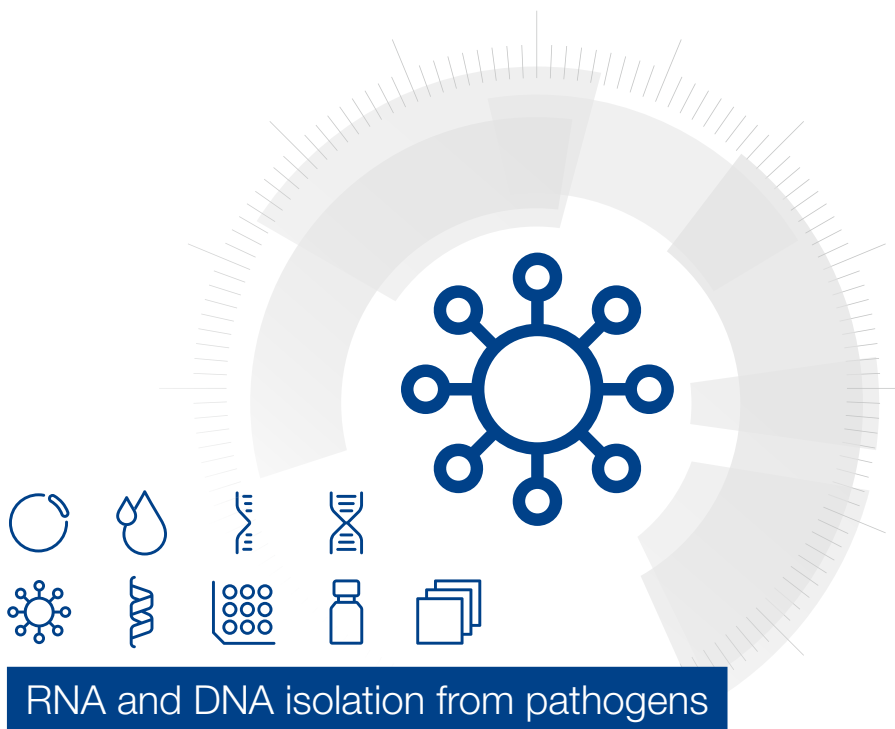


MACHEREY-NAGEL

# User manual



## RNA and DNA isolation from pathogens

- NucleoMag® Pathogen
- NucleoMag® Pathogen Prefilled Plates

April 2025 / Rev. 09

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# 1 Components

## 1.1 Kit contents

| NucleoMag® Pathogen          |                          |                          |
|------------------------------|--------------------------|--------------------------|
| REF                          | 1 × 96 preps<br>744210.1 | 4 × 96 preps<br>744210.4 |
| NucleoMag® B-Beads           | 2 × 1.5 mL               | 10 mL                    |
| Lysis Buffer NPL1            | 30 mL                    | 100 mL                   |
| Binding Buffer NPB2          | 110 mL                   | 3 × 110 mL               |
| Wash Buffer NPW3             | 75 mL                    | 300 mL                   |
| Wash Buffer NPW4             | 75 mL                    | 300 mL                   |
| Elution Buffer NPE5*         | 30 mL                    | 125 mL                   |
| Carrier RNA**                | 400 µg                   | 4 × 400 µg               |
| Carrier RNA Buffer           | 500 µL                   | 4 × 500 µL               |
| Proteinase K (lyophilized)** | 75 mg                    | 3 × 75 mg                |
| Proteinase Buffer PB         | 8 mL                     | 15 mL                    |
| User manual                  | 1                        | 1                        |

| NucleoMag® Pathogen Prefilled Plates                |                        |
|---|------------------------|
| REF   | 6 × 16 preps<br>744211 |
| Lysis Buffer NPL1                                   | 30 mL                  |
| Prefilled 96-well NucleoMag® Pathogen Reagent Plate | 6 pieces               |
| Carrier RNA*  | 400 µg                 |
| Carrier RNA Buffer                                  | 500 µL                 |
| Liquid Proteinase K                                 | 2 mL                   |
| 8-well Tip Combs                                    | 6 × 2 pieces           |
| Leaflet   | 1                      |

