Genomic DNA from Blood

User Manual

NucleoMag® Blood 3 mL

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoMag® Blood 3 mL
	1x 96 preps
REF	**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
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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 ≥ 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
	1 x 96 preps
REF	**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	*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.

Problem	Possible cause and suggestions
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
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.

6.2 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.

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.

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