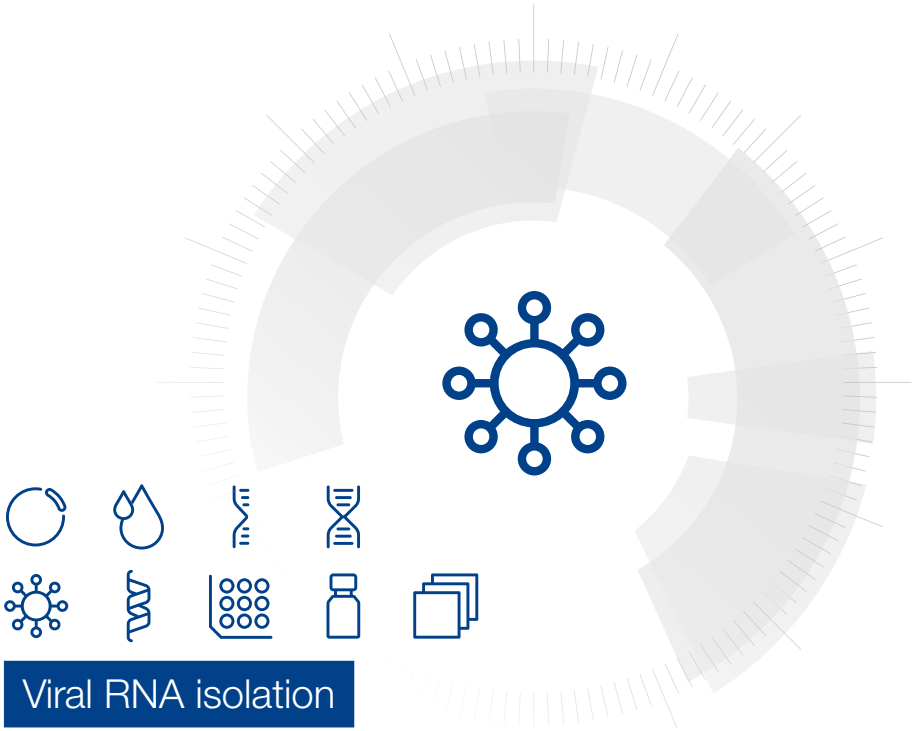
Bioanalysis

MACHEREY-NAGEL

User manual



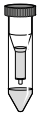
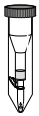
Viral RNA isolation

- NucleoSpin® RNA Virus
- NucleoSpin® RNA Virus F

September 2023 / Rev. 14

Viral RNA isolation

Protocol at a glance (Rev. 14)

	Mini	Funnel
	NucleoSpin® RNA Virus	**NucleoSpin® RNA Virus F**
1 Lysis of viruses	 150 µL sample volume 600 µL RAV1 70 °C, 5 min	 1 mL sample volume 4 mL RAV1 70 °C, 5 min
2 Adjust binding conditions	 600 µL ethanol	 4 mL ethanol
3 Bind viral RNA	 Load sample stepwise  8,000 x *g*, 1 min	 Load sample  3,000 x *g*, 3 – 5 min
4 Wash and dry silica membrane	 1st wash 500 µL RAW 2nd wash 600 µL RAV3 3rd wash 200 µL RAV3  1st and 2nd 8,000 x *g*, 1 min  3rd 11,000 x *g*, 5 min	 1st wash 5 mL RAW 2nd wash 8 mL RAV3 3rd wash 2 mL RAV3  1st and 2nd 3,000 x *g*, 3 min  3rd 3,000 x *g*, 10 min
5 Elute highly pure RNA	 50 µL RNase-free H_2O (70 °C) RT, 1 – 2 min  11,000 x *g*, 1 min	 50 – 100 µL RNase-free H_2O (70 °C) RT, 1 – 2 min  3,000 x *g*, 3 min

