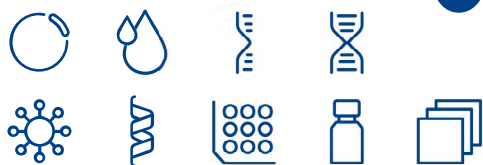


MACHEREY-NAGEL

User manual

Bioanalysis



## RNA and DNA isolation from pathogens

■ NucleoMag® Pathogen

February 2021 / Rev. 05

**MACHEREY-NAGEL**

[www.mn-net.com](http://www.mn-net.com)



## Contact MN

### Germany and international

MACHEREY-NAGEL GmbH & Co. KG  
Neumann-Neander-Str. 6-8 · 52355 Düren · Germany  
Tel.: +49 24 21 969-0  
Toll-free: 0800 26 16 000 (Germany only)  
E-mail: [info@mn-net.com](mailto:info@mn-net.com)

### Technical Support Bioanalysis

Tel.: +49 24 21 969-270  
E-mail: [tech-bio@mn-net.com](mailto:tech-bio@mn-net.com)

### USA

MACHEREY-NAGEL Inc.  
2850 Emrick Blvd. · Bethlehem, PA 18020 · USA  
Tel.: +1 484 821 0984  
Toll-free: 888 321 6224 (MACH)  
E-mail: [sales-us@mn-net.com](mailto:sales-us@mn-net.com)

### France

MACHEREY-NAGEL SARL à associé unique  
1, rue Gutenberg · 67722 Hoerdtsheim · France  
Tel.: +33 388 68 22 68  
E-mail: [sales-fr@mn-net.com](mailto:sales-fr@mn-net.com)

### Switzerland

MACHEREY-NAGEL AG  
Hirsackerstr. 7 · 4702 Oensingen · Switzerland  
Tel.: +41 62 388 55 00  
E-mail: [sales-ch@mn-net.com](mailto:sales-ch@mn-net.com)

[www.mn-net.com](http://www.mn-net.com)

DOMINIQUE DUTSCHER SAS

## Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Material to be supplied by user	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Magnetic separation systems	7
2.4	Adjusting the shaker settings	7
2.5	Handling of beads	8
2.6	Elution procedures	9
3	Storage conditions and preparation of working solutions	10
4	Safety instructions	11
5	Protocol for the isolation of viral RNA and DNA and microbial DNA from blood, tissue homogenates, serum, plasma, other body fluids and washes	13
5.1	Preparation of sample materials	13
5.2	Protocol at a glance	14
5.3	Detailed protocol	16
6	Appendix	18
6.1	Troubleshooting	18
6.2	Ordering information	20
6.3	Product use restriction/warranty	21

# 1 Components

## 1.1 Kit contents

REF	NucleoMag® Pathogen	
	1 x 96 preps 744210.1	4 x 96 preps 744210.4
NucleoMag® B-Beads	2 x 1.5 mL	10 mL
Lysis Buffer NPL1	30 mL	100 mL
Binding Buffer NPB2	110 mL	3 x 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 x 400 µg
Carrier RNA Buffer	500 µL	4 x 500 µL
Proteinase K (lyophilized)**	75 mg	3 x 75 mg
Proteinase Buffer PB	8 mL	15 mL
User manual	1	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>		
e.g., KingFisher® Accessory Kit A (Deep-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® Pathogen preps using KingFisher® Flex platform)	744950	1 set

### Reagents:

- 80 % ethanol

## 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.

### 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 6.2) 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.

**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 6.2). 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.

## 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 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 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 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 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

\* 8-channel pipetting device



## 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® 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.

### 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 (18–25 °C) and are stable for up to one year.

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.

**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 x 96 preps 744210.1	4 x 96 preps 744210.4
Proteinase K (lyophilized)	1 vial (75 mg) Add 3.35 mL Proteinase Buffer	3 vials (75 mg/vial) Add 3.35 mL Proteinase Buffer to each vial
Carrier RNA (lyophilized)	1 vial (400 µg) Add 500 µL Carrier RNA Buffer	4 vials (400 µg/vial) Add 500 µL Carrier RNA Buffer to each vial






## 4 Safety instructions

The following components of the **NucleoMag® Pathogen** kits contain hazardous contents.

