Genomic DNA from Blood

User manual

NucleoMag 96 Blood

February 2003/Rev. 01

MACHEREY-NAGEL

Table of contents

1 Kit contents

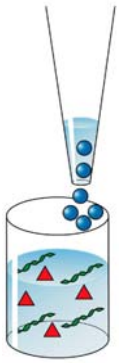
	NucleoMag 96 Blood		
Cat. No.	**1 x 96 preps 744 500.1**	**4 x 96 preps 744 500.4**	**24 x 96 preps 744 500.24**
NucleoMag B-Beads	1.68 ml	6.7 ml	40.3 ml
Lysis Buffer MB1	15 ml	60 ml	360 ml
Binding Buffer MB2	40 ml	2 x 80 ml	8 x 120 ml
Wash Buffer MB3	66 ml	264 ml	2 x 792 ml
Wash Buffer MB4	66 ml	264 ml	2 x 792 ml
Wash Buffer MB5	102 ml	408 ml	3 x 816 ml
Elution Buffer MB6	12 ml	48 ml	288 ml
Elution plate, U-bottom (including one Self-adhering PE foil)	1	4	24
Protocol	1	1	1

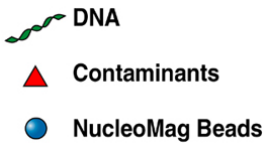
Material to be supplied by user:
Separation plate, e.g. MN Square-well Block (see ordering information).

2 Product description

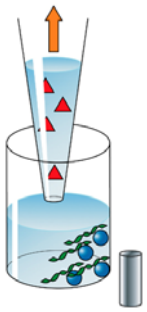
2.1 The basic principle

The **NucleoMag 96 Blood** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Whole blood is lysed with Lysis Buffer MB1 without a proteinase K digestion. Adjusting the binding conditions of nucleic acid with Binding Buffer MB2 and addition of paramagnetic beads can be carried out simultaneously. 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 MB5. Finally, highly purified DNA is eluted with low-salt Elution Buffer MB6 and can directly be used for downstream applications. The **NucleoMag 96 Blood** kit can be used either manually or automated on standard liquid handling instruments.

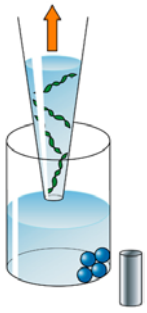




Whole blood is lysed with Lysis Buffer MB1. Binding conditions are adjusted and the NucleoMag Blood Beads are added to the sample.



DNA is bound to the NucleoMag Blood Beads. Beads are held back in the well while contaminants are washed away.



DNA is eluted from the beads and recovered, while beads are held back in the well by the magnet. DNA is ready-to-use in downstream applications.

2.2 Kit specifications

NucleoMag 96 Blood is designed for rapid manual and automated small-scale preparation of highly pure genomic DNA from 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 96 Blood allows easy automation on common liquid handling 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 1 – 4 µg of pure genomic DNA from 100 µl whole blood with an $A_{260/280}$ ratio ≥ 1.6 – 1.9 and typical concentration of 20 – 40 ng/µl. Depending on health status of the donor and the elution volume used concentrations of 10 – 160 ng/µl can be obtained.

Fresh, frozen or blood treated either with EDTA, citrate, or heparin can be used. The procedure is optimized for a sample volume of 100 µl.

NucleoMag 96 Blood can be processed completely at room temperature. Elution at 55°C will increase the yield by about 15 – 20%.

NucleoMag Blood Beads are highly reactive, superparamagnetic beads. The binding capacity is 0.4 µg of gDNA per 1 µl of NucleoMag Blood-Bead Suspension, 1 µl of suspension contains 130 µg of beads.

2.3 Magnetic separation systems

For use of **NucleoMag 96 Blood** the NucleoMag SEP is recommended. Separation is carried out in a MN 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 (Cat. No. 744 900)	Square-well Block (MN, Cat. No. 740 670)
Promega MagnaBot	Square-well Block (MN, Cat. No. 740 670)
Tecan Te-MagS	1.5 ml tubes without lid (Sarstedt)

Static magnetic pins
Separators with static magnetic pins, e.g. NucleoMag SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a plate shaker, e.g. H+P Variomag® Teleshake (H+P Labortechnik AG, Bruckmannring 28, D-85764 Oberschleißheim, Germany, www.hp-lab.de), 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, e.g. 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.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps the speed settings have to be checked carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

- Apply 600 µl dyed water (select desired elution buffer volume) to the wells of the separation plate. Position 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 and elution step.