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Virus		
REF	**10 preps 740956.10**	**50 preps 740956.50**	**250 preps 740956.250**
Lysis Buffer RAV1	10 mL	35 mL	5 × 35 mL
Wash Buffer RAW	6 mL	30 mL	150 mL
Wash Buffer RAV3 (Concentrate)*	6 mL	12 mL	50 mL
RNase-free H_2O	13 mL	13 mL	30 mL
Elution Buffer RE**	13 mL	13 mL	30 mL
Carrier RNA (lyophilized)	300 µg	1 mg	5 × 1 mg
NucleoSpin® RNA Virus Columns (dark blue rings, plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	30	150	750
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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

Kit contents *continued*

	NucleoSpin® RNA Virus F
REF	**25 preps 740958**
Lysis Buffer RAV1	2 × 120 mL
Wash Buffer RAW	150 mL
Wash Buffer RAV3 (Concentrate)*	3 × 25 mL
RNase-free H_2O	13 mL
Elution Buffer RE**	13 mL
Carrier RNA (lyophilized)	2 × 300 µg
NucleoSpin® RNA Virus F Columns (plus Collection Tubes)	25
Collection Tubes (50 mL)	25
Collection Tubes (0.5 mL)	25
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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

Consumables

- 1.5 mL microcentrifuge tubes (NucleoSpin® RNA Virus) or 50 mL tubes (NucleoSpin® RNA Virus F)
- Disposable tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes (NucleoSpin® RNA Virus) or 50 mL tubes (NucleoSpin® RNA Virus F)
- Vortex mixer
- Heating block or water bath for 70 °C incubation
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Virus / RNA Virus F** kit is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is 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 revisions.

2 Product description

2.1 The basic principle

With the **NucleoSpin® RNA Virus** method, RNA viruses are lysed quickly and efficiently by Lysis Buffer RAV1 which is a highly concentrated solution of GITC. DNA viruses (e.g., HBV) are usually more difficult to lyse and require Proteinase K digestion (see support protocol, section 5.2). Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the **NucleoSpin® RNA Virus Columns**. Carrier RNA improves binding and recovery of low-concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with ethanolic buffers **RAW** and **RAV3**. The nucleic acids can be eluted in low salt buffer or water and are ready-for-use in subsequent reactions.

2.2 Kit specifications

NucleoSpin® RNA Virus / Virus F kits are designed for the rapid preparation of highly pure viral nucleic acids (e.g., HCV, HIV, CMV) from fluid biological samples, for example plasma, serum, urine, swab wash solution, liquefied sputum, but not undiluted whole blood (see remarks in section 2.1).

- No cross contamination due to closed systems.
- The **NucleoSpin® RNA Virus** kit works with 150 µL serum, **NucleoSpin® RNA Virus F** funnel columns allow the processing of 1 mL serum.
- The funnel column of the **NucleoSpin® RNA Virus F** kit allows a high loading capacity as well as a simultaneously small elution volume.
- 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, for example in-house nested (RT-) PCR. We highly recommend using internal (low-copy) standards as well as positive and negative controls to monitor the purification, amplification, and detection processes.
- **Carrier RNA** (poly(-A) RNA: poly(A) potassium salt, prepared from ADP with polynucleotide phosphorylase) is included for optimal performance. Carrier RNA enhances binding of viral nucleic acids to the silica membrane and reduces the risk of viral RNA degradation. Please note that eluates of the **NucleoSpin® RNA 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. Thus, other methods for quantification such as specific quantitative PCR or RT-PCR systems are recommended. Furthermore, Carrier RNA may inhibit PCR reactions. The amount of added Carrier RNA may thus be carefully optimized depending on the individual PCR system used.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Virus	NucleoSpin® RNA Virus F
Technology	Silica membrane technology	Silica membrane technology
Format	Mini spin columns	Funnel columns
Sample material	≤ 150 µL serum, plasma, cell-free biological fluids	≤ 1 mL serum, plasma, cell-free biological fluids
Fragment size	100 b–approx. 50 kb	100 b–approx. 50 kb
Typical recovery rates	> 90 %	> 90 %
Typical analysis limit	30 – 60 cp/mL*	30 – 60 cp/mL*
Elution volume	50 µL	50 – 100 µL
Preparation time	30 min/4 – 6 preps	45 min/2 – 4 preps
Binding capacity	40 µg	30 µg
Use	For research use only	

