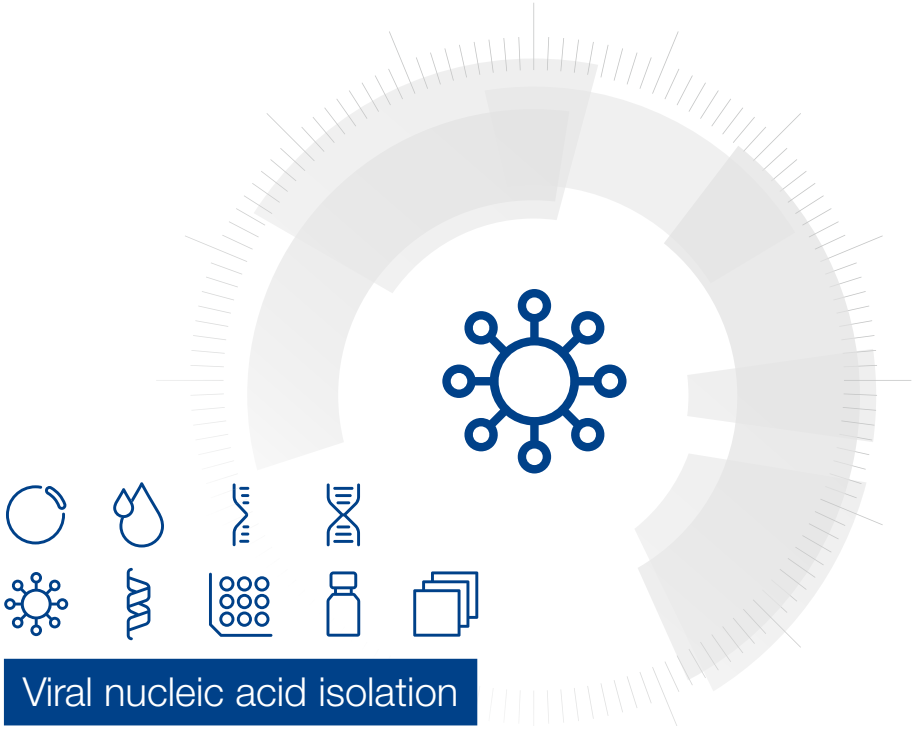
Bioanalysis

MACHEREY-NAGEL

User manual



Viral nucleic acid isolation

■ NucleoSpin® Dx Virus

IVD *In-Vitro* Diagnostic Medical Device

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

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CE

REF 740895.50

Σ 50 preps

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1 Components

1.1 Kit contents

		NucleoSpin® Dx Virus
REF	**Symbol**	**50 preps 740895.50**
Lysis Buffer RAV1	BUF RAV1	35 mL
Wash Buffer RAW	BUF RAW	30 mL
Wash Buffer RAV3 (Concentrate)*	BUF RAV3 Conc.	12 mL
RNase-free H_2O	H_2O	13 mL
Elution Buffer RE**	BUF RE	13 mL
Carrier RNA (lyophilized)*	Carrier RNA	1 mg
Proteinase Buffer PB	BUF PB	1.8 mL
Proteinase K (lyophilized)*	Proteinase K	30 mg
NucleoSpin® Dx Virus Columns (dark blue rings - plus Collection Tubes)	Dx Virus Columns	50
Collection Tubes (2 mL)	Collection Tubes	4 x 50
Lysis Tubes (1.5 mL)	Lysis Tubes	50
Elution Tubes (1.5 mL)	Elution Tubes	50
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* For preparation of working solutions and storage conditions see section 3.

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

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (to adjust nucleic acid binding conditions and to prepare Wash Buffer RAV3)

Consumables

- Disposable pipet tips (aerosol barrier pipet tips are recommended to avoid cross-contamination)

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating block or water bath for 70 °C incubation
- Personal protection equipment (e.g., lab coat, gloves, goggles)

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.

MACHEREY-NAGEL user manuals are available on the internet at ***www.mn-net.com***.

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

Contact information

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Benutzerhandbucher in weiteren Sprachen sind im Download-Bereich auf der Produktseite verfügbar.

