



Viral Nucleic Acid Isolation

User Manual

NucleoSpin® Dx Virus

CE



In-Vitro Diagnostic Medical Device



740895.50, 740895.250



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







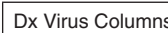


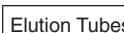

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Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	6
1.3	About this User Manual	6
2	Product description	7
2.1	Intended use	7
2.2	Product use limitations	7
2.3	Quality control	8
2.4	Introduction and kit specifications	8
2.5	Remarks regarding sample quality and preparation	10
2.6	Remarks regarding elution	10
3	Storage conditions and preparation of working solutions	11
4	Safety instructions – risk and safety phrases	13
5	Viral nucleic acid purification with NucleoSpin® Dx Virus	15
5.1	Protocol-at-a-glance	16
5.2	Viral RNA isolation procedure	18
5.3	Viral DNA isolation procedure	20
5.4	Simultaneous viral RNA and DNA isolation procedure	22
6	Appendix	24
6.1	Troubleshooting	24
6.2	Ordering information	25
6.3	Product use restriction/warranty	26

1 Components











1.1 Kit contents











NucleoSpin® Dx Virus				
REF		50 preps 740895.50	250 preps 740895.250	
Lysis Buffer RAV1	 	35 mL	5 x 35 mL	
Wash Buffer RAW	 	30 mL	2 x 75 mL	
Wash Buffer RAV3 (Concentrate)*	  	12.5 mL	3 x 25 mL	
RNase-free H ₂ O		5 mL	25 mL	
Elution Buffer RE**	 	5 mL	25 mL	
Carrier RNA (lyophilized)		1 mg	5 x 1 mg	
Proteinase Buffer PB	 	1.8 mL	8 mL	
Proteinase K (lyophilized)*		30 mg	2 x 75 mg	
NucleoSpin® Dx Virus Columns (dark blue rings - plus Collection Tubes)		50	250	
Collection Tubes (2 mL)		4 x 50	4 x 250	
Lysis Tubes (1.5 mL)		50	5 x 50	
Elution Tubes (1.5 mL)		50	5 x 50	
User Manual		1	1	

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

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

Viral Nucleic Acid Isolation

										
EN	Do not reuse	Use by	Batch code	Catalogue number	Contains sufficient for <n> tests	Manufacturer	In Vitro Diagnostic Medical Device	Consult Instructions for use	Temperature limitation	Irritant
DE	Nicht zur Wiederverwendung	Verwendbar bis	Chargenbezeichnung	Bestellnummer	Inhalt ausreichend für <n> Tests	Hersteller	In-Vitro-Diagnostikum	Gebrauchsanweisung beachten	Temperaturbegrenzung	Reizend
ES	No reutilizar	Fecha de caducidad	Código de lote	Número de catálogo	Contenido suficiente para <n> ensayos	Fabricante	Producto sanitario para diagnóstico in vitro	Consulte las instrucciones de uso	Límite de temperatura	Irritante
IT	Non riutilizzare	Utilizzare entro	Codice del lotto	Numero di catalogo	Contenuto sufficiente per "n" saggi	Fabbricante	Dispositivo medicodiagnostico in vitro	Consultare le istruzioni per l'uso	Limiti di temperatura	Irritant
FR	Ne pas réutiliser	Utiliser jusque	Code du lot	Référence du catalogue	Contenu suffisant pour "n" tests	Fabricant	Dispositif médical de diagnostic in vitro	Consultez les instructions d'utilisation	Limites de température	Irritante
NL	Niet opnieuw gebruiken	Houdbaar tot	Lot nummer	Catalogus nummer	Inhoud voldoende voor "n" testen	Fabrikant	Medisch hulpmiddel voor in-vitro diagnostiek	Raadpleeg de gebruiksaanwijzing	Temperatuurlimiet	Irriterend
DA	Må ikke genbruges	Holdbar til	Lotnummer	Katalognummer	Indeholder tilstrækkeligt til „n“ test	Producent	Medicinsk udstyr til in vitro diagnostik	Se brugsanvisning	Temperaturbegrensning	Lokalirriterende
EL	Μην κάνετε επανα-ναληπτική χρήση	Ημερομηνία λήξης	Αριθμός Παρτίδας	Αριθμός καταλόγου	Περιεχόμενο επαρκές για «n» εξετάσεις	Κατασκευαστής	In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν	Συμβουλευτείτε τις οδηγίες χρήσης	Περιορισμοί θερμοκρασίας	Διαβρωτικό
PT	Não reutilizar	Prazo de validade	Código do lote	Referência de catálogo	Conteúdo suficiente para "n" ensaios	Fabricante	Dispositivo médico para diagnóstico in vitro	Consulte as instruções de utilização	Limites de temperatura	Irritante

										
SV	Återanvänd ej	Använd för	Lot nummer	Katalognummer	Räcker till „n“ antal tester	Tillverkare	Medicintekniska produkter för in vitro diagnostik	Se handhavandebeskrivningen	Temperaturbe-gränsning	Irriterande

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.

