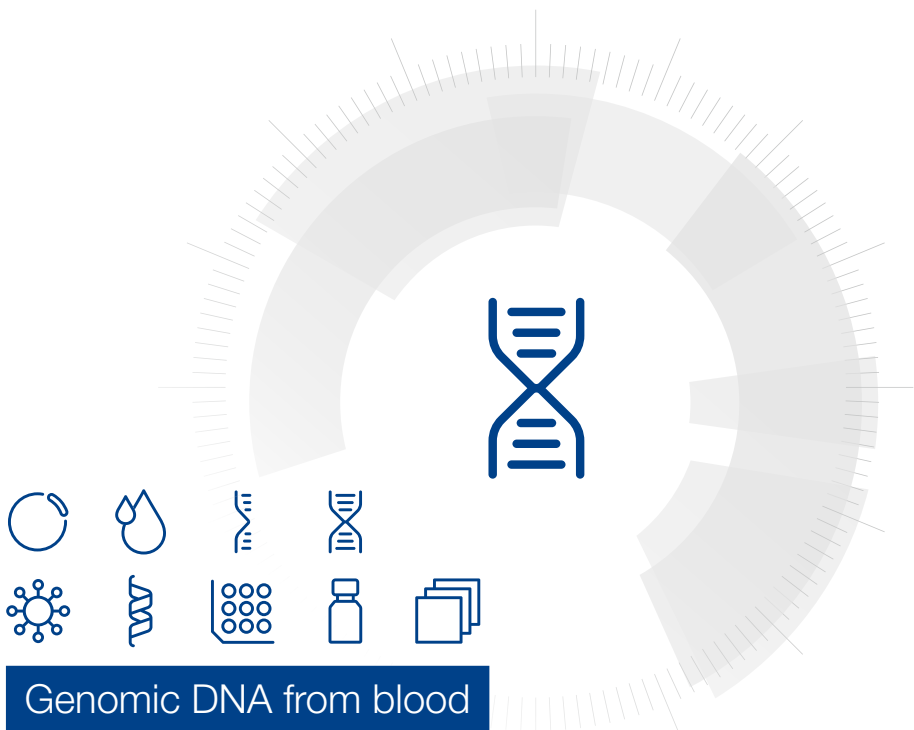


MACHEREY-NAGEL

# User manual



■ NucleoMag® Blood 200 µL

December 2015 / Rev. 04

## Contact MN

### Germany and international

MACHEREY-NAGEL GmbH & Co. KG  
Neumann-Neander-Str. 6-8 · 52355 Düren · Germany  
Tel.: +49 24 21 969-0  
Toll-free: 0800 26 16 000 (Germany only)  
E-mail: [info@mn-net.com](mailto:info@mn-net.com)

### Technical Support Bioanalysis

Tel.: +49 24 21 969-270  
E-mail: [tech-bio@mn-net.com](mailto:tech-bio@mn-net.com)

### USA

MACHEREY-NAGEL Inc.  
2850 Emrick Blvd. · Bethlehem, PA 18020 · USA  
Tel.: +1 484 821 0984  
Toll-free: 888 321 6224 (MACH)  
E-mail: [sales-us@mn-net.com](mailto:sales-us@mn-net.com)

### France

MACHEREY-NAGEL SARL à associé unique  
1, rue Gutenberg · 67722 Hoerdts · France  
Tel.: +33 388 68 22 68  
E-mail: [sales-fr@mn-net.com](mailto:sales-fr@mn-net.com)

### Switzerland

MACHEREY-NAGEL AG  
Hirsackerstr. 7 · 4702 Oensingen · Switzerland  
Tel.: +41 62 388 55 00  
E-mail: [sales-ch@mn-net.com](mailto:sales-ch@mn-net.com)

## Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Magnetic separation systems	7
2.4	Adjusting the shaker settings	8
2.5	Handling of beads	8
2.6	Elution procedures	9
3	Storage conditions and preparation of working solutions	11
4	Safety instructions	12
5	Protocol for the isolation of DNA from blood	14
6	Appendix	20
6.1	Troubleshooting	20
6.2	Ordering information	22
6.3	Product use restriction/warranty	22

# 1 Components

## 1.1 Kit contents

<b>NucleoMag® Blood 200 µL</b>		
<b>REF</b>	<b>1x 96 preps</b>	<b>4 x 96 preps</b>
	<b>744501.1</b>	<b>744501.4</b>
NucleoMag® B-Beads	2 x 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 x 50 mg
Proteinase Buffer PB	8 mL	15 mL
User manual	1	1