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

Los manuales de usuario en otros idiomas estan disponibles en la seccion de descargas de a pagina del producto.

2 Product description

2.1 Intended use

NucleoSpin® Dx Virus is a kit for the isolation of viral nucleic acids from fresh and frozen human serum and plasma, stabilized with either EDTA or citrate from common blood collection systems for the subsequent *in-vitro* analysis. The product provides purified viral nucleic acids to be used for subsequent down-stream analysis such as (RT)-PCR, qPCR, qRT-PCR or sequencing to obtain information about infections with viruses. The product is used by professional users in diagnostic laboratories.

The **NucleoSpin® Dx Virus** kit is not suitable for self-testing or near-patient testing. The user should have experience with molecular biological techniques including experience with serum, plasma 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

The **NucleoSpin® Dx Virus** kit is not for use with human whole blood, tissue, stool samples, or cultured cells.

The kit performance has not been evaluated with other cell-free fluid samples like urine or cerebrospinal fluid.

The kit is also neither specified for the isolation and purification of bacterial, fungal, or parasite nucleic acids from human samples nor for the isolation of viral nucleic acids from human swab samples or other sample collections systems.

Besides human samples also fresh and frozen animal samples can readily be used together with the **NucleoSpin® Dx Virus** kit. Samples include, but are not limited to, serum, plasma, or swabs. It has to be noted that CE IVD labeling of the kit does not apply for animal samples but is limited to human diagnostic use only.

2.3 Quality control

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

2.4 Introduction and kit specifications

NucleoSpin® Dx Virus is based on well-established **NucleoSpin®** silica membrane technology and provides an easy way to isolate viral RNA and viral DNA simultaneously from 150 µL of serum or plasma samples. Purified RNA and DNA are ready to use for downstream amplifications like RT-PCR or PCR.

The **NucleoSpin® Dx Virus** procedure is based on a series of simple steps:

First, the serum or plasma samples are lysed in the presence of chaotropic salts. For the purification of viral DNA, Proteinase K is added to the lysis reaction. Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the **NucleoSpin® Dx Virus Columns**. Carrier RNA improves binding and recovery of low-concentrated viral RNA and DNA. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in washing steps with ethanolic buffers RAW and RAV3. The nucleic acids are finally eluted in 50 µL low salt buffer or water.

Carrier RNA

Carrier RNA is included for optimal performance. Carrier RNA enhances binding of viral nucleic acids to the NucleoSpin Columns and reduces the risk of viral RNA degradation. Please note that eluates of the NucleoSpin Dx Virus 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.

Kit specifications

- **NucleoSpin® Dx Virus** is designed for the rapid preparation of highly pure viral RNA and DNA (e.g., HCV, HIV, HBV, CMV, H1N1) from plasma and serum.
- **NucleoSpin® Dx Virus** is suitable for 150 µL serum or plasma samples.
- The viral nucleic acids isolated and purified with **NucleoSpin® Dx Virus** can be used in qualitative applications (e.g., RT-PCR or PCR for blood screening) as well as in quantitative applications (e.g., detection of viral load by qPCR) employing diagnostic nucleic acid amplification techniques.
- Protocols for isolation of viral RNA, viral DNA, and simultaneous isolation of viral RNA and DNA are included in the user manual.
- The prepared nucleic acids are suitable for applications like automated fluorescent DNA sequencing, RT-PCR, or any kind of enzymatic reaction. The detection limit for certain viruses depends on the individual procedures (e.g., in-house nested (RT-) PCR). To minimize irregularities in diagnostic results, suitable controls for downstream

applications (e.g., extraction controls, positive / negative controls) should be used to monitor the purification, amplification, and detection process.