2.5 Handling of beads

Distribution of beads
A homogenous 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 vortex shortly. Premixing magnetic beads with the binding buffer 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, 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 on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

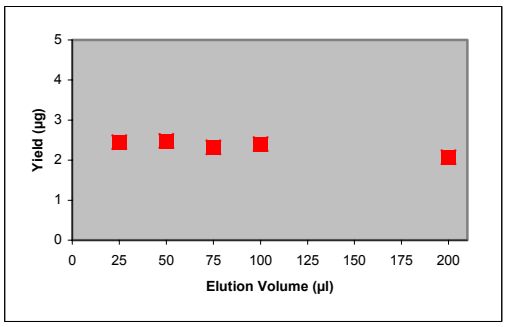
Washing the beads
Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension Efficiency	Speed	Small elution volume possible	Number of tips needed
Magnetic mix	+	++	+	Low
Shaker	++	++	+++	Low
Pipetting	+++	+*	++	High

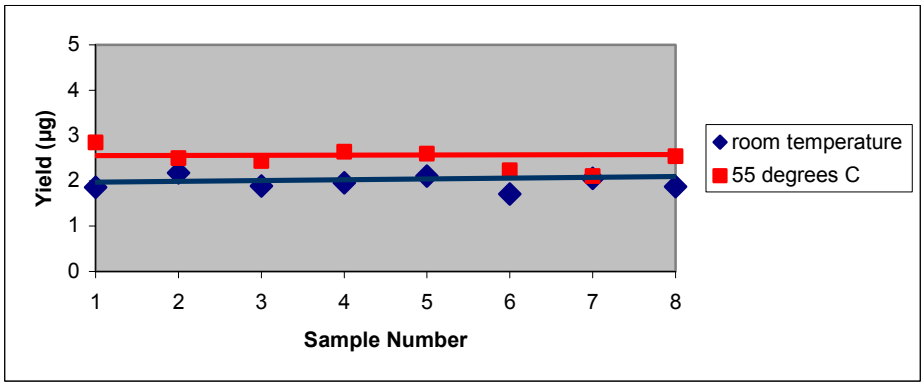
* 8-channel pipetting device

2.6 Elution procedures

Purified genomic DNA can be eluted directly with the supplied Elution Buffer MB6. Elution can be carried out in a volume of ≥ 25 µl. It is essential to cover the NucleoMag Blood Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (the position of the pellet inside the separation plate). For efficient elution the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators high elution volumes might be necessary to cover the whole pellet.



Yield does not depend on elution volume. Averages of 8 samples per elution volume are shown.



Elution is possible at room temperature. Yield can be increased by 15 – 20% if elution is performed at 55°C.

3 Storage conditions and preparation of working solutions

Attention:

Buffers MB1, MB2, MB3, and MB4 contain chaotropic salt! Wear gloves and goggles!

Upon storage, especially at low temperatures, a white precipitate may form in buffer MB3. Dissolve such precipitates by incubation of the bottle at 37°C before use.

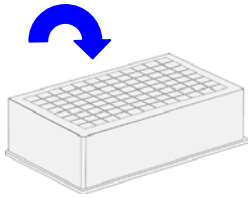
All components of the **NucleoMag 96 Blood** kit should be stored at room temperature (20-25°C) and are stable for up to one year.

All buffers are delivered ready-to-use.

4 General procedure

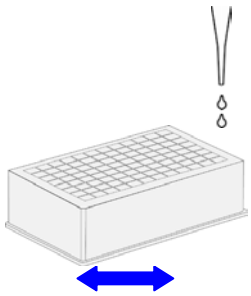
1 Transfer blood to Square-well Block.

100 µl Blood



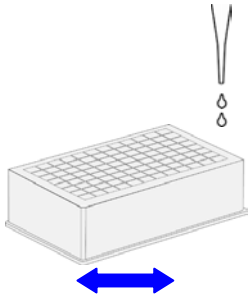
2 Lyse cells.

125 µl MB1
shake 5 min, RT



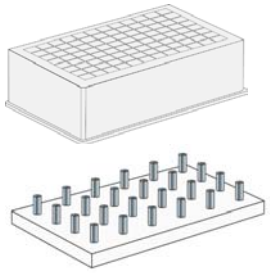
3 Bind DNA to NucleoMag Blood Beads.

14 µl B-Beads
360 µl MB2
shake 5 min, RT



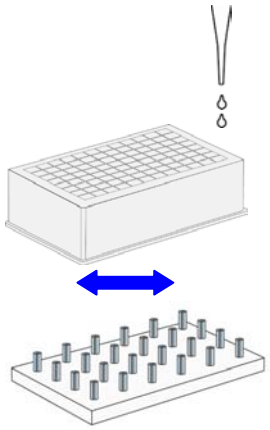
4 Remove supernatant.

