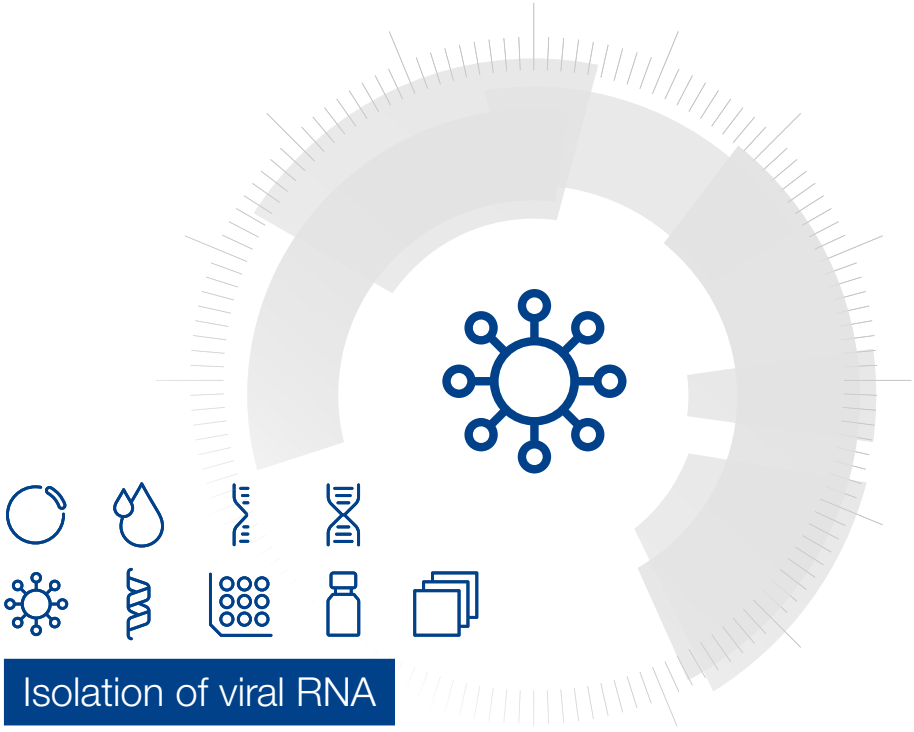
Bioanalysis

MACHEREY-NAGEL

User manual



Isolation of viral RNA

■ NucleoMag® Dx Pathogen

IVD *In-Vitro* Diagnostic Medical Device

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11 · 52355 Düren · Germany

April 2022 / Rev. 03

CE

REF 744215.4

Σ 384 preps

Table of contents

1 Components

1.1 Kit contents

	NucleoMag® Dx Pathogen	
REF	**Symbol**	**4 x 96 preps 744215.4**
NucleoMag® B-Beads	B-Beads	10 mL
Lysis Buffer NPL1	BUF NPL1	100 mL
Binding Buffer NPB2	BUF NPB2	3 x 110 mL
Wash Buffer NPW3	BUF NPW3	300 mL
Wash Buffer NPW4	BUF NPW4	300 mL
Elution Buffer NPE5	BUF NPE5	125 mL
Carrier RNA*	Carrier RNA	4 x 400 µg
Carrier RNA Buffer	Carrier RNA Buffer	4 x 500 µL
Proteinase K (lyophilized)*	Proteinase K	3 x 75 mg
Proteinase Buffer PB	BUF PB	15 mL
User manual		1

1.2 Reagents, consumables and equipment to be supplied by user

The needed equipment may vary depending on processing (e.g. manual or automated) and instrument setup or configuration. Please check with your local platform manufacturer regarding platform-specific consumables. Additionally, refer to section 2.7 for further details regarding the automation of NucleoMag® Dx Pathogen.

Product	**REF**	**Pack of**
Magnet for magnetic beads separation, NucleoMag® SEP	744900	1
Separation plate for magnetic beads separation, Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
Elution plate for collecting purified nucleic acids, Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24

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

Reagents:

- 80 % ethanol (v/v) (absolute or non-denatured ethanol)

Consumables:

- Disposable pipet tips (aerosol barrier and RNase-free pipet tips are recommended to avoid cross-contaminations)

Equipment for manual sample preparation

- Manual pipettors, preferably electronic 8-channel pipettes.
- Suitable shaking device (see section 2.7)
- Personal protection equipment (e.g. lab coat, gloves, googles)

1.3 About this user manual

It is strongly recommended to read the detailed protocol section of this user manual. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

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

Contact information

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Benutzerhandbücher in weiteren Sprachen sind im Downloads-Bereich auf der Produktseite verfügbar.