- Besides human samples also fresh and frozen animal samples can readily be used together with the **NucleoSpin® Dx Virus** kit. Samples include, but are not limited to, serum, plasma, or swabs. It has to be noted that CE IVD labeling of the kit does not apply for animal samples but is limited to human diagnostic use only.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Dx Virus
Technology	Silica membrane technology
Sample material	Serum or plasma
Sample volume	150 µL
Elution volume	50 µL
Preparation time	30 min / 4 – 6 preps
Processing	Centrifugation

2.5 Analytical and clinical performance

The linear range of the **NucleoSpin® Dx Virus** procedure has been determined for HCV RNA and HBV DNA in downstream diagnostic assays (Figure 1 and Figure 2). The kit shows linearity over several orders of magnitude, comprising relevant viral titer for diagnostic purposes. Within run repeatability was tested with RT-qPCR of MS2-RNA and qPCR of T7-DNA. For six spike amounts – each in triplicate, covering several orders of magnitude – the coefficient of variation (CV) of the Cp-values was 0.2 – 0.9 % for T7-DNA and 0.6 – 5.6 % for MS2-RNA. Between run repeatability was tested in 2 independent runs. With six plasma samples each the difference between the average Ct-values of the two runs was 0.1 cycle corresponding to a 0.4 % difference between the average Ct-values of the two runs. Batch-to-batch repeatability was tested with three batches of NucleoSpin Dx Virus. gDNA from plasma samples was isolated for each batch (n=6). The mean Ct-value for the three batches tested was 27.63 Ct with a standard deviation of 0.07 Ct-value. In a similar approach, an MS2-RNA spike was isolated from plasma samples and analyzed by qRT-PCR. The mean Ct-value for the three batches tested was 25.34 Ct with a standard deviation of 0.25 Ct-value.

Reproducibility between operators was tested with RT-qPCR of MS2-RNA. In two runs performed by two operators with six plasma samples each the difference between the average Ct-values of the two operators was 0.6 cycles corresponding to a 3 % difference between the average Ct-values of the two operators.

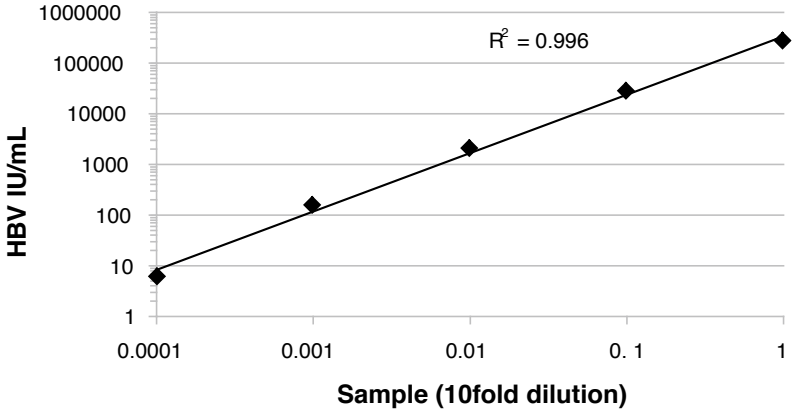

Figure 1 Serial dilution of a plasma sample with high HBV viral load.
Real-time PCR of HBV DNA: Artus RealArt HBV DNA, quantification in Roche LightCycler® 480.

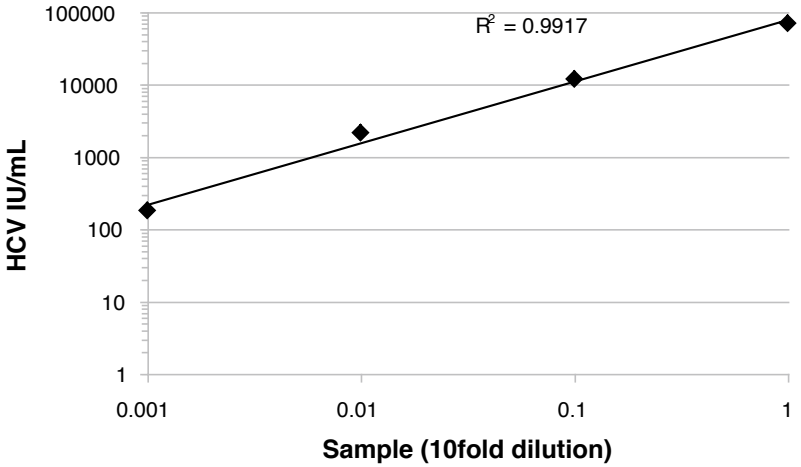

Figure 2 Serial dilution of a plasma sample with high HCV viral load.
Real-time RT-PCR of HCV RNA: Artus RealArt HCV RNA, quantification in Roche LightCycler® 480.