2.3 Remarks regarding sample quality and preparation

Liquid samples

Biological fluids or semi-fluid samples can be processed (e.g., serum, urine, or bronchoalveolar lavage). For successful nucleic acid purification it is important to obtain a homogeneous, clear, and non-viscous sample before loading onto the NucleoSpin® RNA Virus Column. Therefore, check all samples (especially old or frozen ones) for presence of precipitates. Precipitates remaining after lysis with Buffer RAV1 can be removed by centrifugation. Avoid clearing samples before lysis, because viruses of interest may be associated with particles or aggregates. Incubation with Buffer RAV1 can be prolonged in order to dissolve and digest residual cell structures, precipitates and virus particles. RNA, however, is sensitive and prolonged incubation may cause decreased yields.

Solid samples (tissue samples, stool samples)

Prepare a 10 % (w/v) suspension of tissue in buffer (e.g., PBS) using commercial homogenization tools (rotor-stator or bead-based homogenization tools, etc.). Centrifuge the suspension in order to remove particles. Use the clear particle-free supernatant for further processing.

* Nested PCR

Swab material

Incubate swabs with moderate shaking for 30 min in a suitable buffer (e.g., PBS or liquid medium) to release sample material from the swab. Swab heads should be completely submerged in buffer (usually requires 400 – 500 µL buffer). Transfer 150 µL of the swab wash solution to a suitable container (e.g. reaction tube) and proceed with the sample lysis according to the standard protocol (section 5).

Viscous biological fluids (sputum, bronchoalveolar lavage, etc.)

Viscous biological samples, such as sputum and bronchoalveolar lavage, should be liquefied before starting the extraction protocol. Transfer 150 µL sample to a suitable reaction tube. Add 600 µL Buffer RAV1 containing Carrier RNA to the sample and incubate at 70 °C for 10 min with moderate shaking. Allow the sample to cool down, check if the sample is liquefied and proceed with adjusting the binding conditions according to the standard protocol (section 5).

In case the sample is not liquefied after the heat incubation restart the procedure following recommended guidelines (e.g., CDC guidelines for processing sputum samples:

https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf). Transfer 150 µL of the liquefied sample to a suitable reaction tube and proceed with the standard protocol (section 5) starting with the sample lysis.

Blood samples

Processing of blood samples is possible if using blood diluted with PBS buffer. Using undiluted blood may cause clogging of the silica membrane of the NucleoSpin® Virus Binding Plate. The amount of PBS buffer added to blood samples has to be optimized for the individual organism. As a rule of thumb we recommend to start with 50 µL blood diluted with 50 µL PBS buffer.

Proteinase K treatment

Addition of Proteinase K solution (see ordering information) is necessary for the isolation of viral DNA or simultaneous viral RNA/DNA isolation. For isolation of viral RNA, Proteinase K treatment is usually not required. Proteinase K treatment is recommended for viral RNA isolation when viscous samples have to be processed (e.g., sputum samples).

Sample lysis

For isolation of viral RNA in general a lysis of samples in Buffer RAV1 for 10 min at room temperature will be sufficient. For isolation of viral RNA from viscous samples, for example sputum or supernatants of tissue suspensions or stool samples, a lysis at 70 °C may be required. For simultaneous isolation of viral RNA and DNA, incubation time (e.g., 5 – 15 min), and temperature (e.g., RT, 56 °C, or 70 °C) should be optimized and adjusted to the sample material used.

2.4 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).
- Elution can be performed in a single step with water / elution buffer as indicated in the protocol, obtaining at least 80 % of the bound nucleic acids. To improve sensitivity, this eluate can be used in a second elution step increasing the efficiency of elution and concentration of viral nucleic acids slightly. Alternatively, a second elution step can be performed with an additional volume of water / elution buffer releasing practically all bound nucleic acids but resulting in a lower concentrated, combined eluate.
- RNA should be eluted with the water supplied and DNA with Elution Buffer RE. Buffer RE provides better storage conditions for DNA. To elute both types of nucleic acids together, use the pH proofed (pH 6 – 8), RNase-free H_2O preheated to 70 °C.