Les manuels d'utilisation dans d'autres langues sont disponibles dans la section Téléchargements de la page du produit.

Los manuales de usuario en otros idiomas están disponibles en la sección de descargas de la página del producto.

2 Product description

2.1 The intended purpose

NucleoMag® Dx Pathogen is a kit for the isolation of viral RNA from human respiratory swabs and saliva, for the subsequent *in-vitro* analysis. The product provides purified viral RNA to be used for subsequent down-stream analysis such as qRT-PCR or sequencing to obtain information about infections with RNA viruses. The product is used by professional users in diagnostic laboratories. The kit is suitable for automated use on laboratory automation platforms.

The **NucleoMag® Dx Pathogen** kit is not suitable for self-testing or near-patient testing. The user should have experience with molecular biological techniques including experience with swabs, saliva and other potentially infectious human sample materials.

The use of suitable controls such as internal controls, extraction controls, positive / negative controls is recommended.

2.2 Product use limitations

NucleoMag® Dx Pathogen is suitable for human respiratory swabs and saliva. NucleoMag® Dx Pathogen has not been validated for other sample materials. Nasopharyngeal (NP) swabs can be used for testing asymptomatic persons in a healthcare setting. For testing symptomatic patients other sample collection systems may be required. Synthetic fiber swabs with plastic shafts are recommended. Calcium alginate swabs or swabs with wooden shafts are not recommended, as they may contain substances that inhibit PCR testing.

Product performance has been established in SARS-CoV-2 diagnostic workflows from fresh and non-treated human respiratory swabs and saliva. Notwithstanding, performance characteristics for every RNA-virus species in the respective clinical samples or sample stabilization reagent have not been established and must be validated by the user. Additionally, extraction of viral RNA using the NucleoMag® Dx Pathogen on different automation platforms must be validated by the user.

Please refer to applicable guidelines for collection, handling and storage of clinical samples and other pre-analytical requirements.

2.3 Quality control

In accordance with MACHEREY-NAGEL's Quality Management System, each lot of NucleoMag® Dx Pathogen kit is tested against predetermined specifications to ensure consistent product quality.

2.4 The basic principle

The NucleoMag® Dx Pathogen kit is designed for the isolation of viral RNA from human respiratory swabs and saliva. The kit provides reagents and magnetic beads for isolation of 384 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 and NPW4 and 80 % ethanol. Residual ethanol from previous wash steps is removed by air-drying. Finally, highly pure viral RNA is eluted with low salt Elution Buffer NPE5 or water. Purified viral RNA can directly be used for downstream applications. The NucleoMag® Dx Pathogen kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

Carrier RNA

Carrier RNA is included for optimal performance. Carrier RNA enhances binding of viral nucleic acids to the magnetic beads and reduces the risk of viral RNA degradation. Please note that eluates of the NucleoMag® Dx Pathogen kit contain both viral nucleic acids and Carrier RNA with amounts of Carrier RNA that may exceed the amount of viral nucleic acids. Therefore, it is not possible to quantify the nucleic acids isolated with the kit by photometric or fluorometric methods when using the Carrier RNA. Thus, other methods for quantification such as specific quantitative PCR or RT-PCR systems are recommended. Furthermore, Carrier RNA may inhibit in rare cases PCR reactions. The amount of added Carrier RNA may thus be carefully optimized depending on the individual PCR system used.

2.5 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoMag® Dx Pathogen
Technology	Magnetic bead technology
Sample material	Respiratory swabs and saliva (human)
Sample volume	200 µL
Elution volume	50 – 100 µL
Preparation time	Approx. 40 – 120 min/96 preps *
Processing	Manual or automated

Depending on instrument configuration and setup

2.6 Sample quality and preparation

Nasopharyngeal (NP) swabs can be used for testing asymptomatic persons in a healthcare setting. For testing symptomatic patients other sample collection systems may be required. Synthetic fiber swabs with plastic shafts are recommended. Calcium alginate swabs or swabs with wooden shafts are not recommended, as they may contain substances that inhibit PCR testing.