\* Composition of Elution Buffer NPE5: 5 mM Tris/HCl, pH 8.5

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

## 1.2 Material to be supplied by user

| Product  | REF       | Pack of       |
|--|-----------|---------------|
| <b>Magnet for magnetic beads separation,</b>   |           |               |
| e.g., NucleoMag® SEP   | 744900    | 1             |
| NucleoMag® SEP Mini  | 744901    | 1             |
| NucleoMag® SEP Maxi  | 744902    | 1             |
| NucleoMag® SEP 24  | 744903    | 1             |
| <b>Separation plate for magnetic beads separation,</b>   |           |               |
| e.g., Square-well Block (96-well block with 2.1 mL square-wells)   | 740481    | 4             |
|  | 740481.24 | 24            |
| <b>Lysis tubes for incubation of samples and lysis,</b>  |           |               |
| 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    | 4 sets        |
|  | 740477.24 | 24 sets       |
| <b>Elution plate for collecting purified nucleic acids,</b>  |           |               |
| e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)   | 740486.24 | 24            |
| <b>For use of kit on KingFisher® Flex instrument:</b>  |           |               |
|  | 744950    | 1 set         |
| e.g., KingFisher® Accessory Kit A (Deep-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag® Pathogen preps using KingFisher® Flex platform) |           |               |
| <b>For use of kit on MagnetaPure32 Plus or IsoPure Mini:</b>   |           |               |
|  | 744955    | 25 pieces     |
| 96 Deep-well plates for magnetic rod systems   |           |               |
| <b>For use of kit on MagnetaPure32 Plus or IsoPure Mini:</b>   |           |               |
|  | 744960    | 25 × 2 pieces |
| 8-well Tip Combs for magnetic rod systems  |           |               |

Reagents:

- 80% ethanol

## 1.3 About this user manual

It is strongly recommended that first time users of the **NucleoMag® Pathogen** kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used as a tool for quick referencing while performing the purification procedure.

All technical literature is available at [www.mn-net.com](http://www.mn-net.com).

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

## 2 Product description

### 2.1 The basic principle

The **NucleoMag® Pathogen** kit is designed for the isolation of viral RNA and DNA and bacterial DNA from cell-free body fluids such as serum or plasma, blood, saliva, homogenized tissue sample suspensions, stool sample suspensions, and swab washes. This kit provides reagents and magnetic beads for isolation of 96 samples. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a Lysis Buffer NPL1 containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads, Binding Buffer NPB2 and the NucleoMag® B-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers NPW3, NPW4, and 80 % ethanol. Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure pathogen RNA and DNA is eluted with low-salt elution buffer or water. Purified pathogen RNA and DNA can directly be used for downstream applications. It is recommended to use suitable controls for downstream applications (e.g., internal controls, extraction controls, positive/negative controls). The **NucleoMag® Pathogen** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit [www.mn-net.com/automation](http://www.mn-net.com/automation).

### 2.2 Kit specifications

**NucleoMag® Pathogen** kit is designed for rapid manual and automated small-scale preparation of viral RNA and DNA and the DNA of microorganisms from various types of clinical samples. The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information, section 7.2) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA and DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions. For research use only.

