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Genomic DNA from Blood

User Manual

NucleoMag® Blood 200 µL

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoMag® Blood 200 µL	
	1x 96 preps	**4 x 96 preps**
REF	**744501.1**	**744501.4**
NucleoMag® B-Beads	3 mL	12 mL
Lysis Buffer MBL1	10 mL	40 mL
Binding Buffer MBL2	40 mL	160 mL
Wash Buffer MBL3	200 mL	800 mL
Wash Buffer MBL4	125 mL	500 mL
Elution Buffer MBL5*	25 mL	100 mL
Proteinase K, lyophilized**	50 mg	4 x 50 mg
Proteinase Buffer PB	3.6 mL	15 mL
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* Elution Buffer MBL5: 5 mM Tris, pH 8.5

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

1.2 Material to be supplied by user

Consumables

- 80% ethanol (for the washing step)

Equipment

Product	REF	Pack of
• **Separation plate for magnetic beads separation,** e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
• **Elution plate for collecting purified DNA**, e.g., Elution Plate, U-bottom (96-well 0.3 mL microtiterplate with 300 µL U-bottom wells)	740486.24	24
• **For use of kit on KingFisher 96 and KingFisher Flex instrument:** KingFisher® 96 Accessory Kit B (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® 96 Blood 200 µL preps using KingFisher® 96 platform)	740951	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 ≥ 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. See suppliers ordering information for suitable separation plates.

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

Separators with moving magnets, for example Thermo Fisher Scientific KingFisher® instruments: 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.

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 µL 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 µL 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

* 8-channel pipetting device

2.6 Elution procedures

Purified total RNA can be eluted directly with the supplied Elution Buffer MBL5. Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® 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.

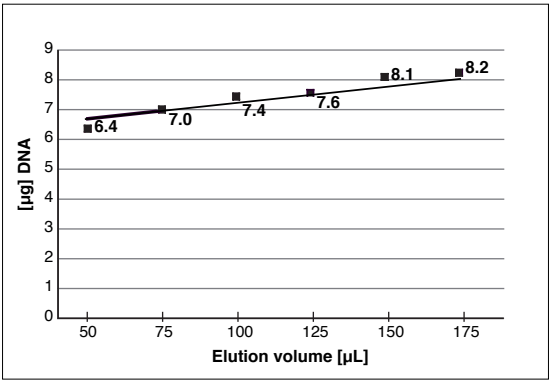


Figure 1: Influence of elution volume on DNA yield (example)

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).

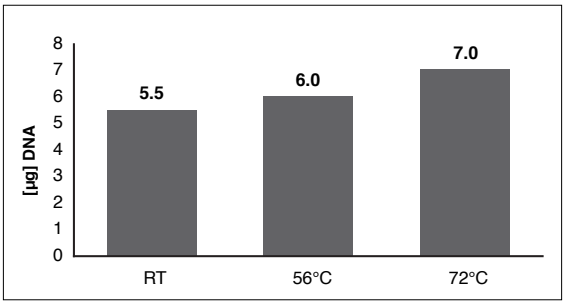


Figure 2: Influence of elution temperature on DNA yield

3 Storage conditions and preparation of working solutions

Attention:

Buffers MBL1, MBL2, and MBL3 contain chaotropic salt! Wear gloves and goggles!

- 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.

	NucleoMag® Blood 200 µL	
	1 x 96 preps	**4 x 96 preps**
REF	**744501.1**	**744501.4**
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 – risk and safety phrases

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.

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
MBL1	Guanidinium hydrochloride <45%	✖ Xn*	Harmful if swallowed - Irritating to eyes and skin	R 20-36/38	S 22
MBL2	Sodium perchlorate <30% + 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

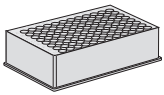
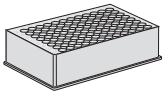
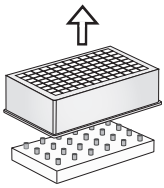
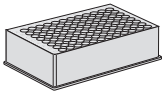
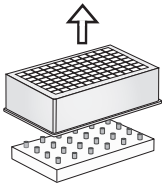
* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

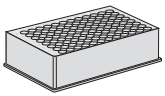
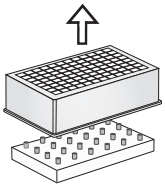
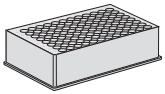
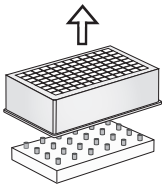
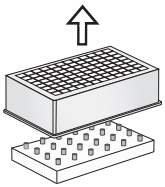
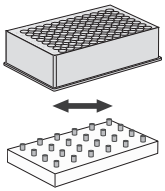
** Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

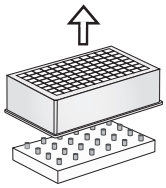
5 Procedure

5.1 General procedure

For details on each step see section 5.2.