2 min separation



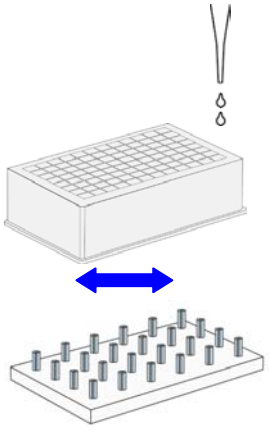
5 MB3 wash step.

600 µl MB3
shake 5 min, RT
2 min separation



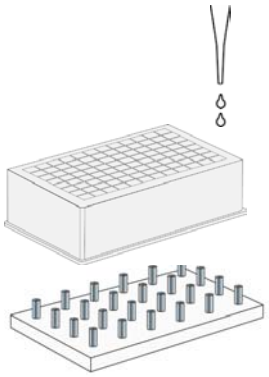
6 MB4 wash step.

600 µl MB4
shake 5 min, RT
2 min separation



7 MB5 wash step.

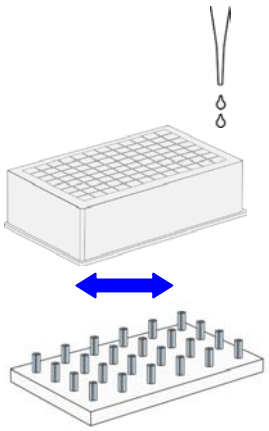
600 µl MB5
90 sec incubation
2 min separation



8 Elute genomic DNA and transfer to Elution Plate.

25 – 100 µl MB6
shake 5 min, RT
2 min separation
transfer

Optional:
Elution at 55°C



4.1 Standard protocol for the purification of genomic DNA

This protocol is designed for magnetic separators with static pins (e.g. NucleoMag SEP) and suitable plate shakers (e.g. H+P Variomag® Teleshake). This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments. For the availability of ready-to-run scripts please contact your local distributor or MN directly.

1 Transfer 100 µl blood (equilibrated to room temperature) to 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.

2 Add 125 µl Lysis Buffer MB1 to each sample and mix by shaking (5 min) at room temperature.

Alternatively, pipette up and down 10 times and incubate 5 min at room temperature.

3 For 96 samples mix at least 1344 µl of NucleoMag B-Beads with 34.56 ml of buffer MB2, vortex briefly and add 374 µl of this mixture to each well of the Square-well Block. Mix immediately by shaking (5 min) at room temperature.

Alternatively, pipette up and down 10 times and incubate 5 min at room temperature.

Note: NucleoMag B-Beads and Binding Buffer MB2 should be premixed. Per well to be processed mix 14 µl of NucleoMag B-Beads with 360 µl Binding Buffer MB2. Vortex briefly. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are necessary.

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.

4 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 to the magnet. Remove and discard 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.

5 Add 600 µl Wash Buffer MB3 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.

Remove the Square-well Block from the magnetic separator.

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

6 Add 600 µl Wash Buffer MB4 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.

Do not remove the Square-well Block from the magnetic separator.

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

7 Add 800 µl Wash Buffer MB5 to each well and incubate for 90 s while the beads are still separated on the magnet. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in Wash Buffer MB 5. This step is to remove traces of ethanol and eliminates a drying step.

8 Add desired volume of Elution Buffer MB6 (25 – 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 yield can be increased by 15 – 20% by using prewarmed elution buffer (55°C) or by incubating the bead/elution buffer suspension at 55°C for 10 min.

5 Appendix

5.1 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 following 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 MB5 already* • Keep the beads on the magnet while dispensing Wash Buffer MB5. Do not resuspend beads in this buffer, 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. *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/Binding Buffer MB2 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, e.g. MN 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 wash buffer MB4* • Be sure to remove all of the ethanolic Wash Buffer MB4, as residual ethanol interferes with downstream applications. *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.
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.

5.2 Ordering information

Product	Cat. No.	Pack of
NucleoMag 96 Blood	744 500.1	1 x 96
NucleoMag 96 Blood	744 500.4	4 x 96
NucleoMag 96 Blood	744 500.24	24 x 96
NucleoMag SEP	744 900	1
Square-well Blocks	740 670	20
Self-adhering PE foil	740 676	50 sheets

6.4 Product Use Restriction / Warranty

NucleoMag 96 Blood kit components were developed, designed 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 96 Blood** 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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