**NucleoMag® Pathogen Prefilled Plates** (REF 744211) are prefilled 96 deep-well plates specifically for the use on MagnetaPure 32 Plus or IsoPure™ Mini systems. Binding Buffer NPB2, NucleoMag® B-Beads, Wash Buffer NPW3, NPW4, 80 % ethanol as well as elution buffer NPE5 are prefilled into their respective wells. Sample preparation/lysis is performed according to the standard procedure of the NucleoMag® Pathogen kit. Subsequently, lysed samples are transferred to the reagent wells containing binding buffer NPB2 and NucleoMag® B-Beads for further processing on the MagnetaPure 32 Plus or IsoPure™ Mini instruments. The purified RNA/DNA, eluted in 100 µL VEL, can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

**NucleoMag® Pathogen** kit 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 an automation platform.

## 2.3 Magnetic separation systems

For use of **NucleoMag® Pathogen**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information, section 7.2). The kit can also be used with other common separators.

| Magnetic separator                  | Separation plate or tube                            |
|-------------------------------------|---|
| NucleoMag® SEP (MN REF 744900)      | Square-well Block (MN REF 740481)                   |
| NucleoMag® SEP Mini (MN REF 744901) | 1.5 mL or 2 mL reaction tubes (Sarstedt)            |
| NucleoMag® SEP Maxi (MN REF 744902) | 50 mL tubes (Falcon)                                |
| NucleoMag® SEP 24 (MN REF 744903)   | 24-Square-well Block U-bottom (MN REF 740448.4/.24) |
| 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. A gripper tool is required for fully automated use on liquid handling workstations. The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times.

### 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

Beads are resuspended by the cover of the magnetic rods. Following the binding, washing and elution steps the beads are collected again with the magnetic rods.

### MagnetaPure32 Plus and IsoPure™ Mini

The **NucleoMag® Pathogen Prefilled Plates** (REF 744211) are specifically designed for the use on MagnetaPure 32 Plus and IsoPure™ Mini magnetic rod systems. Reagents are prefilled in a column-wise manner. Sample preparation and sample lysis is performed externally and lysed samples are transferred to the reagent wells containing binding buffer and magnetic beads.

## 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 washing steps:**

Load 600  $\mu\text{L}$  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 s. Turn off the shaker and check the plate surface for small droplets of dyed water.

Increase speed setting, shake for an additional 30 s, 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  $\mu\text{L}$  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 into the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before dispensing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it briefly on a Vortex. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads into 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 pins, the selected separation plate, distance of the separation plate to 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 different mixing procedures. In contrast to pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous washing of all samples. This reduces the time consumption and the 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 pathogen RNA and DNA can be eluted directly with the supplied elution buffer. Elution can be carried out in a volume of  $\geq 50 \mu\text{L}$ . It is essential to cover the NucleoMag<sup>®</sup> 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.

The NucleoMag<sup>®</sup> Pathogen Prefilled Plates (REF 744211) contain 100  $\mu\text{L}$  of Elution buffer NPE5 supplemented with 0.02 % sodium azide as preservative.

You looking for an automation solution for your NM Pathogen workflow?

Please contact:

MACHEREY-NAGEL GmbH & Co. KG

Tel.: +49 24 21 969-333

support@mn-net.com

\* 8-channel pipetting device

### 3 Storage conditions and preparation of working solutions

#### Attention:

*NPL1, NPB2 and the Carrier RNA Buffer contain chaotropic salt! Wear gloves and goggles!*

All components of the **NucleoMag® Pathogen** kit should be stored at room temperature (15–25 °C) and are stable until: see package label.

The NucleoMag® Pathogen Prefilled Plates should be stored upright at 15–25 °C without direct exposure to UV-light or sunlight. Do not store the NucleoMag® Pathogen Prefilled Plates at temperatures above 25 °C or below 15 °C. Check buffers precipitates and prewarm the plate in order to dissolve precipitates. When stored properly the Prefilled Plates are stable until: see package label.

All buffers are delivered ready-to-use.

Before starting any **NucleoMag® Pathogen** protocol, prepare the following:

**Proteinase K:** Before first use of the kit, add 3.35 mL Proteinase Buffer PB to each vial of the **lyophilized Proteinase K**. Dissolved Proteinase K solution should be stored at -20 °C. (Note: The NucleoMag® Pathogen Prefilled Plates are delivered with liquid proteinase K, which is ready to use.)