2 Product description

2.1 Intended use

The **NucleoSpin® Dx Virus** kit is a generic system for the isolation and purification of viral nucleic acids from human serum or plasma samples for subsequent *in-vitro* diagnostic purposes. The kit can be used with fresh and frozen human serum and plasma, stabilized with either EDTA or citrate from common blood collection systems. 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).

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.

Any diagnostic results generated using nucleic acids isolated with the **NucleoSpin® Dx Virus** kit in conjunction with an *in-vitro* diagnostic assay should be interpreted with regard to additional clinical or laboratory findings. To minimize irregularities in diagnostic results, suitable controls for downstream applications (e.g., extraction controls, positive/negative controls) should be used.

The **NucleoSpin® Dx Virus** kit is intended for use by professional users such as technicians and physicians experienced and trained in molecular biological techniques including experience with serum and plasma samples and viral nucleic acid isolation.

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.

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.

2.2 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.

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.

The linear range of the **NucleoSpin® Dx Virus** procedure has been determined for HCV RNA and HBV DNA in downstream diagnostic assays (Figures 1 and 2). The kits show linearity over several orders of magnitude, comprising relevant viral titer for diagnostic purposes.

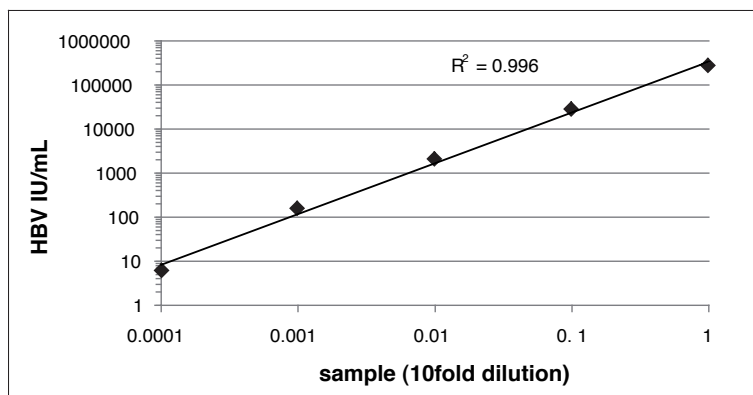


Figure 1: Serial dilution of a plasma sample with high HBV viral load.

Real-time PCR of HBV DNA: Artus RealArt HBV DNA, quantification in Roche LightCycler® 480.

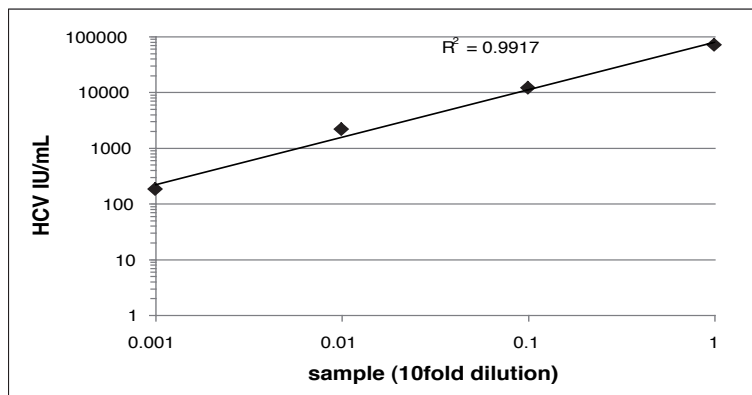


Figure 2: Serial dilution of a plasma sample with high HCV viral load.

Real-time RT-PCR of HCV RNA: Artus RealArt HCV RNA, quantification in Roche LightCycler® 480.

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 Remarks regarding sample quality and preparation

- **NucleoSpin® Dx Virus** is suitable for human serum or plasma samples. For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and non-viscous sample lysate before adjusting binding conditions and loading the sample onto the **NucleoSpin® Dx Virus Column**. Check all samples (especially old or frozen samples) for precipitates.
- 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. 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.