For the evaluation of clinical performance viral nucleic acids were isolated from plasma samples and amplified in qPCR and RT-qPCR assays. Virus load as obtained with NucleoSpin Dx Virus was compared to a reference system (automated nucleic acid isolation system from Abbott). For each virus 8 positive and 2 negative samples as well as 1 positive and 1 negative control were evaluated. For HBV diagnostic sensitivity, and diagnostic specificity were 100 %. For HCV diagnostic sensitivity was 89 %, whereas diagnostic specificity was 100 %. For HIV diagnostic sensitivity was 78 %, and diagnostic specificity was 100 %.

In-vitro diagnostic use of **NucleoSpin Dx® Virus** is exemplified in the following publications:

Raharinosy, V. *et al.* (2019) Fast, Sensitive and Specific Detection of Thailand orthohantavirus and its Variants Using One-Step Real-Time Reverse-Transcription Polymerase Chain Reaction Assay. Viruses, 11(8), 718.

Kassela, K. *et al.* (2019) Intergenotypic 2k/1b hepatitis C virus recombinants in the East Macedonia and Thrace region of Greece. Ann Gastroenterol., 32(1), 88 – 92.

Mousavi, S. H. *et al.* (2019) First Report of Prevalence of Blood-Borne Viruses (HBV, HCV, HIV, HTLV-1 and Parvovirus B19) Among Hemophilia Patients in Afghanistan. Sci Rep., 9(1), 7259.

Hesamizadeh, K. *et al.* (2016) Molecular Epidemiology of Kaposi's Sarcoma-Associated Herpes Virus, and Risk Factors in HIV-infected Patients in Tehran, 2014. Iran Red Crescent Med J., 18(11), e32603.

Lescure, F.-X. *et al.* (2020) Clinical and virological data of the first cases of COVID-19 in Europe: a case series. The Lance Infectious Diseases, 20(6), 697.

Thacker, V. V. *et al.* (2020) Rapid endothelialitis and vascular inflammation characterise SARS-CoV-2 infection in a human lung-on-chip model, BioRxiv, https://doi.org/10.1101/2020.08.10.243220, 2020

Gabaro, F. *et al.* (2020) Introductions and early spread of SARS-CoV-2 in France, BioRxiv, https://doi.org/10.1101/2020.04.24.059576

2.6 Remarks regarding sample quality and preparation

- **NucleoSpin® Dx Virus** is suitable for human serum or plasma samples. It is very important to avoid clearing samples by centrifugation / filtration before the RAV1-lysis step, because viruses may be associated with particles or aggregates.
- For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and nonviscous sample lysate before adjusting binding conditions and loading the sample onto the **NucleoSpin® Dx Virus** Column. Check all lysates (especially of old or frozen samples) for precipitates.
  Incubation with Buffer RAV1 can be prolonged to dissolve and digest residual cell structures, precipitates and virus particles. However, RNA is sensitive and prolonged incubation may cause decreased yields.

2.7 Remarks regarding elution

- Pure nucleic acids are finally eluted under low ionic strength conditions with RNase-free H_2O (pH about 7 – 8) or slightly alkaline Buffer RE (5 mM Tris-HCl, pH 8.5), both are supplied with **NucleoSpin® Dx Virus**.
- RNA should be eluted with the RNase-free H_2O and DNA with Elution Buffer RE.
- To elute both types of nucleic acids together, use RNase-free H_2O provided with the kit, preheated to 70 °C.

Storage of nucleic acids

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

3 Storage conditions and preparation of working solutions

Attention: *Buffer RAV1 contains guanidinium thiocyanate and Buffer RAW contains guanidine hydrochloride 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.*

- Check all components for damages after receiving the kit. If kit contents like buffer bottles or blister packages are damaged, contact MACHEREY-NAGEL technical support and customer service, or your local distributor.
- Do not use damaged kit components.
- Upon arrival the **NucleoSpin® Dx Virus** kit should be stored at room temperature (18 – 25 °C). It is NOT required to open the kit on delivery and remove individual components for separate storage.
- **NucleoSpin® Dx Virus Columns** can be used until the expiration date on the kit box.
- Use RNase-free equipment.