**Carrier RNA:** Before first use of the kit, add 500 µL Carrier RNA Buffer to each vial **lyophilized Carrier RNA**. Dissolve the Carrier RNA and store dissolved Carrier RNA solution in aliquots at -20 °C for up to 6 months.

*Note: Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardly be visible.*

| NucleoMag® Pathogen        |   |   |
|----------------------------|---|---|
| REF                        | 1 × 96 preps<br>744210.1                            | 4 × 96 preps<br>744210.4  |
| Proteinase K (lyophilized) | 1 vial (75 mg)<br>Add 3.35 mL<br>Proteinase Buffer  | 3 vials (75 mg/vial)<br>Add 3.35 mL Proteinase<br>Buffer to each vial     |
| Carrier RNA (lyophilized)  | 1 vial (400 µg)<br>Add 500 µL<br>Carrier RNA Buffer | 4 vials (400 µg/vial)<br>Add 500 µL<br>Carrier RNA Buffer to each<br>vial |

**NucleoMag<sup>®</sup> Pathogen Prefilled Plates**

**REF**

**1 × 96 preps  
744210.1**

Carrier RNA (lyophilized)

1 vial (400 µg)

Add 500 µL Carrier RNA Buffer

## 4 Safety instructions

When working with the **NucleoMag® Pathogen** kit or with the **NucleoMag® Pathogen Prefilled Plates** wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at [www.mn-net.com/msds](http://www.mn-net.com/msds)).



Caution: Guanidine hydrochloride in buffer NPL1, and guanidinium thiocyanate in carrier RNA buffer can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoMag® Pathogen** kit or **NucleoMag® Pathogen Prefilled Plates** has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

### 4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

## 5 Protocol for the isolation of viral RNA and DNA and microbial DNA from blood, tissue homogenates, serum, plasma, other body fluids and washes

### 5.1 Preparation of sample materials

#### a) Blood samples / saliva

A sample volume of 100–200 µL blood is recommended. Do not use higher volumes. When processing less than 200 µL sample adjust with PBS buffer to a final volume of 200 µL.

#### b) Tissue samples

Homogenize tissue samples. Typically 5–10 mg sample material can be homogenized in 400 µL PBS buffer using a bead based homogenizer. If necessary, higher amounts of sample material can be used (up to 25 mg). It should be considered that the copurified total nucleic acids may cause inhibition in the subsequent PCR assays. Centrifuge the homogenized sample and use up to 200 µL clear supernatant for further processing. If using less than 200 µL adjust with PBS buffer to a final volume of 200 µL.

For isolation of viral RNA:

Tissue can also be disrupted in a buffer containing chaotropic salt (e.g., Buffer RA1, see ordering information) and beta-mercaptoethanol or TCEP reducing agent (see ordering information, section 7.2).

#### c) Swab samples

Incubate the swabs in PBS, sodium chloride, or cell culture medium for 30 min with agitation. Then remove the swab pressing it against the walls of the tube to squeeze out most of the liquid or use our NucleoSpin® Forensic Filters (single spin filters. For Lysate clearing in 96-well format use the NucleoSpin® Trace Filter Plate, see ordering information, section 7.2).

#### d) Feces

Mix 1 volume of feces (e.g., 500 µL) with an equal volume of PBS buffer. Mix vigorously by vortexing for 1 min. Allow the particles to settle down or centrifuge with low speed (e.g., at 500 x g). Proceed with the cleared supernatant. For difficult to lyse bacteria mechanical disruption or treatment using suitable beads may be required (e.g., Bead Tubes Type A, see ordering information section 7.2)

#### e) Clean up of TRIzol® purified samples

After phase separation by centrifugation proceed with the aqueous phase (the colorless upper phase; Approximately 400 µL). For further processing start with step 2 of the purification protocol by mixing 400 µL of the aqueous phase with 600 µL Buffer NPB2 and 20 µL NucleoMag® B-Beads.