Please refer to applicable guidelines for collection, handling and storage of clinical samples and other pre-analytical requirements.

2.7 Performance evaluation on automated systems

The NucleoMag® Dx Pathogen can be automated on various liquid handling platforms or automated magnetic separators. However, extraction of viral RNA using the NucleoMag® Dx Pathogen on different automation platforms must be validated by the user in conjunction with a downstream *in-vitro* diagnostic assay depending on the target pathogen and in conjunction with suitable controls for downstream applications (e.g., internal controls, extraction controls, positive / negative controls).

It is highly recommended to use extraction controls, positive / negative controls, internal controls as well as to perform cross-contamination assays during the implementation of NucleoMag® Dx Pathogen on automated platforms.

The following chapters provide a guideline for automating the NucleoMag® Dx Pathogen kit and how to evaluate the performance on an automated system.

2.7.1 General handling of magnetic 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. During automation, a premix step before aspirating the beads from the reservoir / tube is recommended to keep the beads resuspended.

2.7.2 Liquid handling systems

The NucleoMag® Dx Pathogen can be automated on various liquid handling platforms using the NucleoMag® SEP (MN REF: 744900) in combination with the Square-well Block (MN REF: 740481) and with a suitable shaking device for optimal resuspension during binding, washing and elution steps. Additionally, 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. The complete resuspension of the magnetic beads during the extraction is essential for a reliable performance and should be checked during the validation on liquid handling systems. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times.

The following schematic shows the basic principle of the extraction using a static magnetic separator starting from the binding step with the addition of magnetic beads and binding buffer.

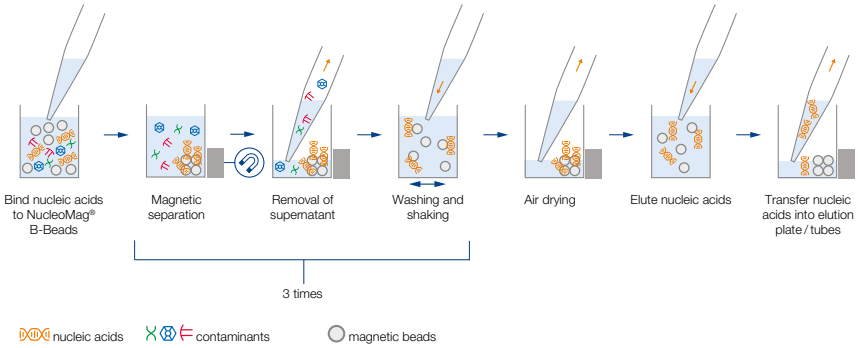

Figure 1 Basic principle of magnetic bead-based extraction using a static magnetic separator

2.7.3 Adjusting the shaker settings

Efficient resuspension of the NucleoMag® B-Beads is essential for reliable extraction and is dependent on the specifications of the used shaking device (e.g. speed and orbit). When using a plate shaker for the binding, washing and elution steps, the speed settings have to be adjusted carefully for the Square-well Block and specific shaking device to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and washing steps:

Load 1030 µL (total volume of binding step) or 600 µL (volume of washing steps) of dyed water to the wells of a 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 a collection plate and proceed as described above.

The following table should give an overview of already tested settings on different platforms and can serve as a first guideline during the automation process. It is highly recommended to use internal controls, extractions as well as positive and negative controls during the validation. It is recommended to loosen the magnetic bead pellet for 30 sec on a shaking device before dispensing buffer easing the resuspension of magnetic beads. Depending on resuspension efficiency, it might be necessary to perform a pipette mixing step.

Liquid handling system / Shaking device	Speed	Time
Thermomixer comfort (Eppendorf)	Lysis: 600 rpm	15 min
	Binding 1000 rpm	5 min
	Washing: 1000 rpm	2 min
	Elution 1000 rpm	5 min
epMotion® 5075t TMX (Eppendorf)	Lysis: 1200 rpm	15 min
	Binding: 1000 rpm	5 min
	Washing: 1200 rpm	2 min
	Elution 1200 rpm	5 min
Te-Shake™ (Tecan)	Lysis 1400 rpm	15 min
	Binding: 1400 rpm*	5 min
	Washing: 1400 rpm **	3 min
	Elution: 1000 rpm	5 min
Hamilton Heater Shaker HHS (Hamilton)	Lysis: 1200 rpm	15 min
	Binding: 1200 rpm	5 min
	Washing: 1200 rpm	2 min
	Elution: 1200 rpm	5 min

It is highly recommended to perform an initial cross-contamination assay (e.g. checkerboard pattern of positive- and negative samples) during the implementation and adjustment of NucleoMag® Dx Pathogen kit on automated platforms.