2.5 Remarks regarding quality control

- Buffers and **NucleoSpin® RNA Virus / RNA Virus F** Columns have been tested with rRNA and MS2 phage RNA. The absence of RNases, and the yield and efficiency of purification have been investigated with RT-PCR.

3 Storage conditions and preparation of working solutions

Attention*: Buffers RAV1 and RAW contain chaotropic salts. Wear gloves and goggles!*

CAUTION: 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.

- All kit components can be stored at room temperature (15 – 25 °C) and are stable until: see package label.
- **Carrier RNA** has a limited shelf life in Buffer RAV1. For this reason, some kits contain several bottles of lyophilized Carrier RNA that should be used successively as required, to avoid degradation of Carrier RNA.

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.

- Before use, add 1 mL **Lysis Buffer RAV1** to the Carrier RNA tube. Dissolve the RNA and transfer it back to the RAV1 bottle.

Storage of Carrier RNA in Buffer RAV1:

- **Lysis Buffer RAV1 including Carrier RNA** can be stored at room **temperature** for **1 – 2 weeks**. Storage at room temperature prevents salt precipitation.
  Lysis Buffer RAV1 including Carrier RNA can be stored at **4 °C** for up to 4 weeks or aliquoted and stored at **-20 °C for longer periods**. **Carrier-RNA dissolved in RAV1 and stored at -20 °C is stable for at least one year.** Storage at 4 °C or below may cause salt precipitation. Therefore, the mixture must be preheated at 40 – 60 °C for a maximum of 5 min in order to redissolve salts.
  Do not warm 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. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular if low-titer samples are used.

Before starting any **NucleoSpin® RNA Virus/RNA Virus F** protocol, prepare the following:

- **Wash Buffer RAV3**: Add the indicated volume 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 for up to one year.

	NucleoSpin® RNA Virus		
REF	**10 preps** **740956.10**	**50 preps** **740956.50**	**250 preps** **740956.250**
Wash Buffer RAV3 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol

	NucleoSpin® RNA Virus F
REF	**25 preps** **740958**
Wash Buffer RAV3 (Concentrate)	3 × 25 mL Add 100 mL ethanol to each vial

4 Safety instructions

When working with the **NucleoSpin® RNA 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 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® RNA** 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 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 NucleoSpin® RNA Virus protocols

5.1 Viral RNA isolation from cell-free biological fluids

Before starting the preparation:

- Check if Lysis Buffer RAV1 and Wash Buffer RAV3 were prepared according to section 3.
- Preheat an aliquot of Elution Buffer RE / RNase-free H_2O to 70 °C.
- Make sure to prepare the sample according to the remarks for sample preparation (section 2.3).

1 Lysis of viruses

Add **600 µL Buffer RAV1** containing Carrier RNA to **150 µL of the sample**. Pipette mixture up and down and vortex well. Incubate for **5 min** at **70 °C**.

Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting for further hints, section 6.1).

*If the resulting solution is still turbid, centrifuge the mixture for **1 min** at **11,000 x g** (to pellet particles and to prevent clogging of the NucleoSpin® RNA Virus Columns). Take off the supernatant and proceed with step 2.*

150 µL sample

+600 µL RAV1

70 °C, 5 min

2 Adjust binding conditions

Add 600 µL ethanol (96 – 100 %) to the clear lysis solution and mix by vortexing (10 – 15 s).

+600 µL EtOH

3 Bind viral RNA

Place NucleoSpin® RNA Virus Columns in Collection Tubes (2 mL) and load **700 µL** lysed sample. Centrifuge for **1 min** at **8,000 x *g***.

The use of new Collection Tubes (2 mL) is recommended if infectious material has to be prepared.

Load the residual lysis solution onto the NucleoSpin® RNA Virus Column. Centrifuge for **1 min** at **8,000 x *g***. Discard flowthrough and place the NucleoSpin® RNA Virus Column into another new Collection Tube (2 mL). More than two loading steps are not recommended.