In the processing of **NucleoZOL**, the aqueous phase can be used in the same way as described above.

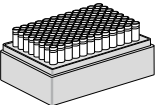
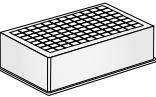

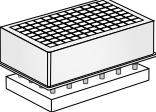
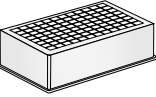

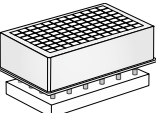
## 5.2 Protocol at a glance

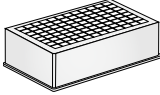

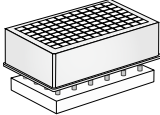
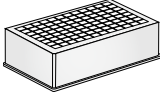

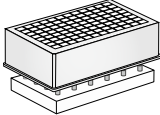
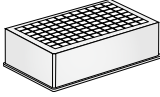
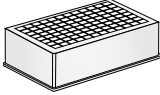

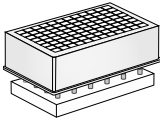
For hardware requirements refer to section 2.3.

For detailed information on each step see page 16.

### Before starting the preparation:

Check if Proteinase K and Carrier RNA were prepared according to section 3.

|  |   |   |
|--|---|---|
| <p><b>1</b> Lyse sample</p>                              | <p><b>200 µL (homogenized) sample</b></p> <p><b>20 µL Proteinase K</b></p> <p><b>4 µL Carrier RNA</b></p> <p><b>180 µL NPL1</b></p> <p><b>Mix</b></p> <p><b>RT, 15 min</b><br/>or<br/><b>56 °C, 15 min</b></p>      |    |
| <p><b>2</b> Bind nucleic acids to NucleoMag® B-Beads</p> | <p><b>600 µL NPB2</b></p> <p><b>20 µL B-Beads</b></p> <p><b>Mix by shaking for 5 – 10 min at RT</b><br/><i>(Optional: Mix by pipetting up and down)</i></p> <p><b>Remove supernatant after 2 min separation</b></p> | <br><br><br><br>      |
| <p><b>3</b> Wash with NPW3</p>                           | <p><b>Remove Square-well Block from NucleoMag® SEP</b></p> <p><b>600 µL NPW3</b></p> <p><b>Resuspend: Shake 1 min at RT</b></p> <p><b>Remove supernatant after 2 min separation</b></p>                             | <br><br><br><br> |

|   |                        |   |   |
|---|------------------------|---|---|
| 4 | Wash with NPW4         | <p><b>Remove Square-well Block from NucleoMag® SEP</b></p> <p>600 µL NPW4</p>         |    |
|   |                        | <p>Resuspend: Shake 1 min at RT</p>   |    |
|   |                        | <p>Remove supernatant after 2 min separation</p>                                      |    |
| 5 | Wash with 80 % Ethanol | <p><b>Remove Square-well Block from NucleoMag® SEP</b></p> <p>600 µL 80 % Ethanol</p> |    |
|   |                        | <p>Resuspend: Shake 1 min at RT</p>   |    |
|   |                        | <p>Remove supernatant after 2 min separation</p>                                      |    |
| 6 | Drying step            | <p>Air dry for 10 min at room-temperature</p>   |    |
| 7 | Elute RNA and DNA      | <p><b>Remove Square-well Block from NucleoMag® SEP</b></p> <p>50-100 µL NPE5</p>      |   |
|   |                        | <p>Shake 5 min at RT</p> <p><i>(Optional: Mix by pipetting up and down)</i></p>       |  |
|   |                        | <p>Separate 2 min and transfer RNA and DNA into elution plate / tubes</p>             |  |

### 5.3 Detailed protocol

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, section 7.2). Alternatively, isolation of pathogen RNA and 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 **20 µL Proteinase K** and **200 µL of sample** to a suitable reaction tube. Add **180 µL Lysis Buffer NPL1** to the reaction tube.