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

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

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

### Reagents

- 80% ethanol (for the washing step)

### Equipment/Consumables

Product	REF	Pack of
<ul style="list-style-type: none"> <li>• <b>Separation plate for magnetic beads separation,</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>Elution plate for collecting purified DNA,</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® Flex instrument:</b> KingFisher® Accessory Kit B (Square-well Blocks, Deep-well tip Combs, Elution Plates for 4 x 96 NucleoMag® Blood 200 µL preps using KingFisher® platform)</li> </ul>	740951	1 set
<ul style="list-style-type: none"> <li>• <b>For use of kit on KingFisher® Duo/Duo Prime instrument:</b> KingFisher® Duo Accessory Kit (KingFisher® Deep-well Blocks, KingFisher® Duo 12 Tip Combs, KingFisher® Duo Elution Strips for 8 x 12 NucleoMag® Blood 200 µL preps using KingFisher® Duo/Duo Prime platform)</li> </ul>	744952	1 set

## 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.

### 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** 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 µL.

**NucleoMag® Blood 200 µL** 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 µg of gDNA per 1 µL of NucleoMag® Blood Bead Suspension, 1 µL of suspension contains 140 µg of beads.

## 2.3 Magnetic separation systems

For use of **NucleoMag® Blood 200 µL**, 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)
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.\*

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

## 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  $\mu$ L 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  $\mu$ L dyed water to the wells of the collection plate and proceed as described above.

## 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

## 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.

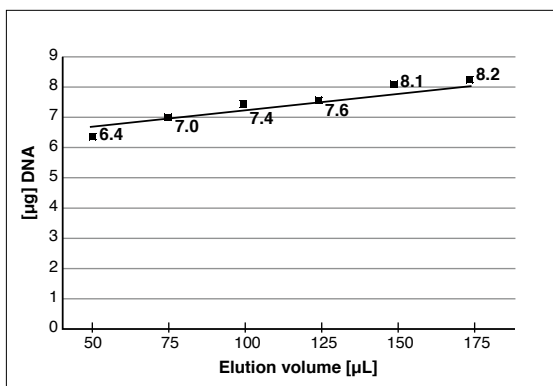
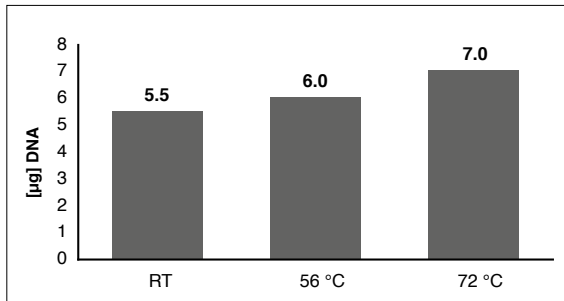


Figure 1: Influence of elution volume on DNA yield (example)

\* Depending on multichannel system

Elution is possible at room temperature. However, DNA yield can be increased by 15–20% if elution is performed at 72 °C (see Figure 2).



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

### 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 room temperature (18–25 °C) and are stable for up to one year.
- All buffers are delivered ready-to-use.

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.

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

## 4 Safety instructions





The following components of the **NucleoMag® Blood 200 µL** kits contain hazardous contents.

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

### GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

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

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
MBL1	Guanidine hydrochloride 50–66 % <i>Guanidinhydrochlorid 50–66 %</i>	 Danger <i>Gefahr</i>	302, 315, 319	280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313
MBL2	Sodium perchlorate 20–40 % + ethanol 35–55 % <i>Natriumperchlorat 20–40 % + Ethanol 35–55 %</i>	 Warning <i>Achtung</i>	226, 302	210, 233, 301+312, 330, 403+235
MBL3	Sodium perchlorate 5–20 % + ethanol 20–35 % <i>Natriumperchlorat 5–20 % + Ethanol 20–35 %</i>	 Warning <i>Achtung</i>	226	210, 233, 403+235
Proteinase K	Proteinase K, lyophilized <i>Proteinase K, lyophilisiert</i>	 Danger <i>Gefahr</i>	315, 319, 334, 335	261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 403+233

### Hazard phrases

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

## Hazard phrases

- H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.  
*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
- H 335 May cause respiratory irritation.  
*Kann die Atemwege reizen.*

## Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
- P 233 Keep container tightly closed.  
*Behälter dicht verschlossen halten.*
- P 261 Avoid breathing dust.  
*Einatmen von Staub vermeiden.*
- P 280 Wear protective gloves / eye protection.  
*Schutzhandschuhe / Augenschutz tragen.*
- P 301+312 IF SWALLOWED: Call a POISON CENTER/ doctor/.../if you feel unwell.  
*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /... anrufen.*
- P 302+352 IF ON SKIN: Wash with plenty of water/...  
*BEI KONTAKT MIT DER HAUT: Mit viel Wasser/... waschen.*
- P 304+340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.  
*BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
- P 305+351+338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing  
*BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.*
- P 312 Call a POISON CENTER/ doctor/.../if you feel unwell.  
*Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /... anrufen.*
- P 330 Rinse mouth.  
*Mund ausspülen.*
- P 332+313 IF skin irritation occurs: Get medical advice / attention.  
*Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 337+313 Get medical advice / attention.  
*Bei anhaltender Augenreizung: Ärztliche Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/...  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/... anrufen.*
- P 403+233 Store in a well ventilated place. Keep cool.  
*Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*
- P 403+235 Store in a well ventilated place. Keep cool.  
*Kühl an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ([www.mn-net.com](http://www.mn-net.com)).  
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ([www.mn-net.com](http://www.mn-net.com)).



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

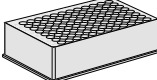
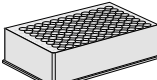

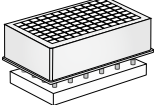

## 5 Protocol for the isolation of DNA from blood

### 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 19.

#### Before starting the preparation:

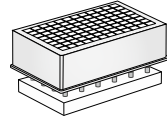
- Check if Proteinase K was prepared according to section 3.

<p>1 Lyse samples</p>	<p><b>Dispense 20 µL Proteinase K into Square-well Block</b></p> <p><b>200 µL blood</b></p> <p><b>80 µL MBL1</b></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> <hr/> <p><b>Mix by shaking for 5 min at RT</b> <i>(Optional: Mix by pipetting up and down)</i></p> <hr/> <p><b>Remove supernatant after 2 min separation</b></p>	    
<p>3 <b>Wash with MBL3 (1<sup>st</sup> wash)</b></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)*



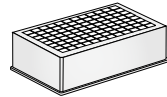
**Remove supernatant after 2 min separation**



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

Remove Square-well Block from NucleoMag® SEP

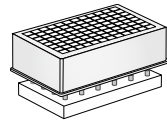
**800 µL MBL3**



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



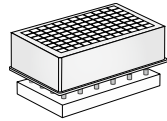
**Remove supernatant after 2 min separation**



5 **Wash with 80 % ethanol**

Remove Square-well Block from NucleoMag® SEP

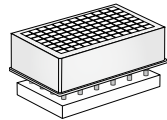
**800 µL 80 % ethanol**



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



**Remove supernatant after 2 min separation**



6 **Wash** with MBL4

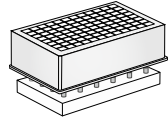
Leave Square-well Block  
on NucleoMag® SEP

**900 µL MBL4**

**Incubate for 45–90 s**

Aspirate and discard supernatant

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



7 **Elute** DNA

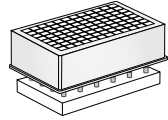
Remove Square-well Block  
from NucleoMag® SEP

**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.*

---

## 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.*

---

## 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.*

---

## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	<p><i>Elution buffer volume insufficient</i></p> <ul data-bbox="309 376 941 397" style="list-style-type: none"> <li>• Beads pellet must be covered completely with elution buffer</li> </ul>
	<p><i>Insufficient performance of elution buffer during elution step</i></p> <ul data-bbox="309 475 981 552" 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>
	<p><i>Beads dried out</i></p> <ul data-bbox="309 630 981 675" style="list-style-type: none"> <li>• Do not let the beads dry as this might result in lower elution efficiencies.</li> </ul>
	<p><i>Partial elution in Wash Buffer MBL4 already</i></p> <ul data-bbox="309 753 981 855" 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>
	<p><i>Aspiration of attracted bead pellet</i></p> <ul data-bbox="309 933 981 1010" 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>
	<p><i>Incubation after dispensing beads to lysate</i></p> <ul data-bbox="309 1088 981 1133" style="list-style-type: none"> <li>• Mix immediately after dispensing NucleoMag® B-Beads and Binding Buffer MBL2 to the lysate.</li> </ul>
Low purity	<p><i>Poor blood quality</i></p> <ul data-bbox="309 1211 981 1287" style="list-style-type: none"> <li>• 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.</li> </ul>
	<p><i>Insufficient washing procedure</i></p> <ul data-bbox="309 1366 981 1442" style="list-style-type: none"> <li>• Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP.</li> </ul>

