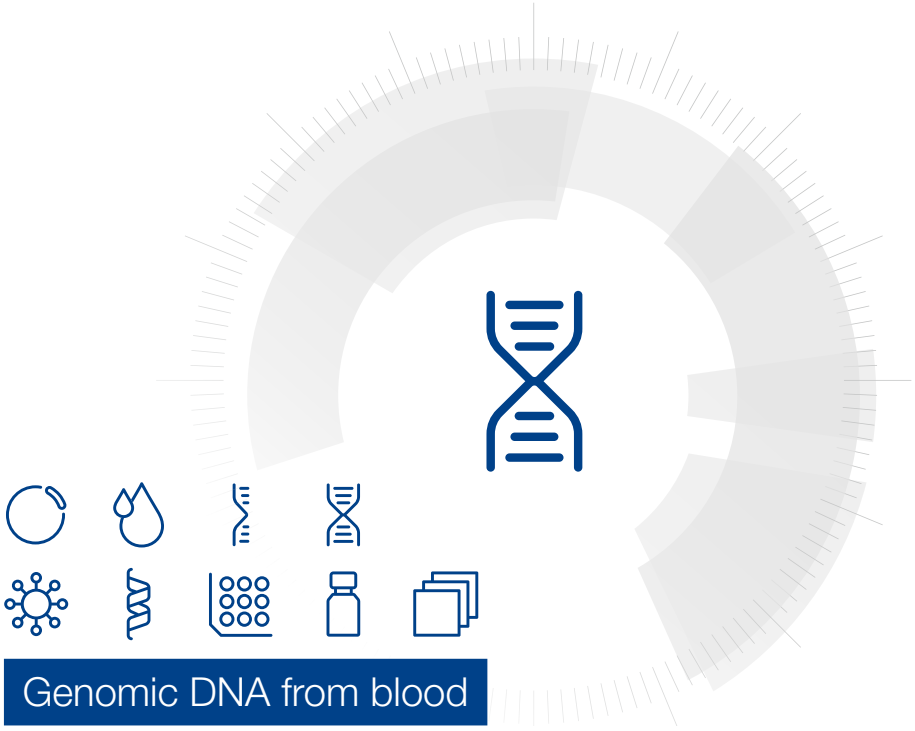
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from blood

■ NucleoMag® Blood 3 mL

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1 Components

1.1 Kit contents

	NucleoMag® Blood 3 mL
REF	**1x 96 preps 744502.1**
NucleoMag® B-Beads	18 mL
Lysis Buffer MBL1	125 mL
Binding Buffer MBL2	500 mL
Wash Buffer MBL3	1000 mL
Wash Buffer MBL4	500 mL
Elution Buffer MBL5*	125 mL
Proteinase K, lyophilized**	12 x 75 mg
Proteinase Buffer PB	2 x 35 mL
User manual	1

1.2 Material to be supplied by the user for use on KingFisher Flex

Reagents

- 80% ethanol (for the washing step)

Equipment / Consumables

Product

- **Magnetic separator,**
  e.g., KingFisher® Flex 24 instrument

- **Separation plates, elution plates**,
  e.g., KingFisher® 24 Deep-well Plates

- **Tip combs,**
  e.g., KingFisher® 24 well Tip Comb

* Elution Buffer MBL5: 5 mM Tris, pH 8.5

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

2 Product description

2.1 The basic principle

The **NucleoMag® Blood 3 mL** 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 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. **NucleoMag® Blood 3 mL** is recommended for use on KingFisher® Flex 24 instrument.

2.2 Kit specifications

The **NucleoMag® Blood 3 mL** kit is made for isolation of genomic DNA from blood samples. This kit provides reagents and magnetic beads for isolation of genomic DNA from 96 samples of up to 3 mL. The purified DNA can be used directly as template for PCR, blotting, or any kind of enzymatic reactions.

The kit provides reagents for the purification of up to 100–130 µg of pure genomic DNA from 3 mL whole blood with an A_{260}/A_{280} ratio ≥ 1.6–1.9.

Fresh, frozen, or blood treated either with EDTA or citrate can be used.

NucleoMag® Blood 3 mL kit can be processed completely at room temperature. Elution at 55 °C will increase the yield by about 15–20 %.

NucleoMag® B-Beads are highly reactive, superparamagnetic beads with a high binding capacity.

NucleoMag® Blood 3 mL kit has been developed for use with ThermoFisher's KingFisher® Flex 24 instrument. A script is available on request from MACHEREY-NAGEL. The maximum sample volume of 3 mL is splitted into two aliquots of 1.5 mL each.

For processing smaller blood sample volumes, use of liquid handling robots other than the KingFisher® Flex 24 or manual extraction, please inquire with MN technical support for details.

For smaller blood sample volumes, MN offers the NucleoMag® Blood 200 µL kit (see ordering information, section 7.2).

2.3 Elution procedures

Purified genomic DNA can be eluted directly with the supplied Elution Buffer MBL5. Elution can be carried out in a volume of > 1 mL. Smaller elution buffer volumes may result in incomplete bead separation. For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer.