*Optional: Add 4 µL of the Carrier RNA stock solution to the reaction tube.*

Mix well by repeated pipetting up and down and incubate at room temperature for 15 min with shaking. Alternatively, lysis step can be performed in Tube Strips (see ordering information, section 7.2).

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.

Lysis incubation can be performed at 56 °C to increase the lysis efficiency e.g. for isolation of bacterial DNA from difficult to lyse bacteria.

*Optionally, lysis can be supported by a pretreatment of the sample with suitable beads for mechanical disruption of difficult to lyse bacteria.*

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#### 2 Bind nucleic acids to magnetic beads

Add **20 µL resuspended NucleoMag® B-Beads** and **600 µL Binding Buffer NPB2** to the lysed sample.

Mix by pipetting up and down 6 times and **shake** for **5 min at room temperature**.

*Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.*

*NucleoMag® B-Beads and Buffer NPB2 can be premixed.*

*Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.*

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

*Do not disturb the attracted beads while aspirating the supernatant.*

---

### 3 Wash with NPW3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer NPW3** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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

---

### 4 Wash with NPW4

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **600 µL Buffer NPW4** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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

---

### 5 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **600 µL 80 % ethanol** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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

---

### 6 Air dry magnetic beads

Air dry the magnetic bead pellet for 10 min at room temperature.

---

### 7 Elute RNA and DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add desired volume of **NPE5 (50 – 100 µL)** to each well of the Square-well Block and resuspend the beads by shaking 5 min at room temperature. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 min at 56 °C**.

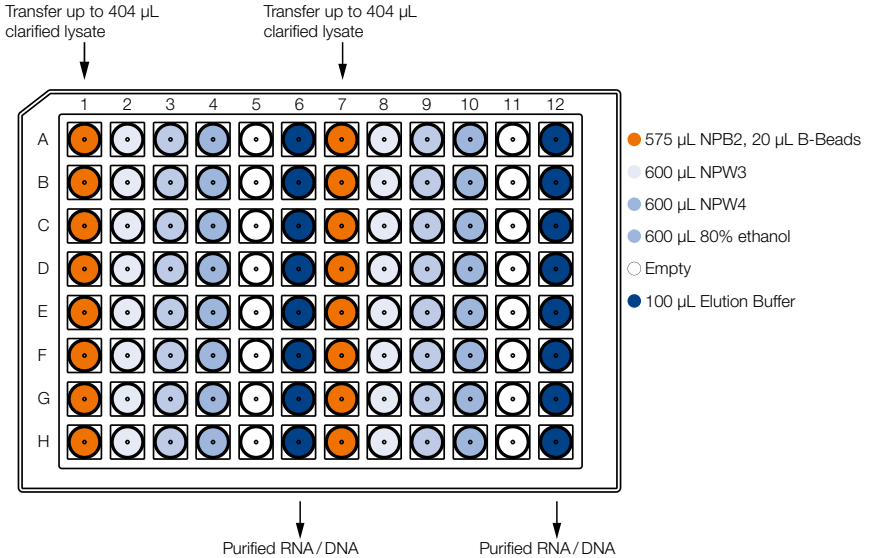
Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all beads have been attracted to the magnets. Transfer the supernatant containing the purified nucleic acids to either microtubes or Tube Strips (see ordering information, section 7.2).

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## 6 Protocol for NucleoMag® Pathogen Prefilled Plates

The NucleoMag® Pathogen Prefilled Plates are specifically designed for the use on MagnetaPure 32 Plus or IsoPure™ Mini instruments only. Please contact our technical support for questions regarding the compatibility of comparable instruments.

Schematic overview of the Prefilled reagent plate.



