covered magnets and transferred to the next plate or tube.\*

### **MagnetaPure32 Plus and IsoPure™ Mini**

The NucleoMag® Blood 200 µL Prefilled Plates (REF 744510) are specifically designed for the use on MagnetaPure 32 Plus and IsoPure™ Mini magnetic rod systems. Reagents are prefilled in a column-wise manner. Sample preparation and sample lysis is performed externally and lysed samples are transferred to the reagent wells containing binding buffer and magnetic beads.

## **2.4 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 wash steps:**

- Load 800 µL 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 plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check plate surface for droplets again.
- Continue increasing 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 µL dyed water to the wells of the collection plate and proceed as described above.

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\* Contact MN Technical Service for optimized program files and support protocols.

## 2.5 Handling of beads

### Distribution of beads

A homogeneous distribution of the magnetic 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 MBL2 allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

### 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, the 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. Complete and homogenous resuspension of the beads in wash buffers MBL3, MBL4, and 80 % ethanol is mandatory for best performance of the kit.

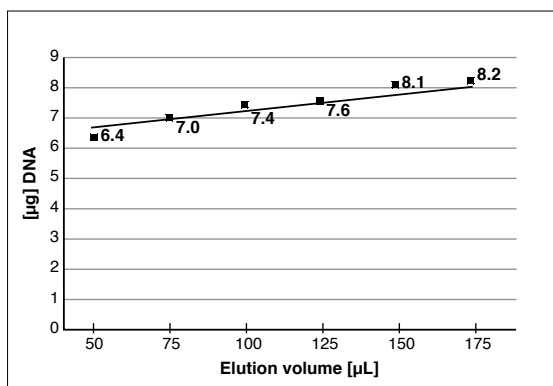
Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

+: acceptable, ++: good, +++: excellent

\* Depending on multichannel system

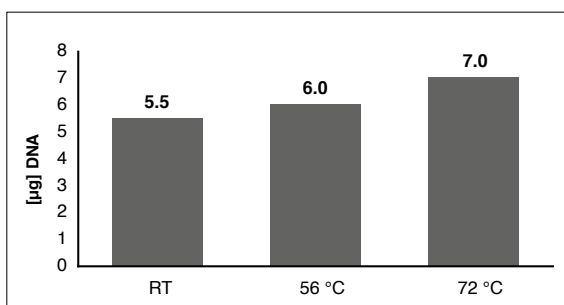
## 2.6 Elution procedures

Purified total DNA can be eluted directly with the supplied Elution Buffer MBL5. Elution can be carried out in a volume of  $\geq 50 \mu\text{L}$ . It is essential to cover the NucleoMag<sup>®</sup> B-Beads completely with Elution Buffer MBL5 during the elution step. The volume of dispensed Elution Buffer MBL5 depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the Elution Buffer MBL5. For some separators high elution volumes might be necessary to cover the whole magnetic bead pellet.



**Figure 1:** Influence of elution volume on DNA yield (example)

Elution is possible at room temperature. However, DNA yield can be increased by 15–20 % if elution is performed at  $72 \text{ }^\circ\text{C}$  (see Figure 2).



**Figure 2:** Influence of elution temperature on DNA yield

The NucleoMag<sup>®</sup> Blood 200  $\mu\text{L}$  Prefilled Plates (REF 744510) contain 100  $\mu\text{L}$  of Elution buffer MBL5 per well. For photometrical UV absorbance measurements eluates can be blanked against  $\text{dH}_2\text{O}$ .

For more information on automating the NucleoMag<sup>®</sup> Blood kit on various manufacturers' platforms, please visit the download section of our website.

<https://www.mn-net.com/de/nucleomag-blood-200-l-for-dna-purification-from-blood-744501.4?c=0>

### 3 Storage conditions and preparation of working solutions

**Attention:** Buffers MBL1, MBL2, and MBL3 contain chaotropic salt! Wear gloves and goggles!

**CAUTION:** Buffer MBL1 contains guanidine 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.

- All components of the **NucleoMag® Blood 200 µL** kit should be stored at 15–25 °C and are stable until: see package label.
- All buffers are delivered ready-to-use.

The **NucleoMag® Blood 200 µL Prefilled Plates** should be stored upright at 15–25 °C without direct exposure to UV-light or sunlight. Do not store the **NucleoMag® Blood 200 µL Prefilled Plates** at temperatures above 25 °C or below 15 °C. Before use please check buffers for precipitates and prewarm the plate in order to dissolve precipitates. When stored properly the Prefilled Plates are stable until: see package label.