Individual protocol steps may vary depending on available consumables, hardware, platform and instrument setup. The automated workflow or script for the extraction of viral RNA using the NucleoMag® Dx Pathogen kit on different automation platforms must be validated by the user in conjunction with the respective downstream analysis.

*Including Pipette-Mixing-steps

**Alternating directions

2.7.4 Automated magnetic separation systems

The NucleoMag® Dx Pathogen can be automated on different automated magnetic separation platforms using the specific consumables of the respective instrument. The magnetic beads are usually resuspended by the movement of the plastic sheath (tip-comb) covering the magnetic rods. Following the binding, washing and elution steps the beads are collected again with the magnetic rods. In most of the cases, buffers and components have to be dispensed individually before starting the purification protocol. Please make sure not to exceed the maximum filling volume per reaction container according to the manufacturers specifications. All settings should be validated by the user in conjunction with their specific platform configuration and down-stream analysis. It is highly recommended to use internal controls, extractions as well as positive and negative controls during the validation of platform settings.

The following schematic shows the basic principle of the extraction using an automated magnetic separation systems.

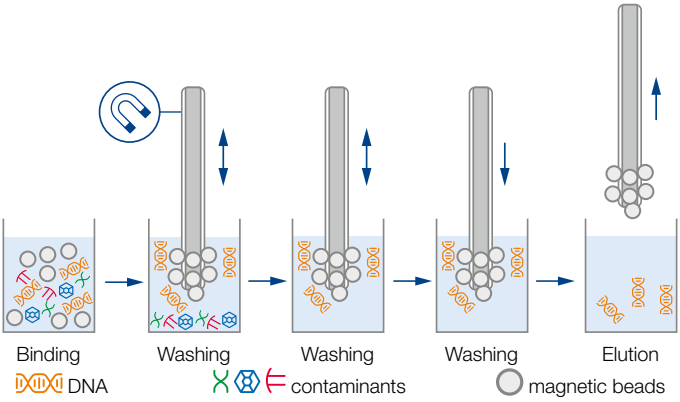

Figure 2 Basic principle showing the magnetic bead based extraction using an automated separation systems.

2.8 Elution procedures

Purified pathogen RNA can be eluted directly with the supplied elution buffer. Elution can be carried out in a volume of 50 – 100 µL. It is essential to cover the NucleoMag® B-Beads completely with elution buffer during the elution step. For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer.

Storage of nucleic acids

Recommendation:
Short term storage (up to 24 h): 2 – 8 °C
Long term storage (over 24 h): -20 °C

2.9 Analytical and clinical performance

Analytical performance of NucleoMag® Dx Pathogen was evaluated with down-stream RT-qPCR of isolated MS2-RNA. Within run repeatability was calculated from parallel isolation of 96 samples. With n=96 a mean Ct-value of 27.92 ± 0.118 and a CV < 1 % was obtained. Between run repeatability was calculated from 3 independent MS2-RNA isolation runs. For 4 MS2-RNA inputs the standard deviation from 3 runs each was less than 0.1 with a CV of 0.26 %. For batch-to-batch repeatability 3 batches of NucleoMag® Dx Pathogen were tested side by side. For 3 MS2-RNA inputs the standard deviation from 3 batches each was less than 0.61 with a CV of 1.49 %.

For the evaluation of reproducibility between operators, four MS2-RNA inputs were isolated in two independent runs done by two different operators. The average ΔΔCt-value for the 4 concentrations between operators was calculated as less than 0.1.

In study with SARS-CoV-2 positive samples log10 dilution series were generated in negative pool material of oral swabs, nasal swabs and saliva. Extracted RNA was tested in two different real-time RT-PCR systems, two different master mixes in combination with two different internal control systems (beta-actin-DNA-mix 2 and IC2-RNA/EGFP-Mix 1).