*Note:* The required method files for processing the NucleoMag® Pathogen Prefilled Plate on the IsoPure™ Mini or MagnetaPure32 Plus instruments can be requested at [support@mn-net.com](mailto:support@mn-net.com)

## 6.1 Detailed protocol for IsoPure™ Mini and MagnetaPure32 Plus

Preparation (see section 5) and lysis of sample material must be performed outside of the instrument. Before starting the preparation:

- Wear protective goggles and suitable protective clothing
  - Check if Carrier RNA was prepared according to section 3.
  - Check buffers for precipitates according to section 3.
  - Check if the correct script is installed on your instrument.
    - IsoPure™ Mini: NMPathogen (run time approx. 30 min)
    - MagnetaPure32 Plus: NMPathogen (run time approx. 30 min)
- 

### 1 Lyse sample(s)

Pre-dispense **20 µL Proteinase K** and **200 µL of sample** to a suitable reaction tube. Add 180 µL Buffer NPL1 to the reaction tube. Optional: add **4 µL of the Carrier RNA** stock solution to the reaction tube. Mix well by repeated pipetting up and down and incubate at **room temperature** for **15 min** with shaking. Alternatively, lysis step can be performed in Tube Strips (see ordering information).

Meanwhile, prepare the **Prefilled 96-well NucleoMag® Pathogen Reagent Plate**.

Following the lysis incubation, spin down to collect any sample from the lysis tube lids.

*Note: Check section 5 for sample preparation.*

---

### 2 Prepare reagent plate(s)

Briefly spin down (e.g. 10–15 s at 1,000 × g) the sealed **Prefilled 96-well NucleoMag® Pathogen Reagent Plate** in a suitable centrifuge to remove droplets from the underside of the seal.

---

### 3 Remove seal(s)

Carefully remove the seal from the reagent plate by pulling on the seal from one side with an equal amount of force.

*Note: Check for residual sealant on the plate and remove with tweezers if necessary.*

---

### 4 Transfer cleared lysate(s)

Transfer up to 400 µL of cleared lysed sample from step 1 to the respective wells of column 1 and 7 of the used plates.

*Note: Do not moisten the upper rim of the 96 deep-well plate.*

*Note: Up to 404 µL of cleared lysate can be transferred if Carrier RNA is used.*

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**5 Select the protocol and start the run**

Load the plate(s) on the instrument.

Insert tip combs on the mounting grooves.

Start the run.

*Note: Please equip all tip combs in order to cover the magnetic rods in used and unused wells.*

---

**6 Transfer eluate**

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

---

## 7 Appendix

### 7.1 Troubleshooting

| <b>Problem</b>               | <b>Possible cause and suggestions</b>  |
|------------------------------|--|
| Poor yield / low sensitivity | <i>Incomplete sample lysis</i> <ul style="list-style-type: none"><li>• Sample mixed with Lysis Buffer and Proteinase K was not thoroughly homogenized and mixed with Lysis buffer, Proteinase K. The mixture has to be shaken continuously. Alternatively, prolong incubation time with Proteinase K.</li></ul>  |
|                              | <i>Insufficient elution buffer volume</i> <ul style="list-style-type: none"><li>• Bead pellet must be covered completely with elution buffer and needs to be fully resuspended.</li></ul>  |
|                              | <i>Insufficient performance of elution buffer during elution step</i> <ul style="list-style-type: none"><li>• Remove all buffer completely from the bead pellet after the binding and wash steps. Remaining buffer decreases the efficiency of the subsequent steps.</li></ul>   |
|                              | <i>Aspiration of attracted bead pellet</i> <ul style="list-style-type: none"><li>• Do not disturb the attracted beads while aspirating the supernatant. This requires special caution when removing the lysate from the beads as the lysate is usually too opaque to allow visual control of the pellet.</li></ul>   |
|                              | <i>Aspiration and loss of beads</i> <ul style="list-style-type: none"><li>• Time for magnetic separation too short or aspiration speed too high.</li></ul>   |
| Low purity / low sensitivity | <i>Insufficient washing procedure</i> <ul style="list-style-type: none"><li>• Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP.</li><li>• 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.</li></ul> |

**Problem**

**Possible cause and suggestions**

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Poor performance of DNA/RNA in downstream applications