Before starting the **NucleoSpin® Dx Virus** protocol prepare the following:

- **Lyophilized Proteinase K** can be stored at room temperature (18 – 25 °C) until the expiration date without decrease in performance. Before first use of the kit, add the indicated volume of **Proteinase Buffer PB** to dissolve lyophilized Proteinase K. Reconstituted Proteinase K should be stored at -20 °C for up to 6 months, but only until the expiration date.
- Carrier RNA: Before first use, add 1 mL **Lysis Buffer RAV1** to the **Carrier RNA** vial. Dissolve the Carrier RNA and pipette the solution back to the RAV1 bottle.
  Note: Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardly be visible.

  Lysis Buffer RAV1 including Carrier RNA can be stored at 4 °C for up to 4 weeks. Storage at 4 °C or below may cause salt precipitation. If precipitates are visible, make sure to dissolve all precipitates before use by heating at 40 – 60 °C for a maximum of 5 min. Carrier RNA dissolved in Buffer RAV1 and stored at -20 °C is stable for at least one year.

  Do not warm up Buffer RAV1 containing Carrier RNA more than 4 times! Frequent warming, temperatures > 80 °C, and extended heat incubation will accelerate the degradation of Carrier RNA.
- **Wash Buffer RAV3:** Add the indicated volume (see table below or on the bottle) of ethanol (96 – 100 %) to **Wash Buffer RAV3 Concentrate**. Mark the label of the bottle to indicate that the ethanol is added. Store Wash Buffer RAV3 at room temperature. Wash Buffer RAV3 can be stored at room temperature (18 – 25 °C) for up to one year but only until the expiration date.

	NucleoSpin® Dx Virus
REF	**50 preps** **740895.50**
Wash Buffer RAV3 (Concentrate)	12 mL Add 48 mL ethanol
Proteinase K	30 mg Add 1.35 mL Proteinase Buffer PB

4 Safety instructions

When working with the **NucleoSpin® DX Virus** 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: Guanidine hydrochloride in Buffer RAW, and guanidinium thiocyanate in Buffer RAV1 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 **NucleoSpin® DX Virus** 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 Viral nucleic acid purification with NucleoSpin® Dx Virus

The procedures below provide instructions for processing a single plasma or serum sample. However, several samples can be processed at the same time; the number depends on the capacity of the microcentrifuge used.

Before starting the preparation:

- Check that Wash Buffer RAV3 and Proteinase K were prepared according to section 3.
- Check that Carrier RNA has been dissolved in Lysis Buffer RAV1 according to section 3.
- Check that 96 – 100 % ethanol (denatured or non-denatured) is available to adjust nucleic acid binding conditions.
- Set an incubator (e.g., heating block) or water bath to 70 °C.
- Equilibrate the plasma/serum samples to room temperature (18 – 25 °C). Make sure that the samples are mixed well.
- If a precipitate has formed in Lysis Buffer RAV1 or Buffer RAW, incubate the buffer at 40 – 60 °C until the precipitate is dissolved.
- Generally, do not mix reagents and columns from different kits and lots.
- Heat RNase-free H_2O / Elution Buffer RE to 70 °C for final elution of nucleic acids.
- Do not add Proteinase K solution directly to Lysis Buffer RAV1. The sample has to be combined with the Lysis Buffer RAV1 before addition of Proteinase K.
- All centrifugation steps should be carried out at room temperature (18 – 25 °C).

5.1 Protocol at a glance

Supplemental protocol-overview:

Carefully read the detailed protocol (section 5.2 5.4) before starting the procedure.

Note: The protocols differ in Proteinase K lysis step (step 3) and elution step (step 24) only.