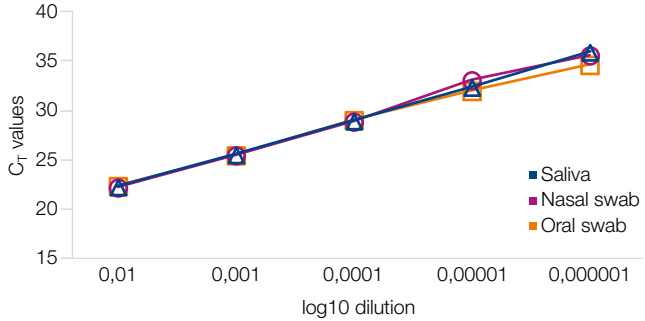

Figure 3 Log10 dilution series of SARS-CoV-2 in saliva, nasal and oral swabs. NucleoMag® Dx Pathogen on KingFisher™ Flex (Thermo Fisher Scientific), AgPath-ID™ One-Step RT-PCR kit (Thermo Fisher Scientific), nCoV-IP4 Assay (Institut Pasteur, Paris). Data kindly provided by Dr. B. Hoffmann, Friedrich-Löffler Institute, Germany.

For the evaluation of clinical performance SARS-CoV2 RNA was isolated from swabs and amplified in SARS-CoV2 RT-qPCR assays. The positive and negative controls were evaluated. In 27 runs with 94 samples per run all 27 positive controls and all 27 negative controls were measured as expected. Diagnostic sensitivity and diagnostic specificity was 100%.

3 Storage conditions and preparation of working solutions

Attention:

NPL1, NPB2, NPW3, NPW4 and the Carrier RNA Buffer contain chaotropic salt (e.g. guanidine hydrochloride and/or sodium perchlorate) which can form highly reactive compounds when combined with bleach (sodium hypochlorite)! DO NOT add bleach or acidic solutions directly to the sample preparation waste. Wear suitable protective clothing, gloves and safety goggles!

- Check all components for damages after receiving the kit. If kit content like buffer bottles, screw cap tubes or glass vials are damaged, contact MACHEREY-NAGEL technical support and customer service, or your local distributor.
- Do not use damaged kit components.
- Use RNase-free equipment.
- Upon arrival, all components of the **NucleoMag® Dx Pathogen** kit should be stored at room temperature (18 – 25 °C). Do not use the product after the expiration date.
- Lyophilized Proteinase K can be stored at room temperature (18 – 25 °C) until the expiration date without decrease in performance. Reconstituted Proteinase K should be stored at -20 °C for up to 6 months, but only until the expiration date.
- Lyophilized Carrier RNA can be stored at room temperature (18 – 25 °C) until the expiration date without decrease in performance. Reconstituted Carrier RNA should be stored at -20 °C for up to 6 months, but only until the expiration date.
- All buffers are delivered ready-to-use.

Before starting any **NucleoMag® Dx 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 and dissolve Proteinase K**. Dissolved Proteinase K solution should be stored at -20 °C for up to 6 months, but only until the expiration date.
- **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, but only until the expiration date. *Note: Due to the production procedure and the small amount of Carrier RNA contained in the vial, the carrier may hardly be visible in the vial.*
- Do not freeze-thaw or warm up reconstituted Carrier RNA repeatedly! Frequent freeze-thaw-cycles, frequent warming, temperatures > 80 °C and extended heat incubation will accelerate the degradation of Carrier RNA. It is recommended to store reconstituted Proteinase K and Carrier RNA in aliquots.

NucleoMag® Dx Pathogen	
REF	**4 x 96 preps** **744215.4**
Proteinase K (lyophilized)	3 vials (75 mg/vial) Add 3.35 mL Buffer PB before first use.
Carrier RNA (lyophilized)	4 vials (400 µg/vial) Dissolve in 500 µL Carrier RNA Buffer before first use.

4 Safety instructions

When working with the **NucleoMag® Dx 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® Dx 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.*

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 from respiratory swabs and saliva

Individual protocol steps may vary depending on available consumables, hardware, platform and instrument setup. The automated workflow or script for the extraction of viral RNA using the NucleoMag® Dx Pathogen on different automation platforms must be validated by the user in conjunction with the respective downstream analysis.

The procedure below provides instructions for manual processing of a single sample in a Square-well Block in combination with the Eppendorf Thermomixer comfort. However, multiple samples (up to 96 samples per Square-well Block) can be processed at the same time in a manual or automated manner. Please read section 2 carefully before starting the extraction or implementation on automated platforms.