*Wear gloves and goggles and follow the safety instructions given in this section.*

### GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.  
*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS-Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
NPL1	Guanidine hydrochloride 36–50 % <i>Guanidinhydrochlorid 36–50 %</i> CAS 50-01-1	 WARNING ACHTUNG	302, 319	264W, 280sh, 301+312, 330
NPB2	Ethanol 35–55 % + sodium perchlorate 15–40 % <i>Ethanol 35–55 % + Natriumperchlorat 15–40 %</i> CAS 64-17-5, 7601-89-0	 WARNING ACHTUNG	226, 302	210, 264W, 301+312, 330
NPW3 + NPW4	Ethanol 20–35 % + sodium perchlorate 15–40 % <i>Ethanol 20–35 % + Natriumperchlorat 15–40 %</i> CAS 64-17-5	 WARNING ACHTUNG	226, 302	210, 264W, 301+312, 330
Carrier RNA Buffer	Guanidinium thiocyanate 30–45 % <i>Guanidinthiocyanat 30–45 %</i> CAS 593-84-0	 WARNING ACHTUNG	302, 412	264W, 273, 301+312, 330
Proteinase K	Proteinase K 90–100 % <i>Proteinase K 90–100 %</i> CAS 39450-01-6	 DANGER GEFAHR	315, 319, 334	261sh, 280sh, 342+311

### Hazard phrases

H 226	Flammable liquid and vapor. <i>Flüssigkeit und Dampf entzündbar.</i>
H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 315	Causes skin irritation. <i>Verursacht Hautreizungen</i>
H 319	Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i>

- H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.  
*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
- H 412 Harmful to aquatic life with long lasting effects.  
*Schädlich für Wasserorganismen, mit langfristiger Wirkung.*

#### Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen*
- P 261sh Avoid breathing dust/vapors  
*Einatmen von Staub/Dampf vermeiden.*
- P 264W Wash with water thoroughly after handling.  
*Nach Gebrauch mit Wasser gründlich waschen.*
- P 273 Avoid release to the environment.  
*Freisetzung in die Umwelt vermeiden.*
- P 280sh Wear protective gloves/eye protection.  
*Schutzhandschuhe/Augenschutz tragen.*
- P 301+312 IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell.  
*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.*
- P 330 Rinse mouth.  
*Mund ausspülen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER / doctor  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM/Arzt anrufen.*



The symbol shown on labels refers to further safety information in this section.  
*Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.*

When working with the NucleoMag<sup>®</sup> Pathogen kit 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 <http://www.mn-net.com/msds>).

Caution: Guanidin hydrochloride in Lysis Buffer NPL1, sodium perchlorate in buffer NPB2, NPW3, NPW4 and guanidine 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<sup>®</sup> Pathogen** kit 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.*

#### 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 6.2).

#### 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 6.2).

#### 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 6.2)

#### 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.

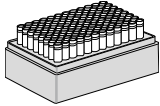

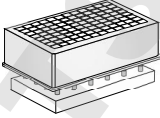
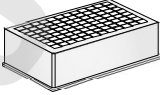
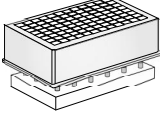
## 5.2 Protocol at a glance



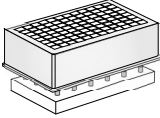


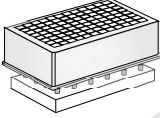
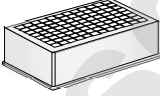
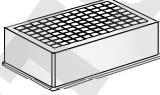

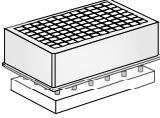
For hardware requirements refer to section 2.3.

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.

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

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

### 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 6.2). 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 6.2).