		Viral **RNA** isolation procedure (section 5.2)	Viral **DNA** isolation procedure (section 5.3)	Viral **RNA + DNA** isolation procedure (section 5.4)
Provide sample, lyse viruses, clear lysate	1	**150 µL sample** in Lysis Tubes	**150 µL sample** in Lysis Tubes	**150 µL sample** in Lysis Tubes
	2	**600 µL Buffer RAV1** containing Carrier RNA	**600 µL Buffer RAV1** containing Carrier RNA	**600 µL Buffer RAV1** containing Carrier RNA
	3	*Note: No Proteinase K is used for the isolation of viral RNA only*	**20 µL Proteinase K** *(Incubate at least 1 min at room temperature)*	**20 µL Proteinase K** *(Incubate at least 1 min at room temperature)*
	4	Pipette mixture up and down and vortex well	Pipette mixture up and down and vortex well	Pipette mixture up and down and vortex well
	5	Incubate at **70 °C** for **5 min**	Incubate at **70 °C** for **5 min**	Incubate at **70 °C** for **5 min**
	6	Short spin to clean the lid	Short spin to clean the lid	Short spin to clean the lid
Adjust binding conditions	7	**600 µL ethanol**	**600 µL ethanol**	**600 µL ethanol**
	8	Mix by vortexing (10 – 15 s)	Mix by vortexing (10 – 15 s)	Mix by vortexing (10 – 15 s)
Bind RNA/ DNA	9	Load **700 µL lysate** onto the NucleoSpin® Dx Virus Column	Load **700 µL lysate** onto the NucleoSpin® Dx Virus Column	Load **700 µL lysate** onto the NucleoSpin® Dx Virus Column
	10	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	11	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube

	12	Load the **residual lysate** (ca. 650 µL) onto the column	Load the **residual lysate** (ca. 650 µL) onto the column	Load the **residual lysate** (ca. 650 µL) onto the column
	13	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	14	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
Wash silica membrane	15	**500 µL RAW**	**500 µL RAW**	**500 µL RAW**
	16	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	17	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
	18	**600 µL RAV3**	**600 µL RAV3**	**600 µL RAV3**
	19	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	20	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
	21	**200 µL RAV3**	**200 µL RAV3**	**200 µL RAV3**
	22	11,000 x *g*, 3 min	11,000 x *g*, 3 min	11,000 x *g*, 3 min
Elute RNA/ DNA	23	Transfer the NucleoSpin® Dx Virus Column to an Elution Tube	Transfer the NucleoSpin® Dx Virus Column to an Elution Tube	Transfer the NucleoSpin® Dx Virus Column to an Elution Tube
	24	**50 µL RNase-free H_2O** (70 °C); Incubate 1 – 2 min	**50 µL Buffer RE** (70 °C); Incubate 1 – 2 min	**50 µL RNase-free H_2O** (70 °C); Incubate 1 – 2 min
	25	11,000 x *g*, 1 min	11,000 x *g*, 1 min	11,000 x *g*, 1 min

5.2 Viral RNA isolation procedure

1 Provide **150 µL sample** in a Lysis Tube (1.5 mL, provided).

2 Add **600 µL Buffer RAV1** containing Carrier RNA to the Lysis Tube.

3 *Note: No Proteinase K is used for the isolation of viral RNA only.*

4 Pipette mixture up and down and vortex well.

5 Incubate for **5 min** at **70 °C**.

6 **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).

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7 Add **600 µL ethanol** (96 – 100 %) to the clear lysate.

8 Mix by vortexing (10 – 15 s).

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9 Carefully load **700 µL of the lysate** onto the **NucleoSpin® Dx Virus Column** placed in a Collection Tube and close the lid.

10 **Centrifuge 1 min** at **8,000 x *g***.

11 Place the **NucleoSpin® Dx Virus Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

12 Load the **residual lysate** (approx. 650 µL) onto the NucleoSpin® Dx Virus Column and close the lid.

13 **Centrifuge 1 min** at **8,000 x *g***.

14 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

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15 Add **500 µL Buffer RAW** to the NucleoSpin® Dx Virus Column.

16 **Centrifuge 1 min** at **8,000 x *g***.

17 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

18 Add **600 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

19 **Centrifuge 1 min** at **8,000 x *g***.