5.1 Preparation of sample materials

The NucleoMag® Dx Pathogen kit is suitable for fresh and non-treated human respiratory swabs and saliva. Nasopharyngeal (NP) swabs can be used for testing asymptomatic persons in a healthcare setting. For testing symptomatic patients other sample collection systems may be required. Synthetic fiber swabs with plastic shafts are recommended. Calcium alginate swabs or swabs with wooden shafts are not recommended, as they may contain substances that inhibit PCR testing.

Please refer to applicable guidelines for collection, handling and storage of clinical samples and other pre-analytical requirements.

a) Swab samples

Incubate the swabs in PBS, sodium chloride, or cell culture medium for 30 min with agitation. Make sure that the swab head is completely submerged. Transfer 200 µL of the solution for further processing. If necessary, press the swab against the wall of the tube to squeeze out the liquid.

b) Saliva

Fresh and non-treated saliva samples can be directly subjected to extraction procedure. In case of highly viscous samples it is recommended to dilute the saliva samples with sterile PBS. Transfer 100 µL or 200 µL of the solution for further processing. When processing less than 200 µL sample adjust with PBS buffer to a finale volume of 200 µL.

Before starting the preparation:

- Check that Proteinase K was prepared according to section 3.
- Check that Carrier RNA was prepared according to section 3.
- Check that 80 % ethanol (non-denatured) is available.
- Check that the automated platform or shaking device has been set up appropriately.
- Equilibrate the samples to room temperature (18 – 25 °C). Make sure that the samples are mixed well.
- Generally, do not mix reagents from different kits and lots.
- Do not add Proteinase K directly to Lysis Buffer NPL1. The sample has to be combined with the Lysis Buffer NPL1 before addition of Proteinase K.
- All processing steps should be carried out at room temperature (18 – 25 °C)

5.2 Protocol at a glance

Supplemental protocol-overview:

Carefully read the detailed protocol (section 5.3) as well as section 2 before starting the procedure. The procedure below provides instructions for manual processing of a single sample in a Square-well Block in combination with the Eppendorf Thermomixer comfort.

Do not moisten the rims of the well.

1	**Provide sample and lyse viruses**	
	1	200 µL sample in Square-well Block
	2	180 µL Lysis Buffer NPL1
	3	4 µL Carrier RNA stock solution
	4	20 µL Proteinase K solution
	5	Mix well by repeated pipetting up and down or shaking.
	6	Incubate at RT for 15 min at 600 rpm
2	**Bind RNA**	
	7	20 µL NucleoMag® B-Beads
	8	600 µL Binding Buffer NPB2
	9	Mix well by repeated pipetting up and down or shaking and incubate for 5 min at 1000 rpm.
	10	Separate magnetic beads for 2 min
	11	Remove supernatant
	12	Remove Square-well Block from NucleoMag® SEP

3	**Wash magnetic beads**	
	13	600 µL Wash Buffer NPW3
	14	Mix well by repeated pipetting up and down or shaking and incubate for 2 min at 1000 rpm.
	15	Separate magnetic beads for 2 min
	16	Remove supernatant
	17	Remove Square-well Block from NucleoMag® SEP
	18	600 µL Wash Buffer NPW4
	19	Mix well by repeated pipetting up and down or shaking and incubate for 2 min at 1000 rpm.
	20	Separate magnetic beads for 2 min
	21	Remove supernatant
	22	Remove Square-well Block from NucleoMag® SEP
	23	600 µL 80 % Ethanol
	24	Mix well by repeated pipetting up and down or shaking and incubate for 2 min at 1000 rpm.
	25	Separate magnetic beads for 2 min
	26	Remove supernatant
4	**Drying**	
	27	Air dry the magnetic beads for 10 min at RT
5	**Elute RNA**	
	28	Remove Square-well Block from NucleoMag® SEP
	29	100 µL Elution Buffer NPE5
	30	Mix well by repeated pipetting up and down or shaking.
	31	Incubate for 5 min at 1000 rpm.
	32	Separate magnetic beads for 2 min
	33	Transfer supernatant of the eluted sample to the elution plate

5.3 Detailed protocol

The procedure below provides instructions for manual processing of a single sample in a Square-well Block in combination with the NucleoMag® SEP and the Eppendorf Thermomixer comfort. Individual protocol steps may vary depending on available consumables, hardware, platform and instrument setup and must be validated by the user.