Load sample stepwise

8,000 x *g*, 1 min

4 Wash and dry silica membrane

1 st wash

Add **500 µL Buffer RAW** to the NucleoSpin® RNA Virus Column. Centrifuge for **1 min** at **8,000 x *g***. Discard flowthrough.

+500 µL RAW

8,000 x *g*, 1 min

This washing step removes contaminants and PCR inhibitors.

+600 µL RAV3

2nd wash

Add **600 µL Buffer RAV3** to the NucleoSpin® RNA Virus Column. Centrifuge for **1 min** at **8,000 x *g***. Discard flowthrough with Collection Tube.

8,000 x *g*, 1 min

3rd wash

Place the NucleoSpin® RNA Virus Column in a new Collection Tube (2 mL) and add **200 µL Buffer RAV3**. Centrifuge for **2 – 5 min** at **11,000 x *g*** to remove ethanolic Buffer RAV3 completely.

+200 µL RAV3

11,000 x *g*, 5 min

<u>Optional:</u> Residual Buffer RAV3 may inhibit subsequent reactions. Therefore, for subsequent reactions which are extremely ethanol-sensitive, we recommend repeating the centrifugation with a new Collection Tube (2 mL). Or alternatively, incubate the NucleoSpin® RNA Virus Columns for 1 min at 70 °C to remove any remaining traces of ethanol.

5 Elute viral RNA

Place the NucleoSpin® RNA Virus Column into a new, sterile 1.5 mL microcentrifuge tube (not provided). Add **50 µL RNase-free H_2O (preheated to 70 °C)** and incubate for **1 – 2 min**. Centrifuge for **1 min** at **11,000 x *g***.

To elute viral DNA which was prepared according to the support protocol 5.2, we recommend using Buffer RE, preheated to 70 °C (also see section 2.4).

50 µL RNase-free H2O (70 °C)

RT, 1 – 2 min

11,000 x *g*, 1 min

5.2 Isolation of viral RNA and DNA from cell-free biological fluids

This protocol is recommended for the purification of viral RNA and viral DNA for all types of DNA viruses like HBV and CMV from small samples of up to 150 µL.

Before starting the preparation:

- Check if Lysis Buffer RAV1 and Wash Buffer RAV3 were prepared according to section 3.
- Check if Proteinase K solution (not included, see ordering information) was prepared.
- Preheat an aliquot of Elution Buffer RE / RNase-free H_2O to 70 °C.
- Make sure to prepare the sample according to the remarks for sample preparation (section 2.3).

1 Lysis of viruses

Add **600 µL Buffer RAV1** containing Carrier RNA to **150 µL of the sample**. Add **20 µL Proteinase K** (20 mg/mL stock solution), to the lysis mixture. Pipette mixture up and down and vortex for 10 – 15 s. Incubate for **5 min** at **70 °C**.

Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting).

*Proteinase K is not included in this kit, but can be ordered separately (see ordering information). For the isolation of viral DNA and genomic DNA from other matrices (**not cell-free**) like blood we recommend the **NucleoSpin® Blood** or **NucleoSpin® Tissue** kit (see ordering information).*

*If the resulting solution is still turbid, centrifuge the mixture for **1 min** at **11,000 x g** to pellet particles and to prevent clogging of the NucleoSpin® RNA Virus Columns. Take off the supernatant and continue with step 2 of protocol 5.1.*

6 NucleoSpin® RNA Virus F protocols

6.1 Viral RNA isolation from cell-free biological fluids

Before starting the preparation:

- Check if Lysis Buffer RAV1 and Wash Buffer RAV3 were prepared according to section 3.
- Preheat an aliquot of Elution Buffer RE/RNase-free H_2O to 70 °C.
- Make sure to prepare the sample according to the remarks for sample preparation (section 2.3).

1 Lysis of viruses

Add **4 mL Buffer RAV1** containing Carrier RNA to **1 mL of the sample**. Pipette mixture up and down and vortex well. Incubate for **5 min** at **70 °C**.

Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting for further hints).