2.6 Remarks regarding elution

- Pure nucleic acids are finally eluted under low ionic strength conditions with RNase-free H₂O (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 H₂O and DNA with Elution Buffer RE.
- To elute both types of nucleic acids together, use RNase-free H₂O provided with the kit, preheated to 70°C.

3 Storage conditions and preparation of working solutions

Attention:

- 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** has a limited shelf life in Buffer RAV1. For this reason, **NucleoSpin® Dx Virus** (250 preps) kit (REF 740895.250) contains several bottles of lyophilized Carrier RNA that should be used successively as required, to avoid degradation of 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.

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.

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 RA3 at room temperature. Wash Buffer RA3 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	250 preps 740895.250
Wash Buffer RAV3 (Concentrate)	12.5 mL Add 50 mL ethanol	3 x 25 mL Add 100 mL ethanol to each bottle
Proteinase K	30 mg Add 1.35 mL Proteinase Buffer PB	2 x 75 mg Add 3.35 mL Proteinase Buffer PB to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® Dx Virus** kits contain hazardous contents.

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

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
RAV1	Guanidinium thiocyanate	✘ Xn*		Harmful by inhalation, in contact with the skin and if swallowed	R 20/21/22 S 13
RAW	Guanidine hydrochloride + ethanol <50%	✘ Xn*		Flammable - Harmful if swallowed - Irritating to eyes and skin	R 10-22-36/38 S 7-16
Proteinase K	Proteinase K, lyophilized	✘ Xn*		Irritating to eyes, respiratory system, and skin, may cause sensitization by inhalation	R 36/37/38-42 S 22-24-26-36/37

Risk phrases

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

Safety phrases

S 7	Keep container tightly closed
S 13	Keep away from food, drink, and animal feedstuffs
S 16	Keep away from sources of ignition - No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water, and seek medical advice
S 36/37	Wear suitable protective clothing and gloves

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

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: Guanidinium thiocyanate in Lysis Buffer RAV1 and guanidine hydrochloride in Wash Buffer RAW 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.

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 H₂O/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	<i>Note: No Proteinase K is used for the isolation of viral RNA only</i>	20 µL Proteinase K <i>(Incubate at least 1 min at room temperature)</i>	20 µL Proteinase K <i>(Incubate at least 1 min at room temperature)</i>
	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 H₂O (70°C); Incubate 1 – 2 min	50 µL Buffer RE (70°C); Incubate 1 – 2 min	50 µL RNase-free H₂O (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).
-
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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through from the previous step.
 24. Add **50 µL RNase-free H₂O** (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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through 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 flow-through from the previous step.
 24. Add **50 µL RNase-free H₂O** (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.
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6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	<i>Low viral load in the sample</i> <ul style="list-style-type: none">• The nucleic acid yield depends on the viral load in the sample.
	<i>Problems with Carrier RNA</i> <ul style="list-style-type: none">• Carrier RNA not added.• See remarks concerning storage of Buffer RAV1 with Carrier RNA (section 3).
	<i>Proteinase K digestion may be necessary</i> <ul style="list-style-type: none">• Choose the appropriate protocol for viral RNA or viral DNA isolation, see section 5.1.
Problems with subsequent detection	<i>Viral nucleic acids degraded</i> <ul style="list-style-type: none">• 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.
	<i>Reduced sensitivity</i> <ul style="list-style-type: none">• Change the volume of eluate added to the PCR/RT-PCR.
	<i>Ethanol carry-over</i> <ul style="list-style-type: none">• Prolong centrifugation step (step 22) in order to remove Buffer RAV3 completely.

6.2 Ordering information

Product	REF	Pack of
<u>CE-IVD marked kits</u>		
NucleoSpin® Dx Virus	740895.50/.250	50/250
NucleoSpin® Dx Blood	740899.50/.250	50/250
<u>Kits for research purposes</u>		
NucleoSpin® RNA Virus	740956.10/.50/.250	10/50/250
NucleoSpin® RNA Virus F	740958	25
NucleoSpin® FFPE RNA	740969.10/.50/.250	10/50/250
NucleoSpin® FFPE RNA/DNA	740978.10/.50/.250	10/50/250
NucleoSpin® FFPE DNA	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.

6.3 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.

The **NucleoSpin® Dx Virus** kit is intended for use by professional users such as technicians and physicians experienced and trained in molecular biological techniques including experience with serum and plasma samples and viral nucleic acid isolation.

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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