Viral RNA/DNA Isolation

User Manual

NucleoMag 96 Virus

July 2009/ Rev. 02

MACHEREY-NAGEL

Table of contents

1 Components

1.1 Kit contents

	NucleoMag 96 Virus	
	1x 96 preps	**4 x 96 preps**
Cat. No.	**744800.1**	**744800.4**
NucleoMag V-Beads	3 ml	4 x 3 ml
Lysis Buffer MV1	20 ml	80 ml
Binding Buffer MV2	60 ml	2 x 120 ml
Wash Buffer MV3	50 ml	2 x 100 ml
Wash Buffer MV4	50 ml	2 x 100 ml
Wash Buffer MV5	55 ml	2 x 110 ml
Elution Buffer MV6	10 ml	40 ml
Carrier RNA*	400 µg	4 x 400 µg
Carrier RNA Buffer	500 µl	4 x 500 µl
Proteinase K (lyophilized)*	22 mg	4 x 22 mg
Proteinase Buffer PB	3.6 ml	8 ml
User Manual	1	1

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

1.2 Material to be supplied by user

Product	Cat. No.	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
Lysis tubes for incubation of samples and lysis, e.g. Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 ml wells) each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Elution plate for collecting purified nucleic acids, e.g. Elution Plate, U-bottom (96-well 0.3 ml microtiterplate with 300 µl u-bottom wells) e.g. Elution Plate, Flat-bottom (96-well 0.3 ml microtiterplate with 300 µl flat-bottom wells)	740486.24 740673	24 20
For use of kit on KingFisher 96 instrument: KingFisher 96 Accessory Kit A (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag 96 Virus preps using King Fisher 96 platform)	744950	1 set

2 Product description

2.1 The basic principle

The **NucleoMag 96 Virus** kit is designed for the isolation of viral DNA or RNA from cell free body fluids such as serum or plasma. This kit provides reagents and magnetic beads for isolation of 96 samples from 200 µl serum or plasma. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation in a solution containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads Binding Buffer MV2 and the NucleoMag V-Beads are added to the lysate. After magnetic separation the paramagnetic beads are washed to remove contaminants and salts using the Wash Buffers MV3 and MV4. Residual ethanol from previous wash steps is removed by a short incubation of the beads in Wash Buffer MV5. Finally, highly pure viral RNA/DNA is eluted with low-salt Elution Buffer MV6 or water. Purified viral RNA/DNA can directly be used for downstream applications. The **NucleoMag 96 Virus** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag 96 Virus is designed for rapid manual and automated small-scale preparation of viral RNA/DNA from cell-free body fluids such as serum or plasma samples. The kit is designed for use with NucleoMag SEP magnetic separator plate (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 purified RNA/DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

NucleoMag 96 Virus allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the 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.

2.3 Magnetic separation systems

For use of **NucleoMag 96 Virus** 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.

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 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: 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.

Automated separators

Separators with moving magnets: 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 binding and wash steps:

- Load 1000 µl (for checking the settings for the binding step) or 600 µl (for checking the settings for the washing steps) 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 the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the 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 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 and adjusted on each system. It is recommended using 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.

Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

2.6 Elution procedures

Purified viral RNA/DNA can be eluted directly with the supplied Elution Buffer MV6. Elution can be carried out in a volume of ≥ 50 µl. It is essential to cover the NucleoMag Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer 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. For some separators high elution volumes might be necessary to cover the whole pellet.

* 8-channel pipetting device

3 Storage conditions and preparation of working solutions

Attention:

Buffers MV1, MV2, and MV3 contain chaotropic salt! Wear gloves and goggles!

- All components of the **NucleoMag 96 Virus** 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.
- **Lysis Buffer MV1**:
  Lysis Buffer MV1 may form a salt precipitate upon storage. To re-dissolve the salt precipitate incubate the buffer bottle at 40°C until all of the precipitate is re-dissolved.
  Lysis Buffer MV1 with Carrier RNA: Lysis Buffer MV1 with added Carrier RNA can be stored at room temperature for 1 - 2 weeks.
  Frequent warming, temperatures >80°C and extended heat incubation will cause degradation of the Carrier RNA. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular if low-titer samples are used. Do not warm Buffer MV1 containing Carrier RNA more than 6 times!

Before starting any **NucleoMag 96 Virus** protocol prepare the following:

- **Proteinase K:** Before first use of the kit add 1.1 ml Proteinase Buffer PB to each vial of the **lyophilized Proteinase K**. Dissolved Proteinase K solution should be stored in aliquots at - 20°C.
- **Carrier RNA:** Before first use of the kit add 500 µl Carrier RNA Buffer to each vial **lyophilized Carrier RNA**. Store dissolved Carrier RNA solution in aliquots at - 20°C.