*Carry-over of ethanol from wash buffers*

- Be sure to remove all of the 80% ethanolic wash solution from the final wash, 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.
-

## 7.2 Ordering information

| Product  | REF                                       | Pack of                      |
|--|---|------------------------------|
| NucleoMag® Pathogen  | 744210.1<br>744210.4                      | 1 × 96 preps<br>4 × 96 preps |
| NucleoMag® Pathogen Prefilled Plates   | 744211                                    | 6 × 16 preps                 |
| NucleoMag® SEP Mini  | 744901                                    | 1                            |
| NucleoMag® SEPMaxi   | 744902                                    | 1                            |
| NucleoMag® SEP 24  | 744903                                    | 1                            |
| Square-well Blocks   | 740481<br>740481.24                       | 4<br>24                      |
| Self-adhering PE Foil  | 740676                                    | 50 sheets                    |
| Rack of Tube Strips<br>(set consists of 1 Rack, 12 Tube Strips<br>with 8 tubes each, and 12 Cap Strips)  | 740477<br>740477.24                       | 4 sets<br>24 sets            |
| NucleoSpin® Forensic Filters<br>(Semipermeable mini spin filter; individually<br>blistered)  | 740988.50<br>740988.250                   | 50<br>250                    |
| NucleoSpin® Forensic Filters (Bulk)<br>(Semipermeable mini spin filter; bulk packed)   | 740988.50B<br>740988.250B<br>740988.1000B | 50<br>250<br>1000            |
| NucleoSpin® Trace Filter Plate<br>(96-well filter plate to e.g., separate swabs from<br>the lysate)  | 740677                                    | 20                           |
| NucleoSpin® Bead Tubes Type A  | 740786.50                                 | 50                           |
| 96-well Accessory Kit A for KingFisher<br>Square-well Blocks, Deep-well tip combs,<br>Elution Plates for 4 × 96 NucleoMag® Pathogen<br>preps using KingFisher® Flex platform | 744950                                    | 1 set                        |
| Buffer RA1 (60 mL)   | 740961                                    | 60 mL                        |
| Reducing agent TCEP  | 740395.107                                | 107 mg                       |
| 96 Deep-well plates for magnetic rod systems   | 744955                                    | 25                           |
| 8-well Tip Combs for magnetic rod system   | 744960                                    | 50                           |
| 8-well Accessory Kit magnetic rod systems  | 744961                                    | 1 set                        |

Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information.

### 7.3 Product use restriction/warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

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Last updated: 08/2022, Rev. 04

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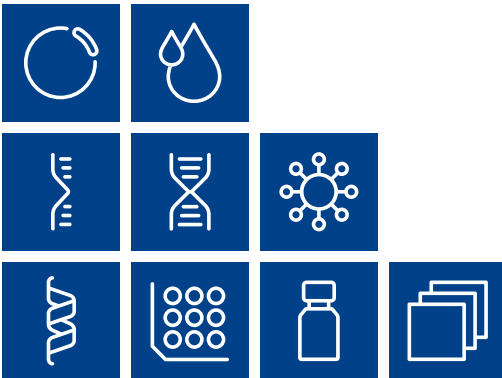
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Plasmid DNA

Clean up

RNA

DNA

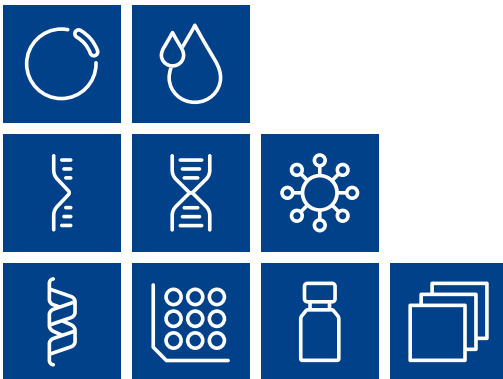
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



# MACHEREY-NAGEL

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