



Genomic DNA from blood

User manual

NucleoMag[®] Blood 3 mL

March 2018 / Rev. 04

Table of contents

1 Components	4
1.1 Kit contents	4
1.2 Material to be supplied by the user	4
2 Product description	5
2.1 The basic principle	5
2.2 Kit specifications	5
2.3 Elution procedures	5
3 Storage conditions and preparation of working solutions	6
4 Safety instructions	7
5 Protocol for the isolation of genomic DNA from 3 mL blood samples using KingFisher® Flex 24	9
6 Appendix	11
6.1 Troubleshooting	11
6.2 Ordering information	12
6.3 Product use restriction/warranty	13

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

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 6.2).

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	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	P-Sätze
MBL1	guanidine hydrochloride 50–66 % <i>Guanidinhydrochlorid 50–66 %</i> CAS 50-01-1	 WARNING ACHTUNG	302, 315, 319	264W, 280sh, 301+312, 330
MBL2, MBL3	Ethanol 35–55 % and sodium perchlorate 20–40 % <i>Ethanol 35–55 % und Natriumperchlorat 20–40 %</i> CAS 64-17-5, 7601-89-0	  WARNING ACHTUNG	226, 302	210, 264W, 301+312, 330
Proteinase K	Proteinase K 90–100 % <i>Proteinase K 90–100 %</i> CAS 39450-01-6	  DANGER GEFAHR	315, 319, 334	261sh, 280sh, 342+311

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

Precaution phrases

P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. <i>Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.</i>
P 261sh	Avoid breathing dust/vapors. <i>Einatmen von Staub/Dampf vermeiden.</i>

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



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 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	<i>Elution buffer volume insufficient</i>
	Beads pellet must be covered completely with elution buffer.
	<i>Insufficient performance of elution buffer during elution step</i>
	Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of subsequent washing and elution steps.
	<i>Beads dried out</i>
Low purity	Do not let the beads dry as this might result in lower elution efficiency.
	<i>Partial elution in Wash Buffer MBL4 already</i>
	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.
Suboptimal performance of DNA in downstream applications	<i>Incubation after dispensing beads to lysate</i>
	Mix immediately after dispensing NucleoMag® B-Beads and Binding Buffer MBL2 to the lysate.
	<i>Poor blood quality</i>
Low purity	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.
	<i>Incomplete magnetic bead separation</i>
Suboptimal performance of DNA in downstream applications	High amounts of eluted DNA increase the viscosity and prevent the beads from being attracted completely to the magnets. Increase elution buffer volume.
	<i>Carry-over of ethanol from ethanol wash step</i>
	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.
Suboptimal performance of DNA in downstream applications	<i>Low purity</i>
	See above

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

6.2 Ordering information

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

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

6.3 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.

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

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



www.mn-net.com

MACHEREY-NAGEL



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

DE/International:

Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:

Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:

Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:

Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com



A039595/0380.15