	NucleoMag 96 Virus	
	1 x 96 preps	**4 x 96 preps**
Cat. No.	**744800.1**	**744800.4**
Proteinase K (lyophilized)	1 vial (22 mg)	4 vials (22 mg/vial)
	Add 1.1 ml Proteinase Buffer	Add 1.1 ml Proteinase Buffer to each vial
Carrier RNA (lyophilized)	1 vial (400 µg)	4 vials (400 µg/vial)
	Add 500 µl Carrier RNA Buffer	Add 500 µl Carrier RNA Buffer to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoMag 96 Virus** 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
MV1	Guanidine thiocyanate	Xn*	Harmful by inhalation, in contact with the skin, and if swallowed	R 20/21/22	S 13
MV2	Sodium perchlorate <15% + ethanol <50%	*	Flammable	R 10	
MV3	Sodium perchlorate <15% + ethanol <24%	*	Flammable	R 10	
MV4	Ethanol <60%	F*	Highly flammable	R11	S 7-16
Carrier RNA Buffer	Guanidine thiocyanate	Xn*	Harmful by inhalation, in contact with the skin, and if swallowed	R 20/21/22	S 13
Proteinase K	Proteinase K, lyophilized	Xn Xi*	Irritating to eyes, respiratory system and skin - May cause senzitization by inhalation	R 36/37/38-42	S 22-24-26-36/37

Risk phrases

R 10	Flammable
R 11	Highly flammable
R 20/21/22	Harmful by inhalation, in contact with the skin, and if swallowed
R 36/37/38	Irritating to eyes, respiratory system, and skin
R 42	May cause senzitization by inhalation

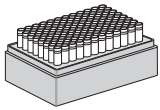
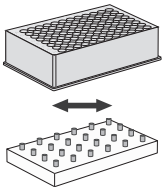
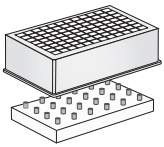
* Hazard labeling not necessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

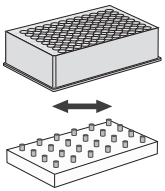
Safety phrases

S 7	Keep container tightly closed
S 13	Keep away from food, drink, and animal feedstuffs
S 16	Keep away from sources of ignition - No smoking!
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

5 Procedure

5.1 General procedure

1	Lyse sample	**200 µl sample** **10 µl Proteinase K** **4 µl Carrier RNA** **200 µl MV1** **Mix** **56°C, 10 min**	
2	Bind viral RNA/DNA to NucleoMag V-Beads	**600 µl MV2** **30 µl V-Beads** **Mix** **RT, 5 min** *(Optional: Mix by shaking)* **Separate, 2 min and remove supernatant**	
3	MV3 wash	**500 µl MV3** **Resuspend, separate, 2 min** **Aspirate and discard supernatant**	
4	MV4 wash	**500 µl MV4** **Resuspend, separate, 2 min** **Aspirate and discard supernatant**	
5	MV5 wash	**550 µl MV5** **RT, 45 s** **Aspirate and discard supernatant**	

6	Elution	**50 - 100 µl MV6** **Shake 5 min at 56°C** ***(Optional: Mix by pipetting up and down)*** **Separate, 2 min and transfer viral RNA/DNA**	

5.2 Protocol for the isolation of viral RNA/DNA from cell free body fluids

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 viral RNA/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.

1 Lyse sample

Pre-dispense **10 µl Proteinase K** and **200 µl of sample** to a suitable reaction tube. Add **200 µl Buffer MV1** (with added **Carrier RNA**) to the reaction tube (If Carrier RNA is not premixed with the Buffer MV1, add 4 µl of the stock solution to the reaction tube). Mix well by repeated pipetting up and down and incubate at **56°C** for **10 min**. Alternatively, lysis step can be performed in Tube Strips (see ordering information).

For higher convenience a premix of Proteinase K, Buffer MV1 and Carrier RNA can be prepared. This premix should be added to the sample immediately (within 15 min after preparation).

Following the lysis incubation, spin down to collect any sample from the lysis tube lids and transfer each lysate to the wells of a Square-well Block.

2 Bind viral RNA/DNA to magnetic beads

Add **30 µl resuspended V-Beads** and **600 µl Buffer MV2** to the lysed sample. Mix by pipetting up and down 6 times and incubate for **5 min** at **room temperature**. NucleoMag V-Beads and Buffer MV2 can be pre-mixed.

Be sure to resuspend the NucleoMag V-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 NucleoMag SEP a magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Do not disturb the attracted beads while aspirating the supernatant.

3 MV3 wash

Remove the Square-well Block from the NucleoMag SEP magnetic separator. Add **500 µl Buffer MV3** and resuspend the beads by pipetting up and down. Incubate for **1 min.**

Separate the magnetic beads by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

4 MV4 wash

Remove the Square-well Block from the NucleoMag SEP magnetic separator. Add **500 µl Buffer MV4** and resuspend the beads by pipetting up and down. Incubate for **1 min.**

Separate the magnetic beads by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

5 MV5 wash

Leave the Square-well Block on the NucleoMag SEP magnetic separator. Gently add **550 µl Buffer MV5** to each well and incubate for **45 - 60 s** while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

Do not resuspend the beads in Buffer MV5. This step is to remove traces of ethanol and eliminates a drying step. Do not exceed incubation time of max. 1 min.

6 Elution

Add desired volume of **Buffer MV6 (50 - 100 µl)** to each well of the Square-well Block and resuspend the beads by pipetting up and down.

Incubate the suspension for **5 min** at **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified viral RNA/DNA to either microtubes or Tube Strips (see ordering information).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor yield/ low sensitivity	*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 the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation too short or aspiration speed too high.
Low purity/ low sensitivity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, e.g. MN Square-well Block in combination with NucleoMag SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
Poor performance of RNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution Buffer MV4, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* • Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.

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.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoMag 96 Virus	744800.1 744800.4	1 x 96 preps 4 x 96 preps
NucleoMag SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Self-adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
KingFisher 96 Accessory Kit A Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag 96 Virus preps using King Fisher 96 platform	744950	1 set

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

6.3 Product use restriction / warranty

NucleoMag 96 Virus 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 96 Virus** 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