20 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

21 Add **200 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

22 **Centrifuge 3 min** at **11,000 x *g***.

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23 Place the NucleoSpin® Dx Virus Column into an Elution Tube (1.5 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

24 Add **50 µL RNase-free H_2O** (preheated to 70 °C) and incubate for 1 – 2 min.

25 **Centrifuge 1 min** at **11,000 x *g*** to elute nucleic acid from the column.

5.3 Viral DNA isolation procedure

1 Provide **150 µL sample** in a Lysis Tube (1.5 mL, provided).

2 Add **600 µL Buffer RAV1** containing Carrier RNA to the Lysis Tube.

3 Add **20 µL Proteinase K** solution to the Lysis Tube.

Note: Proteinase K is necessary for lysis of DNA viruses.

4 Pipette mixture up and down and vortex well.

Note: Make sure that the mixture incubates at least 1 min at room temperature before starting the heat incubation.

5 Incubate for **5 min** at **70 °C**.

6 **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).

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7 Add **600 µL ethanol** (96 – 100 %) to the clear lysate.

8 Mix by vortexing (10 – 15 s).

---

9 Carefully load **700 µL of the lysate** onto the **NucleoSpin® Dx Virus Column** placed in a Collection Tube and close the lid.

10 **Centrifuge 1 min** at **8,000 x *g***.

11 Place the **NucleoSpin® Dx Virus Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

12 Load the **residual lysate** (approx. 650 µL) onto the NucleoSpin® Dx Virus Column and close the lid.

13 **Centrifuge 1 min** at **8,000 x *g***.

14 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

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15 Add **500 µL Buffer RAW** to the NucleoSpin® Dx Virus Column.

16 **Centrifuge 1 min** at **8,000 x *g***.

17 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

18 Add **600 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

19 **Centrifuge 1 min** at **8,000 x *g***.

20 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

21 Add **200 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

22	**Centrifuge 3 min** at **11,000 x *g***.
23	Place the NucleoSpin® Dx Virus Column into an Elution Tube (1.5 mL, provided) and discard the Collection Tube with flowthrough from the previous step.
24	Add **50 µL Buffer RE** (preheated to 70 °C) and incubate for 1 – 2 min.
25	**Centrifuge 1 min** at **11,000 x *g*** to elute nucleic acid from the column.

5.4 Simultaneous viral RNA and DNA isolation procedure

1 Provide **150 µL sample** in a Lysis Tube (1.5 mL, provided).

2 Add **600 µL Buffer RAV1** containing Carrier RNA to the Lysis Tube.

3 Add **20 µL Proteinase K** solution to the Lysis Tube.

Note: Proteinase K is necessary for lysis of DNA viruses.

4 Pipette mixture up and down and vortex well.

Note: Make sure that the mixture incubates at least 1 min at room temperature before starting the heat incubation.

5 Incubate for **5 min** at **70 °C**.

6 **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).

---

7 Add **600 µL ethanol** (96 – 100 %) to the clear lysate.

8 Mix by vortexing (10 – 15 s).

---

9 Carefully load **700 µL of the lysate** onto the **NucleoSpin® Dx Virus Column** placed in a Collection Tube and close the lid.

10 **Centrifuge 1 min** at **8,000 x *g***.

11 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

12 Load the **residual lysate** (approx. 650 µL) onto the NucleoSpin® Dx Virus Column and close the lid.

13 **Centrifuge 1 min** at **8,000 x *g***.

14 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

---

15 Add **500 µL Buffer RAW** to the NucleoSpin® Dx Virus Column.

16 **Centrifuge 1 min** at **8,000 x *g***.

17 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

18 Add **600 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

19 **Centrifuge 1 min** at **8,000 x *g***.

20 Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flowthrough from the previous step.