Before starting **NucleoMag® Blood 200 µL** protocol prepare the following:

- Add the indicated volume of Proteinase Buffer PB to dissolve lyophilized Proteinase K (see table below). Proteinase K solution is stable at -20 °C for up to 6 months.

*Note:* The **NucleoMag® Blood 200 µL Prefilled Plates (REF 744510)** are delivered with liquid Proteinase K, which is ready to use.

NucleoMag® Blood 200 µL		
REF	1 × 96 preps	4 × 96 preps
	744501.1	744501.4
Proteinase K	50 mg	4 × 50 mg
	Add 2.5 mL Proteinase Buffer PB	Add 2.5 mL Proteinase Buffer PB to each vial

## 4 Safety instructions

When working with the **NucleoMag® Blood 200 µL** kit **NucleoMag® Blood 200 µL Prefilled Plates** 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 [www.mn-net.com/msds](http://www.mn-net.com/msds)).



Caution: Guanidin hydrochloride in Buffer MBL1, sodium perchlorate in buffer MBL2 and MBL3 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 **NucleoMag® Blood 200 µL** kit or **NucleoMag® Blood 200 µL Prefilled Plates** 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.

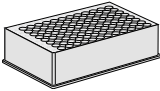
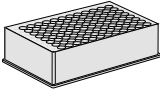

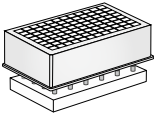
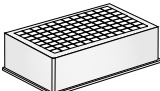
## 5 Protocol for the isolation of DNA from blood

### Protocol-at-a-glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 25.

#### Before starting the preparation:

- Check if Proteinase K was prepared according to section 3.

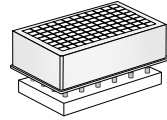
<p>1 Lyse samples</p>	<p><b>Dispense 20 µL Proteinase K into Square-well Block</b></p> <p>200 µL blood</p> <p>80 µL MBL1</p> <p><b>Mix 3–5 times</b></p> <p><b>Shake 10 min at RT</b></p>	
<p>2 <b>Bind DNA to NucleoMag® B-Beads</b></p>	<p><b>25 µL B-Beads</b></p> <p><b>300 µL MBL2</b></p> <p><b>Mix by shaking for 5 min at RT</b> <i>(Optional: Mix by pipetting up and down)</i></p>	 
	<p><b>Remove supernatant after 2 min separation</b></p>	
<p>3 <b>Wash with MBL3</b> (1<sup>st</sup> wash)</p>	<p>Remove Square-well Block from NucleoMag® SEP</p> <p><b>800 µL MBL3</b></p>	

**Resuspend: Shake 5 min at RT**

*(Optional: Mix by pipetting up and down)*



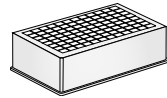
**Remove supernatant after 2 min separation**



4 **Wash** with MBL3  
(2<sup>nd</sup> wash)

Remove Square-well Block from NucleoMag® SEP

**800 µL MBL3**

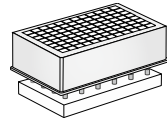


**Resuspend: Shake 5 min at RT**

*(Optional: Mix by pipetting up and down)*



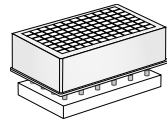
**Remove supernatant after 2 min separation**



5 **Wash** with  
80 % ethanol

Remove Square-well Block from NucleoMag® SEP

**800 µL 80 % ethanol**

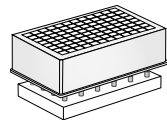


**Resuspend: Shake 5 min at RT**

*(Optional: Mix by pipetting up and down)*



**Remove supernatant after 2 min separation**



6 **Wash with MBL4**

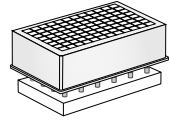
Leave Square-well Block  
on NucleoMag® SEP

**900 µL MBL4**

**Incubate for 45–90 s**

Aspirate and discard supernatant

*Note: Do not resuspend  
the beads in Buffer MBL4!*



7 **Elute DNA**

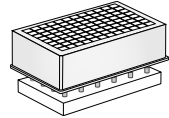
Remove Square-well Block  
from NucleoMag® SEP

**50-100 µL MBL5**  
(Optional: Elute at 55 °C)

**Shake 5 – 10 min at RT**  
(Optional: Mix by pipetting  
up and down)



**Separate 2 min and transfer  
DNA into elution plate / tubes**



## Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

### Before starting the preparation:

- Check if Proteinase K was prepared according to section 3.
- 

#### 1 Lyse samples

Dispense **20 µL of Proteinase K** solution into each well of a Square-well Block.