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 3 mL** 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 3 mL** 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.

	NucleoMag® Blood 3 mL
REF	**1 x 96 preps** **744502.1**
Proteinase K	Add 3.75 mL Proteinase Buffer PB to each vial

4 Safety instructions

The following components of the NucleoMag® Blood 3 mL kits contain hazardous contents.

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

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

Component ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**GHS symbol** ***GHS-Symbol***	**Hazard phrases** ***H-Sätze***	**Precaution phrases** ***P-Sätze***
MBL1	guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %* CAS 50-01-1	 WARNING *ACHTUNG*	302, 315, 319	264W, 280sh, 301+312, 330
MBL2, MBL3	Ethanol 35–55 % and sodium perchlorate 15–40 % *Ethanol 35–55 % und Natriumperchlorat 15–40 %* CAS 64-17-5, 7601-89-0	 WARNING *ACHTUNG*	226, 302	210, 264W, 301+312, 330
Proteinase K	Proteinase K 90–100 % *Proteinase K 90–100 %* CAS 39450-01-6	 DANGER *GEFAHR*	315, 319, 334	261sh, 280sh, 342+311

Hazard phrases

H 226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.

H 302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

H 315 Causes skin irritation.
Verursacht Hautreizungen.

H 319 Causes serious eye irritation.
Verursacht schwere Augenreizung.

H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.

Precaution phrases

P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.

P 261sh Avoid breathing dust/vapors.
Einatmen von Staub/Dampf vermeiden.

P 264W	Wash with water thoroughly after handling. *Nach Gebrauch mit Wasser gründlich waschen.*
P 280sh	Wear protective gloves/eye protection. *Schutzhandschuhe/Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER / doctor / … / if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt / … anrufen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / … *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM/Arzt / … anrufen.*

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

5 Protocol for the isolation of genomic DNA from 3 mL blood samples using KingFisher® Flex 24

The script necessary to run the NucleoMag® Blood 3 mL kit on the KingFisher® Flex 24 is available through MN technical support.

Before starting the preparation:

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

1 Lyse sample

Prepare KingFisher® 24 Deep-well Plate with buffers (label deep-well blocks before use).

Wash and elution buffers

Fill **1 mL Elution Buffer MBL5** to each well of an empty KingFisher® 24 Deep-well Plate.

Fill **4.8 mL Wash Buffer MBL4** to each well of an empty KingFisher® 24 Deep-well Plate.

Fill **4.8 mL 80 % ethanol** to each well of an empty KingFisher® 24 Deep-well Plate.

Fill **4.8 mL Wash Buffer MBL3** to each well of an empty KingFisher® 24 Deep-well Plate.

Fill **4.8 mL Wash Buffer MBL3** to each well of a second empty KingFisher® 24 Deep-well Plate.

Fill **150 µL of Proteinase K working solution** to each well of the two lysis plates (KingFisher® 24 deep-well plates).

Samples

Please note that 3 mL blood samples have to be split and distributed into two plates (1.5 mL for each plate)!

Fill **1.5 mL blood sample** to a well of the lysis plate (KingFisher® 24 Deep-well Plate with 150 µL Proteinase K per well). Fill **1.5 mL blood** of the same sample to the well at the same position of the second lysis plate.

Make sure that one sample is distributed into the same position of each Deep-well Plate (e.g., sample 1 to position A1 of lysis plate 1 and position A1 of lysis plate 2; sample 2 to position A2 of lysis plate 1 and position A2 of lysis plate 2, etc.)

After adding the samples add **575 µL Buffer MBL1** to each well of the two lysis plates.

2 Start isolation on King Fisher® Flex 24 instrument

Start method “NucleoMag®_Blood_3mL” (method is available from MN on request).

Insert plates as indicated on the KingFisher® instrument display.

Method starts with a mixing step (sample lysis) after setting up the last plate to the instrument.

After mixing steps for lysis (approx. 10 min) the instrument will ask for addition of Buffer MBL2 and NucleoMag® B-Beads.

3 Addition of Binding Buffer MBL2 and NucleoMag® B-Beads to lysis plate 1

Add **2.3 mL Buffer MBL2** and **150 µL NucleoMag® B-Beads** to each well of the lysis plate 1.

Mix up NucleoMag® B-Beads before use.

Return lysis plate 1 to the instrument and continue.

4 Addition of Binding Buffer MBL2 to lysis plate 2

Add **2.3 mL Buffer MBL2** to each well of the lysis plate 2.

Return lysis plate 2 to the instrument and continue.

All further steps are now processed without further user interaction.

5 Remove eluted DNA

The instrument stops after the final elution step. Follow the instructions on instrument display and unload the plates from the instrument.

Purified DNA should be centrifuged before UV measurement!

6 Support protocol - DNA from 1 mL whole blood

This support protocol describes the process of manually isolating DNA from 1 mL whole blood samples using a scaled down protocol of the NucleoMag® Blood 3 mL Kit. In order to process this kit manually a magnet for magnetic beads seperation e.g. NucleoMag® SEP 24 (REF 744903) and a suitable 24-well deep well plate is needed.