1	Sample lysis	**Dispense 20 µL Proteinase K into Square-well Block** **200 µL blood** **80 µL MBL1** **Mix 3 – 5 times** **Shake 10 min at RT**	
2	Binding of DNA to NucleoMag® B-Beads	**25 µL B-Beads** **300 µL MBL2**	
		Mix by shaking for 5 min at RT	
		Remove supernatant after 2 min separation	
3	1st MBL3 wash	**800 µL MBL3**	
		Shake 5 min at RT	
		Remove supernatant after 2 min separation	

4	2nd MBL3 wash	**800 µL MBL3**	
		Shake 5 min at RT	
		Remove supernatant after 2 min separation	
5	Ethanol wash	**800 µL ethanol (80%)**	
		Shake 5 min at RT	
		Remove supernatant after 2 min separation	
6	MBL4 wash	**900 µL MBL4** **Incubate 45 – 90 s** ***Note: Do not resuspend beads in Buffer MBL4!*** **Remove supernatant**	
7	Elution	**50 – 100 µL MBL5** ***(Optional: Mix by pipetting up and down)*** **Transfer eluted DNA after 2 min separation**	

Shake 5 – 10 min, RT (*Optional: 55 – 72°C)*	
Transfer supernatant after 2 min separation into elution plate	

5.2 Protocol for the isolation of DNA from blood

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers. It is recommended using a Square-well Block for separation (see ordering information). 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.

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1 Lyse sample

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.

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2 Binding of 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 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.

Remove the Square-well Block from the magnetic separator.

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 1st MBL3 wash

Add **800 µL Buffer MBL3** to each well and wash the bead/DNA complex by **shaking** (5 min) at **room temperature**.

Alternatively, pipette 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 all of the magnetic beads against the side of the well by placing the Square-well Block on the magnetic separator. Aspirate and discard the supernatant.

Remove the Square-well Block from the magnetic separator.

Note: Supernatant has a brownish color, magnetic bead pellet is now visible.

4 2nd MBL3 wash

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, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the Square-well Block on the magnetic separator. Aspirate and discard the supernatant.

Remove the Square-well Block from the magnetic separator.

Note: Supernatant is colorless, magnetic bead pellet is clearly visible.

5 Ethanol wash

Add **800 µL 80% ethanol** to each well and wash the bead/DNA complex by **shaking** (5 min) at **room temperature**.

Alternatively, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the Square-well Block on the magnetic separator. Aspirate and discard the supernatant.

Note: Supernatant is colorless, magnetic bead pellet is visible now.

6 MBL4 wash

Leave Square-well Block on the NucleoMag® SEP separator.

Slowly 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 Elution

Add desired volume of **Buffer MBL5 (50 – 100 µL)** to each well and resuspend the bead/DNA complex by **shaking (5 – 10 min)**.

Alternatively, pipette up and down 10 times and incubate 5 – 10 min.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the magnetic separator. Wait 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: The DNA yield can be increased by 15 – 20% by using prewarmed 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	*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 wash steps and elution step. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Partial elution in Wash Buffer MBL4 already* • 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. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *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 well. Blood can be stored at 2 – 8°C for two weeks. Freeze samples if stored for longer periods. *Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example Square-well Block in combination with NucleoMag® SEP.

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. Use of Buffer MBL4 or introduce on air-drying 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 magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry over. Reduce aspiration speed for elution step. • To remove magnetic beads from the eluates, put the elution plate on the magnetic separator and aspirate the supernatant after sufficient beads separation.
Cross contamination	*Contamination of the rims* • 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.

6.2 Ordering information

Product	Cat. No.	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

Product	Cat. No.	Pack of
Elution Plates U-bottom	740486.24	24
Self-adhering PE Foil	740676	50 sheets
KingFisher® 96 Accessory Kit B Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® Blood 200 µL preps using KingFisher® 96 platform	744951	1 set

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

6.3 Product use restriction / warranty

NucleoMag® Blood 200 µL 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 200 µL** 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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