Transfer **200 µL blood** (equilibrated to room temperature) to each well of a Square-well Block. Do not moisten the rims of the well.

*Note: See recommendations for suitable plates or tubes and compatible magnetic separators (section 2.3).*

Add **80 µL Buffer MBL1** to each sample and **mix** by repeated pipetting up and down (3–5 times) **and shaking** for **5–10 min** at **room temperature**.

*Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate 5–10 min at room temperature.*

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#### 2 Bind DNA to NucleoMag® B-Beads

Add **25 µL NucleoMag® B-Beads** to each sample. Mix magnetic beads thoroughly before dispensing to the samples.

Add **300 µL Buffer MBL2** to each sample and **mix** by pipetting up and down 3–5 times and **shake** for **5 min** to allow the DNA to bind to the magnetic beads. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate 5 min at room temperature.

*Note: NucleoMag® B-Beads and Buffer MBL2 can be premixed. For each sample to be processed, mix 25 µL of NucleoMag® B-Beads with 300 µL Buffer MBL2. Vortex briefly. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are necessary. Mix the solution several times to avoid the beads to settle within the premix distribution step. Do not store the premix of the NucleoMag® B-Beads and Buffer MBL2 longer than 12 h.*

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the magnetic separator. Wait at least **2 min** until all the beads have been attracted by the magnet. Remove and discard the supernatant by pipetting.

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

---

### 3 Wash with MBL3 (1<sup>st</sup> wash)

Remove the Square-well Block from the magnetic separator.

Add **800 µL Buffer MBL3** to each well and resuspend the bead/DNA complex by **shaking** at room temperature until the beads are resuspended completely (**5 min**). Alternatively, resuspend the beads by pipetting up and down (15 times).

*Note: Make sure that the magnetic beads are resuspended completely and form a brownish suspension. If necessary increase shaking incubation time or number of mixing cycles. Incomplete mixing may result in low purity of eluted DNA.*

Separate the magnetic beads by placing the Square-well Block on the magnetic separator. Wait for at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

*Note: Supernatant has a brownish color, magnetic bead pellet is now visible.*

---

### 4 Wash with MBL3 (2<sup>nd</sup> wash)

Remove the Square-well Block from the magnetic separator.

Add **800 µL Buffer MBL3** to each well for a second wash step with Buffer MBL3. Wash the bead/DNA complex by **shaking (5 min)** at **room temperature**. Alternatively, resuspend the beads by pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the magnetic separator. Wait for at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

*Note: Supernatant is colorless, magnetic bead pellet is clearly visible.*

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## 5 Wash with 80 % ethanol

Remove the Square-well Block from the magnetic separator.

Add **800 µL 80 % ethanol** to each well and wash the bead/DNA complex by **shaking** (5 min) at **room temperature**. Alternatively, resuspend the beads by pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the magnetic separator. Wait for at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

*Note: Supernatant is colorless, magnetic bead pellet is visible now.*

---

## 6 Wash with MBL4

Leave Square-well Block on the magnetic separator.

**!** Gently add **900 µL Buffer MBL4** to each well and **incubate** for **45–90 s** while the beads are still attracted to the magnet.

Then aspirate and discard the supernatant.

*Note: Do **not** resuspend the beads in Buffer MBL4. This step is to remove traces of ethanol and eliminates a drying step.*

*Optional: Washing the magnetic beads with Buffer MBL4 may decrease the DNA yield slightly. Alternatively, replace this washing step by air-drying of the magnetic beads for 10–15 min until all of the ethanol from previous washing step has evaporated. Beads with remaining ethanol appear to be glossy. Moderate heating (37 °C) can support and shorten the air-drying step. Over drying the beads may result in low yield in the final elution step.*

---

## 7 Elute DNA

Remove the Square-well Block from the magnetic separator.

Add desired volume of Buffer MBL5 (50–100 µL) to each well of the Square-well Block and resuspend the bead/DNA complex by shaking (5–10 min). Alternatively, resuspend the beads by pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the magnetic separator. Wait for at least **2 min** until all the beads have been attracted to the magnet. Transfer the supernatant containing the purified genomic DNA to the Elution Plate.