21 Add **200 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

22	**Centrifuge 3 min** at **11,000 x *g***.
23	Place the NucleoSpin® Dx Virus Column into an Elution Tube (1.5 mL, provided) and discard the Collection Tube with flowthrough from the previous step.
24	Add **50 µL RNase-free H_2O** (preheated to 70 °C) and incubate for 1 – 2 min.
25	**Centrifuge 1 min** at **11,000 x *g*** to elute nucleic acid from the column.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	*Low viral load in the sample* • The nucleic acid yield depends on the viral load in the sample. *Problems with Carrier RNA* • Carrier RNA not added. • See remarks concerning storage of Buffer RAV1 with Carrier RNA (section 3). *Proteinase K digestion may be necessary* • Choose the appropriate protocol for viral RNA or viral DNA isolation, see section 5.1. *Viral nucleic acids degraded* • Samples should be processed immediately. Ensure appropriate storage conditions up to the processing. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer RAV1, Carrier RNA and Elution Buffer RE.
Problems with subsequent detection	*Reduced sensitivity* • Change the volume of eluate added to the PCR/RT-PCR. *Ethanol carry-over* • Prolong centrifugation step (step 22) in order to remove Buffer RAV3 completely.

Please contact:
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Tel.: +49 (0) 24 21 969 270
e-mail: TECH-BIO@mn-net.com

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, ISBN 3-527-31471-7.

Sawoo, O. *et al.* (2014) Cleavage of Hemagglutinin-Bearing Lentiviral Pseudotypes and Their Use in the Study of Influenza Virus Persistence. PLoS One. 9(8), e106192. Published online 2014 Aug 28. doi: 10.1371/journal.pone.0106192.

Sundarrajan S. *et al.* (2018) Addressing false negatives in viral diagnostic polymerase chain reactions: A new approach. International Journal of Applied Microbiology and Biotechnology Research, IJAMBR 6, 32 – 49.

6.4 Ordering information

Product	REF	Pack of
CE-IVD marked kits		
NucleoSpin® Dx Virus	740895.50	50
NucleoSpin® Dx Blood	740899.50/.250	50/250
Kits for research purposes		
NucleoSpin® Virus	740983.10/.50/.250	10/50/250
NucleoSpin® RNA Virus F	740958	25
NucleoSpin® totalRNA FFPE XS	740969.10/.50/.250	10/50/250
NucleoSpin® totalRNA FFPE	740982.10/.50/.250	10/50/250
NucleoSpin® DNA FFPE XS	740980.10/.50/.250	10/50/250
NucleoSpin® Blood	740951.10/.50/.250	10/50/250
NucleoSpin® Tissue	740952.10/.50/.250	10/50/250
NucleoSpin® Tissue XS	740901.10/.50/.250	10/50/250
NucleoSpin® miRNA	740971.10/.50/.250	10/50/250
Proteinase K	740506	100 mg
Collection Tubes (2 mL)	740600	1000

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

6.5 Explanation of symbols

REF	Item number	Σ	Sufficient for < n> tests
LOT	Batch identification		Permitted storage temperature range
	Manufacturer		Use by
IVD	*In-vitro* diagnostic products		Caution: Further information in user manual
	Please read instructions for use		Do not reuse

6.6 Product use restriction / warranty

The **NucleoSpin® Dx Virus** kit is a generic system for the isolation and purification of viral nucleic acids from human plasma or serum samples for subsequent *in-vitro* diagnostic purposes.

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

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

The **NucleoSpin® Dx Virus** 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. **NucleoSpin® Dx Virus** 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 **NucleoSpin® Dx Virus** 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.

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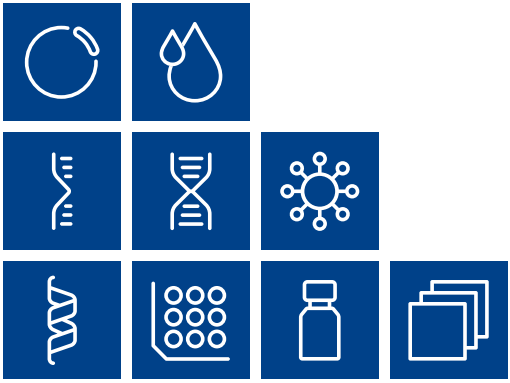
Last updated: April 2022 / Rev. 07

Reason for revision:

Addition of analytical and clinical performance data to chapter 2.5. Reference to new languages of the user manual (chapter 1.3).



www.mn-net.com

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