<b>Problem</b>	<b>Possible cause and suggestions</b>
Suboptimal performance of DNA in downstream applications	<p><i>Carry-over of ethanol from ethanol wash step</i></p> <ul style="list-style-type: none"><li>• 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 on air-drying step.</li></ul> <p><i>Low purity</i></p> <ul style="list-style-type: none"><li>• See above</li></ul>
Carry-over of beads	<p><i>Time for magnetic separation too short</i></p> <ul style="list-style-type: none"><li>• Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.</li></ul> <p><i>Aspiration speed too high (elution step)</i></p> <ul style="list-style-type: none"><li>• High aspiration speed during the elution step may cause bead carry over. Reduce aspiration speed for elution step.</li><li>• To remove magnetic beads from the eluates, put the elution plate on the magnetic separator and aspirate the supernatant after sufficient beads separation.</li></ul>
Cross contamination	<p><i>Contamination of the rims</i></p> <ul style="list-style-type: none"><li>• 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.</li></ul>

## 6.2 Ordering information

Product	REF	Pack of
NucleoMag® Blood 200 µL	744501.1 744501.4	1 x 96 preps 4 x 96 preps
NucleoMag® SEP	744900	1
Square-well Blocks	740481.4 740481.24	4 24
Elution Plates U-bottom	740486.24	24
Self-adhering PE Foil	740676	50 sheets
KingFisher® Accessory Kit B (set consists of Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® Blood 200 µL preps using KingFisher® platform)	744951	1 set
KingFisher® Duo Accessory Kit (set consists of KingFisher® Deep-well Blocks, KingFisher® Duo 12 Tip Combs, KingFisher® Duo Elution Strips for 8 x 12 NucleoMag® Blood 200 µ preps using KingFisher® Duo/Duo Prime platform)	744952	1 set

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

## 6.3 Product use restriction/warranty

**NucleoMag® Blood 200 µL** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective

Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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

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

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

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

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

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

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

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

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent 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 the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications 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.

Last updated: 07/2010, Rev. 03

Please contact:  
MACHEREY-NAGEL GmbH & Co. KG  
Tel.: +49 24 21 969-270  
tech-bio@mn-net.com

---

**Trademarks:**

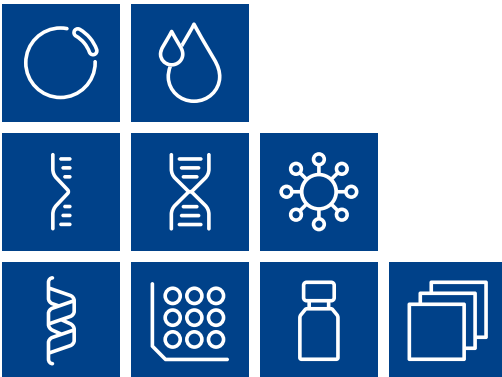
KingFisher® is a registered trademark of Thermo Fisher Scientific  
NucleoMag® is a registered trademark of MACHEREY-NAGEL GmbH & Co KG  
Te-MagS™ is a trademark of Tecan Group Ltd., Switzerland

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.









Plasmid DNA

Clean up

RNA

DNA

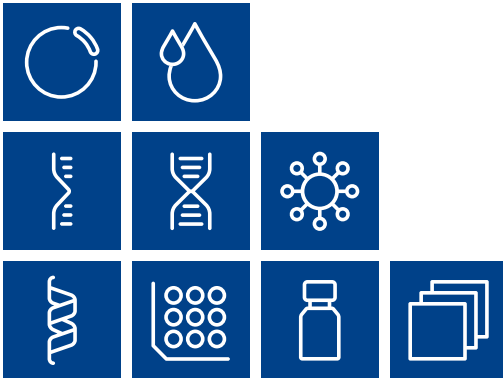
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



[www.mn-net.com](http://www.mn-net.com)

**MACHEREY-NAGEL**



MACHEREY-NAGEL GmbH & Co. KG  
Neumann-Neander-Str. 6-8  
52355 Düren · Germany

DE Tel.: +49 24 21 969-0  
CH Tel.: +41 62 388 55 00  
FR Tel.: +33 388 68 22 68  
US Tel.: +1 484 821 0984

info@mn-net.com  
sales-ch@mn-net.com  
sales-fr@mn-net.com  
sales-us@mn-net.com

A039476/1200.3