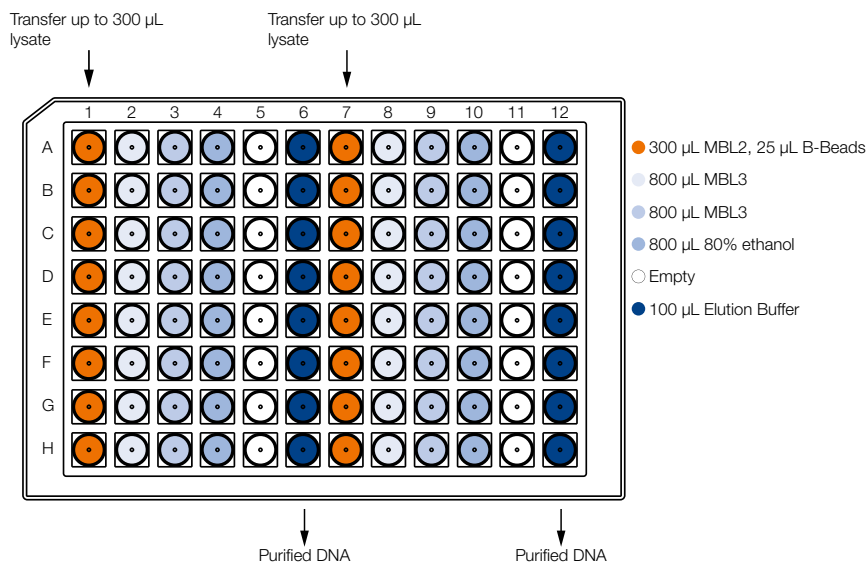
*Note: Yield can be increased by 15–20 % by using pre-heated elution buffer (55–72 °C) or by incubating the bead/elution buffer suspension at 55–72 °C for 10 min.*

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## 6 Protocol for NucleoMag® Blood 200 µL Prefilled Plates

The **NucleoMag® Blood 200 µL Prefilled Plates** are specifically designed for the use on MagnetaPure 32 Plus or IsoPure™ Mini instruments only. Please contact our technical support for questions regarding the compatibility of comparable instruments.

Schematic overview of the Prefilled reagent plate.



*Note: The required method files for processing the NucleoMag® Blood 200 µL Prefilled Plates on the IsoPure™ Mini or MagnetaPure32 Plus instruments can be requested at [support@mn-net.com](mailto:support@mn-net.com)*

### Detailed protocol for IsoPure™ Mini and MagnetaPure32 Plus

Preparation and lysis of sample material must be performed outside of the instrument.

#### Before starting the preparation:

- Wear protective goggles and suitable protective clothing
- Check buffers for precipitates according to section 3.
- Check if the correct script is installed on your instrument.
- IsoPure™ Mini: NMBlood200PFP
- MagnetaPure32 Plus: NMBlood200PFP

## 1 Lyse samples

Dispense **20 µL of Proteinase K** solution into each well of a Square-well Block.

Transfer **200 µL blood** (equilibrated to room temperature) to each well of a Square-well Block. Do not moisten the rims of the well.

*Note: See recommendations for suitable plates or tubes and compatible magnetic separators (section 2.3).*

Add **80 µL Buffer MBL1** to each sample and **mix** by repeated pipetting up and down (3–5 times) **and shaking** for **5–10 min** at **room temperature**.

*Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate 5–10 min at room temperature.*

---

## 2 Prepare reagent plate(s)

Briefly spin down (e.g. 10–15 s at 1,000 × g) the sealed Prefilled 96-well **NucleoMag® Blood 200 µL Reagent Plate** in a suitable centrifuge to remove droplets from the underside of the seal.

## 3 Remove seal(s)

Carefully remove the seal from the reagent plate by pulling on the seal from one side with an equal amount of force.

*Note: Check for residual sealant on the plate and remove with tweezers if necessary.*

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## 4 Transfer cleared lysate(s)

Transfer up to 300 µL lysate from step 1 to the respective wells of column 1 and 7 of the used plates.

*Note: Do not moisten the upper rim of the 96 deep-well plate.*

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## 5 Select the protocol and start the run

**Load the plate(s) on the instrument.**

**Insert tip combs on the mounting grooves.**

**Start the run.**

*Note: Please equip all tip combs in order to cover the magnetic rods in used and unused wells.*