*If the resulting solution is still turbid, centrifuge the mixture for **1 min** at **11,000 x g** (to pellet particles and to prevent clogging of the NucleoSpin® RNA Virus F Columns). Take off the supernatant and proceed with step 2.*

1 mL sample

+4 mL RAV1

70 °C, 5 min

2 Adjust binding conditions

Add **4 mL ethanol** (96 – 100 %) to the clear lysis solution and mix by vortexing (10 – 15 s).

+4 mL EtOH

3 Bind viral RNA

Take the NucleoSpin® RNA Virus F Column placed in a Collection Tube and load lysed sample. Centrifuge for **3 – 5 min** at **3,000 x *g***.

The use of new 50 mL tubes for every step is recommended if infectious material has to be prepared. This avoids cross-contamination and contamination of centrifuge units. For non-infectious samples, we recommend discarding the flowthrough and reusing the 50 mL tube for loading and washing steps. Additional 50 mL tubes have to be ordered separately.

The maximum loading capacity of the NucleoSpin® RNA Virus F Column is about 10 mL in order to work cross-contamination free. If more sample has to be loaded discard flowthrough and put the NucleoSpin® RNA Virus F Column into a new 50 mL tube.

Load sample

3,000 x *g*, 3 – 5 min

*Load the residual lysis solution onto the NucleoSpin® RNA Virus F Column. Centrifuge for **3 – 5 min** at **3,000 x g**. Discard flowthrough and place the NucleoSpin® RNA Virus F Column into another new 50 mL tube. More than two loading steps are not recommended.*

4 Wash and dry silica membrane

1 st wash

+5 mL RAW

3,000 x *g*, 3 min

Add **5 mL Buffer RAW** to the NucleoSpin® RNA Virus F Column. Centrifuge for **3 min** at **3,000 x *g***. Discard flowthrough.

This washing step removes contaminants and PCR inhibitors.

2nd wash

+8 mL RAV3

3,000 x *g*, 3 min

Add **8 mL Buffer RAV3** to the NucleoSpin® RNA Virus F Column. Centrifuge for **3 min** at **3,000 x *g***. Discard flowthrough with Collection Tube.

3rd wash

+2 mL RAV3

3,000 x *g*, 10 min

Place the NucleoSpin® RNA Virus F Column in a new Collection Tube (50 mL) and add **2 mL Buffer RAV3**. Centrifuge for **10 min** at **3,000 x *g*** to remove ethanolic Buffer RAV3 completely.

<u>Optional:</u> Residual Buffer RAV3 may inhibit subsequent reactions. Therefore, for subsequent reactions, which are extremely ethanol-sensitive, we recommend repeating the centrifugation with a new Collection Tube (50 mL). Or alternatively, incubate the NucleoSpin® Virus F Columns for 1 min at 70 °C to remove any remaining traces of ethanol.

5 **Elute viral RNA**

50 – 100 µL RNase-free H2O (70 °C)

RT, 1 – 2 min

3,000 x *g*, 3 min

Attach the supplied Collection Tube (0.5 mL) with the adaptor to the NucleoSpin® RNA Virus F Column. Place the assembly in a 50 mL tube (not provided). Add **50 – 100 µL RNase-free H_2O (preheated to 70 °C)** and incubate for **1 – 2 min** at **room temperature**. Centrifuge for **3 min** at **3,000 x *g***.

6.2 Isolation of viral RNA and DNA from cell-free biological fluids

This protocol is recommended for the purification of viral RNA and viral DNA for all types of DNA viruses like HBV and CMV for samples of up to 1 mL.

Before starting the preparation:

- Check if Lysis Buffer RAV1 and Wash Buffer RAV3 were prepared according to section 3.
- Check if Proteinase K solution (not included, see ordering information) was prepared.
- Preheat an aliquot of Elution Buffer RE/RNase-free H_2O to 70 °C.
- Make sure to prepare the sample according to the remarks for sample preparation (section 2.3).

1 Lysis of viruses

Add **4 mL Buffer RAV1** containing Carrier RNA to **1 mL of the fluid sample**. Add **133 µL Proteinase K** (20 mg/mL stock solution), to the lysis mixture. Pipette mixture up and down and vortex for 10 – 15 s. Incubate for **5 min** at **70 °C**.

Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting).

*Proteinase K is not included in this kit, but can be ordered separately (see ordering information). For the isolation of viral DNA and genomic DNA from other matrices (not cell-free) like blood we recommend the **NucleoSpin® Blood** or **NucleoSpin® Tissue** kit (see ordering information).*

*If the resulting solution is still turbid, centrifuge the mixture for **1 min** at **11,000 x g** to pellet particles and to prevent clogging of the NucleoSpin® RNA Virus Columns. Take off the supernatant and continue with step 2 of protocol 5.1.*

1 mL sample

+4 mL RAV1

+133 µL Prot. K

70 °C, 5 min

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	*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* • Use and compare protocols with and without Proteinase K digestion or prolong incubation time to 10 min. *Viral nucleic acids degraded* • Samples should be processed immediately. If necessary, add RNase inhibitor to the sample and 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. • Incubation time and temperature are critical for lysis as well as RNA stability. For sensitive RNA preparations, incubation at room temperature is sufficient without significant loss of sensitivity. For parallel isolation of viral RNA and DNA incubation time (5 – 15 min) and temperature (RT / 56 °C / 72 °C) may be adapted in order to get optimal recovery rates for both species. *Ethanol carry-over* • Prolong centrifugation steps in order to remove Buffer RAV3 completely.
General problems	*Clogged membrane* • Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding NucleoSpin® RNA Virus Columns.

7.2 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Virus	740956.10/.50/.250	10/50/250
NucleoSpin® RNA Virus F	740958	25
NucleoSpin® Funnel Columns	740959	30 sets
Proteinase K	740506	100 mg
rDNase Set (Recombinant DNase and Reaction Buffer for rDNase; sufficient for 50 mini preps)	740963	1 set
NucleoSpin® Dx Virus	740895.50/.250	50/250
NucleoSpin® Blood	740951.10/.50/.250	10/50/250
NucleoSpin® RNA Blood	740200.10/.50	10/50
NucleoSpin® Tissue	740952.10/.50/.250	10/50/250
Collection Tubes (2 mL)	740600	1000

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

7.3 References

M.L. Villahermosa, M.Thomson, E.Vazques de Parga, M.T. Cuevas, G. Contreas, L.Perez-Alvarez, E.Delgado, N.Manjon, L.Medrano and R.Najera. Improved Conditions for Extraction and Amplification of Human Immunodeficiency Virus Type 1 RNA from Plasma samples with low viral load. Journal of Human Virology 3: 27 – 34, (2000)

7.4 Product use restriction / warranty

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

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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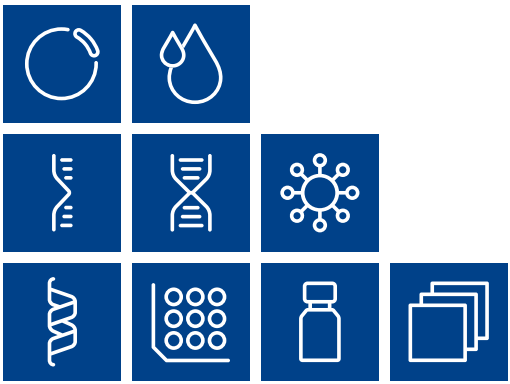

Plasmid DNA
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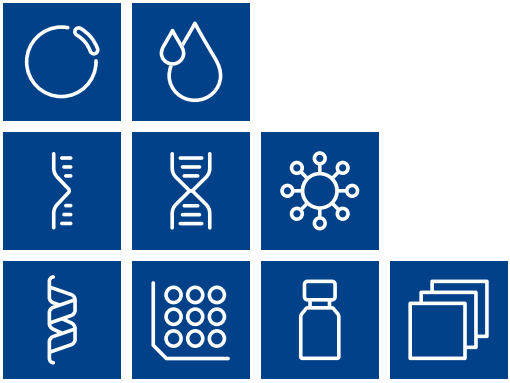

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