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.*

#### 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 6.2).

---

## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
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>
Low purity / low sensitivity	<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>
Low purity / low sensitivity	<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>
	<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>

<b>Problem</b>	<b>Possible cause and suggestions</b>
Poor performance of DNA / RNA in downstream applications	<i>Carry-over of ethanol from wash buffers</i> <ul style="list-style-type: none"><li>• Be sure to remove all of the 80% ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications.</li></ul>
	<i>Ethanol evaporation from wash buffers</i> <ul style="list-style-type: none"><li>• 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.</li></ul>
Carry-over of beads	<i>Time for magnetic separation too short</i> <ul style="list-style-type: none"><li>• Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.</li></ul>
	<i>Aspiration speed too high (elution step)</i> <ul style="list-style-type: none"><li>• High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.</li></ul>

## 6.2 Ordering information

Product	REF	Pack of
NucleoMag® Pathogen	744210.1 744210.4	1 x 96 preps 4 x 96 preps
NucleoMag® SEP	744900	1
NucleoMag® SEP Mini	744901	1
NucleoMag® SEPMaxi	744902	1
NucleoMag® SEP 24	744903	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
NucleoSpin® Forensic Filters (Semipermeable mini spin filter; individually blistered)	740988.50 740988.250	50 250
NucleoSpin® Forensic Filters (Bulk) (Semipermeable mini spin filter; bulk packed)	740988.50B 740988.250B 740988.1000B	50 250 1000
NucleoSpin® Trace Filter Plate (96-well filter plate to e.g., separate swabs from the lysate)	740677	20
NucleoSpin® Bead Tubes Type A	740786.50	50
96-well Accessory Kit A for KingFisher Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® Pathogen preps using KingFisher® Flex platform	744950	1 set
Buffer RA1 (60 mL)	740961	60 mL
Reducing agent TCEP	740395.107	107 mg

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

### 6.3 Product use restriction/warranty

**NucleoMag® Pathogen** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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 to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for 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; defects in products or

components not manufactured by MACHEREY-NAGEL, or 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.

Last updated: 07/2010, Rev. 03

Please contact:

MACHEREY-NAGEL GmbH & Co. KG

Tel.: +49 24 21 969-270

tech-bio@mn-net.com

---

Trademarks:

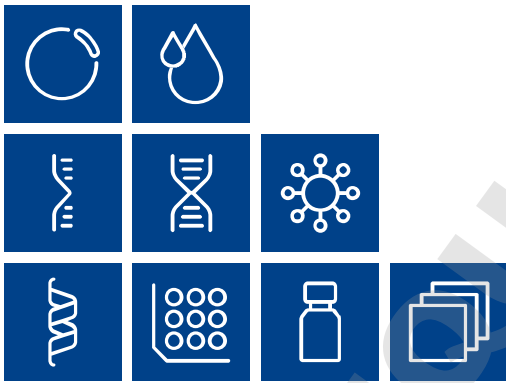
TRIzol is a registered trademark of Molecular Research Center, Inc.

KingFisher is a registered trademark of Thermo Fisher Scientific

NucleoMag is a registered trademark of MACHEREY-NAGEL GmbH & Co KG

Te-MagS is a trademark of Tecan Group Ltd., Switzerland

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation



Plasmid DNA

Clean up

RNA

DNA

Viral RNA and DNA

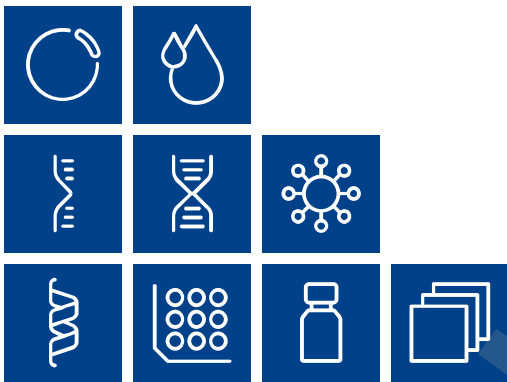
Protein

High throughput

Accessories

Auxiliary tools

DOMINIQUE DUTSCHER SAS



[www.mn-net.com](http://www.mn-net.com)

**MACHEREY-NAGEL**



MACHEREY-NAGEL GmbH & Co. KG  
Neumann-Neander-Str. 6-8  
52355 Düren · Germany

DE	Tel.: +49 24 21 969-0	<a href="mailto:info@mn-net.com">info@mn-net.com</a>
CH	Tel.: +41 62 388 55 00	<a href="mailto:sales-ch@mn-net.com">sales-ch@mn-net.com</a>
FR	Tel.: +33 388 68 22 68	<a href="mailto:sales-fr@mn-net.com">sales-fr@mn-net.com</a>
US	Tel.: +1 484 821 0984	<a href="mailto:sales-us@mn-net.com">sales-us@mn-net.com</a>

A058243/xxxx