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## **6 Transfer eluate**

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

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## 7 Appendix

### 7.1 Troubleshooting

<b>Problem</b>	<b>Possible cause and suggestions</b>
	<i>Elution buffer volume insufficient</i> <ul style="list-style-type: none"><li>• Beads pellet must be covered completely with elution buffer</li></ul>
	<i>Insufficient performance of elution buffer during elution step</i> <ul style="list-style-type: none"><li>• Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of subsequent wash steps and elution step.</li></ul>
	<i>Beads dried out</i> <ul style="list-style-type: none"><li>• Do not let the beads dry as this might result in lower elution efficiencies.</li></ul>
Poor DNA yield	<i>Partial elution in Wash Buffer MBL4 already</i> <ul style="list-style-type: none"><li>• Keep the separation plate on the magnet while dispensing Wash Buffer MBL4. Do not resuspend beads in this buffer, and do not incubate beads in this buffer for more than 2 min, as Buffer MBL4 promotes DNA elution.</li></ul>
	<i>Aspiration of attracted bead pellet</i> <ul style="list-style-type: none"><li>• Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate.</li></ul>
	<i>Incubation after dispensing beads to lysate</i> <ul style="list-style-type: none"><li>• Mix immediately after dispensing NucleoMag® B-Beads and Binding Buffer MBL2 to the lysate.</li></ul>

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<b>Problem</b>	<b>Possible cause and suggestions</b>
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*Poor blood quality*

- Be sure that no blood clots are transferred to the well. Blood can be stored at 2–8 °C for two weeks. Freeze samples if stored for longer periods.

Low purity

*Insufficient washing procedure*

- Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP.
- 

Suboptimal performance of DNA in downstream applications

*Carry-over of ethanol from ethanol wash step*

- Be sure to remove all of the ethanol from the ethanol wash step. Carry-over of ethanol may interfere with downstream applications. Use of Buffer MBL4 or introduce an air-drying step.

*Low purity*

- See above
- 

*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.

Carry-over of beads

*Aspiration speed too high (elution step)*

- High aspiration speed during the elution step may cause bead carry over. Reduce aspiration speed for elution step.
  - To remove magnetic beads from the eluates, put the elution plate on the magnetic separator and aspirate the supernatant after sufficient beads separation.
- 

*Contamination of the rims*

Cross contamination

- Do not moisten the rims of the Square-well Block when transferring the blood. 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.
-

## 7.2 Ordering information

Product	REF	Pack of
NucleoMag® Blood 200 µL	744501.1 744501.4	1 x 96 preps 4 x 96 preps
NucleoMag® Blood 200 µL Prefilled Plates	744510	6 x 16 preps
NucleoMag® SEP	744900	1
Square-well Blocks	740481.4 740481.24	4 24
Self-adhering PE Foil	740676	50 sheets
Elution Plates U-bottom	740486.24	24
Elution Plate Flat-bottom	740673	20
96-well Accessory Kit A for KingFisher® (set consists of Square-well Blocks, Deep-well Tip Combs, Elution Plates; for 4 x 96 NucleoMag® Blood 200 µL preps using KingFisher® Flex platform)	744950	1 set
96 Deep-well plates for magnetic rod systems	744955	25
8-well Tip Combs for magnetic rod system	744960	50
8-well Accessory Kit magnetic rod systems	744961	1 set

Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information.

## 7.3 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 customer 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

Please contact:

MACHEREY-NAGEL GmbH & Co. KG

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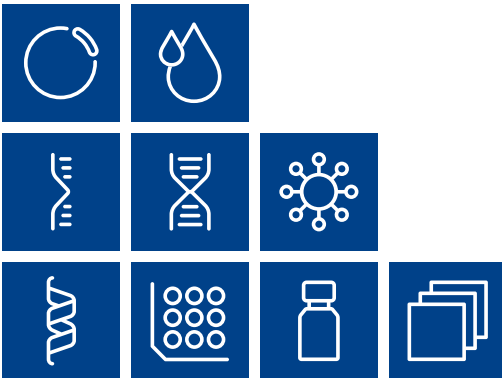
**Trademarks:**

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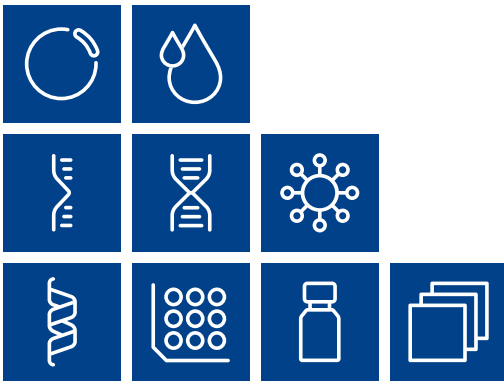
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Plasmid DNA  
Clean up  
RNA  
DNA  
Viral RNA and DNA  
Protein  
High throughput  
Accessories  
Auxiliary tools



# MACHEREY-NAGEL

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