Alternatively, isolation of pathogen RNA 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 automation platforms instruments.

Do not moisten the rims of the well.

1	Provide **200 µL sample** in each well of the Square-well Block
2	Add **180 µL Lysis Buffer NPL1** to each sample.
3	Add **4 µL of Carrier RNA** stock solution to each sample.
4	Add **20 µL Proteinase K** solution to each sample.
5	Mix well by repeated pipetting up and down or shaking. Optional: Seal the plate with a suitable adhesive foil
6	Incubate for **15 min at room temperature** with at 600 rpm Optional: In case of a sealed or closed plate. Briefly spin down to collect any samples from the lid.
7	Add **20 µL resuspended NucleoMag® B-Beads** to each sample. *Note: Make sure that the NucleoMag® B-Beads are completely resuspended before removing them from the storage bottle. Vortex storage bottle until a homogenous bead suspension is obtained.*
8	Add **600 µL Binding Buffer NPB2** to each sample.
9	Mix well by pipetting up and down or shaking. and incubate for 5 min at 1000 rpm. *Note: A homogenous solution should be visible.*
10	Place the Square-well Block on the NucleoMag® SEP magnetic separator.
11	Wait at least 2 min until all beads have been attracted to the magnet. *Note: Depending on sample type, a clear solution should be visible.*
12	Remove and discard the supernatant carefully by pipetting. *Note: Do not disturb the attracted magnetic beads while aspirating the supernatant.*
13	Remove the Square-well Block from the NucleoMag® SEP magnetic separator.
14	Add **600 µL Buffer NPW3** to each sample.
15	Move the Square-well Block onto the Thermomixer.
16	Resuspend the magnetic beads by shaking at 1000 rpm for 2 min until the beads are completely resuspended
17	Place the Square-well Block on the NucleoMag® SEP a magnetic separator.
18	Wait at least 2 min until all beads have been attracted to the magnet

19	Remove and discard the supernatant carefully by pipetting.
20	Remove the Square-well Block from the NucleoMag® SEP magnetic separator.
21	Add **600 µL Buffer NPW4** to each sample.
22	Move the Square-well Block onto the Thermomixer.
23	Resuspend the magnetic beads by shaking at 1000 rpm for 2 min until the beads are completely resuspended
24	Place the Square-well Block on the NucleoMag® SEP magnetic separator.
25	Wait at least 2 min until all beads have been attracted to the magnet
26	Remove and discard the supernatant carefully by pipetting.
27	Remove the Square-well Block from the NucleoMag® SEP magnetic separator.
28	Add **600 µL of 80 % ethanol** to each sample.
29	Move the Square-well Block onto the Thermomixer.
30	Resuspend the magnetic beads by shaking at 1000 rpm for 2 min until the beads are completely resuspended
31	Place the Square-well Block on the NucleoMag® SEP a magnetic separator.
32	Wait at least 2 min until all beads have been attracted to the magnet
33	Remove and discard the supernatant carefully by pipetting.
34	Air dry the magnetic bead pellet for 10 min at room temperature. *Note: Be sure to remove all of the 80 % ethanolic wash solution from the final wash before the drying step.*
35	Remove the Square-well Block from the NucleoMag® SEP magnetic separator.
36	Add **100 µL of elution buffer NPE5** to each reaction container
37	Move the Square-well Block onto the Thermomixer
38	Resuspend the magnetic beads by moderate shaking until the beads are completely resuspended for 5 min at room temperature at 1000 rpm..
39	Place the Square-well Block on the NucleoMag® SEP magnetic separator.
40	Wait at least 2 min until all beads have been attracted to the magnet
41	Carefully transfer the eluate to a suitable elution plate *Note: Do not disturb the magnetic bead pellet.*

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor yield / low sensitivity	*Incomplete sample lysis* • 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. *Insufficient elution buffer volume* • Bead pellet must be covered completely with elution buffer and needs to be fully resuspended. *Insufficient performance of elution buffer during elution step* • Remove all buffers completely from the bead pellet after the binding and wash steps. Remaining buffer decreases the efficiency of the subsequent steps. *Aspiration of attracted bead pellet* • 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. *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, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely (e.g. evenly brownish liquid) during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.

