



Plesmanlaan 1d
2333 BZ Leiden
The Netherlands
T. +31 (0)71 568 10 00
T. Belgium: 0800 71640
F. +31 (0)71 568 10 10
info@bioke.com
www.bioke.com

Genomic DNA from Blood

User Manual

NucleoMag[®] Blood 3 mL

September 2010/Rev.01

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	6
3	Storage conditions and preparation of working solutions	7
4	Safety instructions – risk and safety phrases	8
5	Standard procedure 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	550 mL
Wash Buffer MBL3	1 000 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

- Magnetic separator
KingFisher® Flex 24 instrument
- Separation plates, elution plates
KingFisher® 24 deep well plates
- KingFisher® 24 well tip comb
- 80% ethanol (for the washing step)

* 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 $\geq 1.6 - 1.9$.

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).

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!

- 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 – risk and safety phrases

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.

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
MBL1	Guanidinium hydrochloride	✘ Xn*	Harmful if swallowed - Irritating to eyes and skin	R 22-36/38	S 22
MBL2	Sodium perchlorate + ethanol <50%	*	Flammable	R 10	
MBL3	Sodium perchlorate <15% + ethanol <24%	*	Flammable	R 10	
Proteinase K	Proteinase K, lyophilized	✘ Xn Xi**	Irritating to eyes, respiratory system, and skin - May cause sensitization by inhalation	R 36/37/38-42	S 22-24-26-36/37

Risk phrases

R 10	Flammable
R 22	Harmful if swallowed
R 36/37/38	Irritating to eyes, respiratory system, and skin
R 36/38	Irritating to eyes and skin
R 42	May cause sensitization by inhalation

Safety phrases

S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 36/37	Wear suitable protective clothing and gloves

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

**Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 Standard procedure 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.

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 Thermo KingFisher® 24 deep well plate.

Fill **4.8 mL Wash Buffer MBL4** to each well of an empty Thermo KingFisher® 24 deep well plate.

Fill **4.8 mL 80 % ethanol** to each well of an empty Thermo KingFisher® 24 deep well plate.

Fill **4.8 mL Wash Buffer MBL3** to each well of an empty Thermo KingFisher® 24 deep well plate.

Fill **4.8 mL Wash Buffer MBL3** to each well of a second empty Thermo KingFisher® 24 deep well plate.

Fill **150 µL of Proteinase K working solution** to each well of the two lysis plates (Thermo 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 (Thermo 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	<p><i>Elution buffer volume insufficient</i></p> <ul style="list-style-type: none"> • Beads pellet must be covered completely with elution buffer.
	<p><i>Insufficient performance of elution buffer during elution step</i></p> <ul style="list-style-type: none"> • Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of subsequent washing and elution steps.
	<p><i>Beads dried out</i></p> <ul style="list-style-type: none"> • Do not let the beads dry as this might result in lower elution efficiency.
	<p><i>Partial elution in Wash Buffer MBL4 already</i></p> <ul style="list-style-type: none"> • 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.
Low purity	<p><i>Incubation after dispensing beads to lysate</i></p> <ul style="list-style-type: none"> • Mix immediately after dispensing NucleoMag® B-Beads and Binding Buffer MBL2 to the lysate.
	<p><i>Poor blood quality</i></p> <ul style="list-style-type: none"> • 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.
	<p><i>Incomplete magnetic bead separation</i></p> <ul style="list-style-type: none"> • High amounts of eluted DNA increase the viscosity and prevent the beads from being attracted completely to the magnets. Increase elution buffer volume.

Problem	Possible cause and suggestions
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"> • 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.
	<p><i>Low purity</i></p> <ul style="list-style-type: none"> • See above
Carry-over of beads	<p><i>Time for magnetic separation too short</i></p> <ul style="list-style-type: none"> • Increase separation time to allow the beads to be completely attracted to the magnets.
	<p><i>Incomplete magnetic bead separation</i></p> <ul style="list-style-type: none"> • 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	<p><i>Overfilling of wells from the 24-well separation plate</i></p> <ul style="list-style-type: none"> • 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 were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY**. They are suitable **FOR IN-VITRO USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoMag® Blood 3 mL** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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 an extra copy.

MACHEREY-NAGEL does not warrant against 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; against defects in products or components not manufactured by MACHEREY-NAGEL, or against 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.

Please contact:

MACHEREY-NAGEL Germany

Tel.: +49 (0) 24 21 969 270

e-mail: TECH-BIO@mn-net.com

Last updated: 12/2006, Rev.02

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 or services we can not grant any guarantees regarding selection, efficiency, or operation.