Procedure

1 Lyse samples

Dispense 100 µL Proteinase K and 1 mL blood to each well of a 24-Square-well block.

Add 400 µL Buffer MBL1 to each well of a 24-Square-well block and mix by shaking for 5–10 min at room temperature.

Note: Prepare Proteinase K working solution according to the NucleoMag® Blood 3 mL user manual section 3. Blood samples should be equilibrated to room temperature before lysis.

2 Bind DNA to NucleoMag® B Beads

Dispense 100 µL NucleoMag® B Beads and 1.5 mL Buffer MBL2 to each well of a 24-Square-well block 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: 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 24-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 (1st wash)

Remove the 24-Square-well block from the magnetic separator.

Add 1600 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 against the side of the wells by placing the 24-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.

4 Wash with MBL3 (2nd wash)

Remove the 24-Square-well block from the magnetic separator.

Add 1600 µL Buffer MBL3 to each well for a second wash step with Buffer MBL3. Resuspend the bead/DNA complex by shaking at room temperature until the beads are resuspended com-pletely (5 min). Alternatively, resuspend the beads by pipetting up and down (15 times).

Separate the magnetic beads against the side of the wells by placing the 24-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: Supernatant should be colorless, magnetic bead pellet is clearly visible.

5 Wash with 80 % ethanol

Remove the 24-Square-well block from the magnetic separator.

Add 1600 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 24-Square-well block on the magnetic separator. Wait for at least 2 min until all the beads have been attrected to the magnet. Remove and discard supernatant by pipetting.

6 Wash with MBL4

Leave 24-Square-well block on the magnetic separator.

Gently add 1600 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 24-Square-well block from the magnetic separator.

Add desired volume of Buffer MBL5 (500–1000 µL) to each well of the 24-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 24-Square-well block on the magnetic separator. Wait for at least 2 min until all the beads have been attrected 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

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Elution buffer volume insufficient* • Beads 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 subsequent washing and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiency. *Partial elution in Wash Buffer MBL4 already* • Do not resuspend beads in Buffer MBL4 and do not incubate beads in this buffer for more than 2 min, as this buffer is water-based and might elute the DNA already. *Incubation after dispensing beads to lysate* • Mix immediately after dispensing NucleoMag® B-Beads and Binding Buffer MBL2 to the lysate.
Low purity	*Poor blood quality* • Be sure that no blood clots are transferred to the lysis plates. Blood can be stored at 2–8 °C for two weeks. Freeze samples if stored for longer periods. *Incomplete magnetic bead separation* • High amounts of eluted DNA increase the viscosity and prevent the beads from being attracted completely to the magnets. Increase elution buffer volume.
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. Typically washing the beads in Buffer MBL4 is sufficient to remove ethanol. However, if necessary include a 10 min airdrying step following the Buffer MBL4 wash step. *Low purity* • See above

Problem	Possible cause and suggestions
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnets. *Incomplete magnetic bead separation* • High amounts of eluted DNA increase the viscosity and prevent the beads from being attracted completely to the magnets. Increase elution buffer volume.
Cross contamination	*Overfilling of wells from the 24-well separation plate* • Do not overfill the wells of the separation plates to avoid cross contamination by splashing.

7.2 Ordering information

Product	REF	Pack of
NucleoMag® Blood 3 mL	744502.1	1 x 96 preps
NucleoMag® Blood 200 µL	744501.1 744501.4	1 x 96 preps 4 x 96 preps

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

7.3 Product use restriction / warranty

NucleoMag® Blood 3 mL kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

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

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

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

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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.

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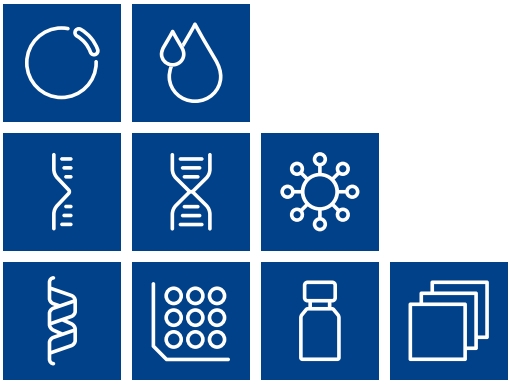

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RNA
DNA
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Protein
High throughput
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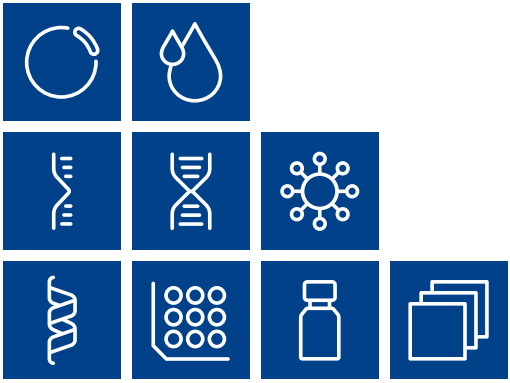

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