Problem	Possible cause and suggestions
Poor performance of RNA in downstream applications / RT-qPCR inhibition	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the 80 % ethanolic wash solution from the final wash. Residual ethanol interferes with downstream applications. • Be sure to aspirate the supernatant completely after each magnetic separation. Double-check for residual wash buffer after each supernatant removal. Adjust aspiration heights in case of an excess residual buffer volume after supernatant removal. *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 or suboptimal aspiration heights during the elution step may cause bead carry-over. Reduce aspiration speed and adjust aspiration height for elution step.

6.2 Notification requirement

Please note that any serious incident that has occurred in relation to the product shall be reported immediately to the manufacturer and the competent authority of the European member state in which the incident occurred. European vigilance contact points: https://ec.europa.eu/health/md_sector/contact_en

6.3 General literature

Thiemann F. et al. (2006) Leitfaden Molekulare Diagnostik - Grundlagen, Gesetze, Tipps und Tricks, WILEY-VCH, 1st ed., ISBN 3-527-31471-7.

Paysic J. et al- (2015). Standardization of Nucleic Acid Tests for Clinical Measurements of Bacteria and Viruses. J Clin Microbiol., 53(7), 2008 – 14.

Cohen, J. et al. (2016) Infectious Diseases, Elsevier, 4th ed., ISBN: 9780702062858.

Vemula S. V. et a. (2016) Current Approaches for Diagnosis of Influenza Virus Infections in Humans, Viruses 8(4), 96.

6.4 Ordering information

Product	REF	Pack of
CE-IVD marked kits		
NucleoMag® Dx Pathogen	744215.4	384 preps
NucleoSpin® Dx Virus	740895.50	50 preps
NucleoSpin® Dx Blood	740899.50	50 preps
	740899.250	250 preps
Kit for research purposes		
NucleoMag® Pathogen (RUO)	744210.1 744210.4	1 x 96 preps 4 x 96 preps
Accessory products		
NucleoMag® SEP	744900	1
Square-well Blocks	740481 740481.24	4 24

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

6.5 Explanation of symbols

REF Item number

LOT Batch identification

Manufacturer

IVD *In-vitro* diagnostic products

Please read instructions for use

Sufficient for <n> tests

Permitted storage temperature range

Use by

Caution: Further information in user manual

Do not reuse

384

6.6 Product use restriction / warranty

The **NucleoMag® Dx Pathogen** kit is a generic system for the isolation and purification of viral RNA from human respiratory samples and saliva for subsequent *in-vitro* diagnostic purposes.

The kit is designed to be used with any downstream application employing enzymatic amplification and detection of RNA (e.g., RT-PCR).

Any and all diagnostic results generated using nucleic acids isolated with the **NucleoMag® Dx Pathogen** kit in conjunction with a diagnostic assay should be interpreted with regard to additional clinical or laboratory findings.

The **NucleoMag® Dx Pathogen** kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in-vitro* diagnostic assay. ONLY MACHEREY-NAGEL products specially labeled as IVD are suitable for In-vitro-diagnostic use.

For safety instructions please refer to the respective chapter in the user manual or the MSDS file. **NucleoMag® Dx Pathogen** kit shall exclusively be used in an adequate test environment, i.e. a suitable laboratory setting. The respective user is liable for any and all damages resulting from application of the **NucleoMag® Dx Pathogen** kit for use deviating from the intended use as specified in the user manual.

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.

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Reason for revision: Addition of analytical and clinical performance data to section 2.9. Reference to new languages of the user manual.

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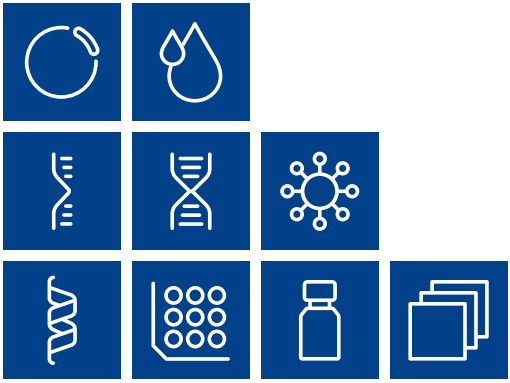

www.mn-net.com

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