

Viral RNA isolation

User manual

NucleoSpin® RNA Virus

NucleoSpin® RNA Virus F

March 2012/Rev. 10

Viral RNA isolation

Protocol-at-a-glance (Rev. 10)







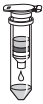



	Mini	Funnel
	NucleoSpin® RNA Virus	NucleoSpin® RNA Virus F
1	Lysis of viruses	1
	 <p>150 µL sample volume 600 µL RAV1</p> <p>70 °C, 5 min</p>	 <p>1 mL sample volume 4 mL RAV1</p> <p>70 °C, 5 min</p>
2	Adjust binding conditions	2
	 <p>600 µL ethanol</p>	 <p>4 mL ethanol</p>
3	Bind viral RNA	3
	 <p>Load sample stepwise</p> <p>8,000 x <i>g</i>, 1 min</p>	 <p>Load sample</p> <p>3,000 x <i>g</i>, 3–5 min</p>
4	Wash and dry silica membrane	4
	 <p>1st wash 500 µL RAW 2nd wash 600 µL RAV3 3rd wash 200 µL RAV3</p> <p>8,000 x <i>g</i>, 1 min</p> <p>1st and 2nd</p> <p>3rd 11,000 x <i>g</i>, 5 min</p>	 <p>1st wash 5 mL RAW 2nd wash 8 mL RAV3 3rd wash 2 mL RAV3</p> <p>3,000 x <i>g</i>, 3 min</p> <p>1st and 2nd</p> <p>3rd 3,000 x <i>g</i>, 10 min</p>
5	Elute highly pure RNA	5
	 <p>50 µL RNase-free H₂O (70 °C)</p> <p>RT, 1–2 min</p> <p>11,000 x <i>g</i>, 1 min</p>	 <p>50–100 µL RNase-free H₂O (70 °C)</p> <p>RT, 1–2 min</p> <p>3,000 x <i>g</i>, 3 min</p>

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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 x 35 mL
Wash Buffer RAW	6 mL	30 mL	2 x 75 mL
Wash Buffer RAV3 (Concentrate)*	12.5 mL	12.5 mL	3 x 25 mL
RNase-free H ₂ O	5 mL	5 mL	25 mL
Elution Buffer RE**	5 mL	5 mL	25 mL
Carrier RNA (lyophilized)	300 µg	1 mg	5 x 1 mg
NucleoSpin® RNA Virus Columns (dark blue rings, plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	30	150	750
User manual	1	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

1.1 Kit contents *continued*

NucleoSpin® RNA Virus F	
REF	25 preps 740958
Lysis Buffer RAV1	2 x 120 mL
Wash Buffer RAW	2 x 80 mL
Wash Buffer RAV3 (Concentrate)*	3 x 25 mL
RNase-free H ₂ O	6 mL
Elution Buffer RE**	5 mL
Carrier RNA (lyophilized)	2 x 300 µg
NucleoSpin® RNA Virus F Columns (plus Collection Tubes)	25
Collection Tubes (50 mL)	25
Collection Tubes (0.5 mL)	25
User manual	1

* 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, but not 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 by 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	<u>Mini spin columns</u>	<u>Funnel columns</u>
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

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 swab in a suitable buffer (e.g., PBS) or cell-culture medium for 30 min. Proceed with particle-free buffer or medium.

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 (20–25 °C) 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 H₂O (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 H₂O 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 contain 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 (18–25 °C) and are stable up to one year.
- **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**. 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 (18–25 °C) for up to one year.

NucleoSpin® RNA Virus			
REF	10 preps	50 preps	250 preps
	740956.10	740956.50	740956.250
Wash Buffer RAV3 (Concentrate)	12.5 mL Add 50 mL ethanol	12.5 mL Add 50 mL ethanol	3 x 25 mL Add 100 mL ethanol to each vial

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

4 Safety instructions – risk and safety phrases

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

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

4.1 Risk and safety phrases

Component	Hazard contents	Hazard symbol	Risk phrases	Safety phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>Gefahrstoff-symbol</i>	<i>R-Sätze</i>	<i>S-Sätze</i>
RAV1	Guanidinium thiocyanate 30–60 % <i>Guanidiniumthiocyanat 30–60 %</i>	✘ Xn*	R 20/21/22-32-52/53	S 13-61
RAW	Guanidine hydrochloride 24–36 % + ethanol 35-55 % <i>Guanidinhydrochlorid 24–36 % + Ethanol 35–55 %</i>	✘ Xn*	R 10-22	S 16

Risk phrases

- R 10 Flammable.
Entzündlich.
- R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.
- R 22 Harmful if swallowed.
Gesundheitsschädlich beim Verschlucken.
- R 32 Contact with acids liberates very toxic gas.
Entwickelt bei Berührung mit Säure sehr giftige Gase. Sensibilisierung durch Einatmen und Hautkontakt möglich.
- R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.

Safety phrases



- S 13 Keep away from food, drink and animal foodstuffs.
Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.
- S 16 Keep away from sources of ignition – No smoking.
Von Zündquellen fernhalten – Nicht rauchen!
- S 61 Avoid release to the environment. Refer to special instructions / safety data sheet.
Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen / Sicherheitsdatenblätter zu Rate ziehen.

* 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.

4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS Symbol	H-Sätze	P-Sätze
RAV1	Guanidinium thiocyanate 30–60 % <i>Guanidiniumthiocyanat</i> 30–60 %	 Warning Achtung	302, 412, EUH031	260, 273, 301+312, 330
RAW	Guanidine hydrochloride 24–36 % + ethanol 35- 55 % <i>Guanidinhydrochlorid 24–36 %</i> <i>+ Ethanol 35–55 %</i>	 Warning Achtung	226, 302	210, 233, 301+312, 330, 403+235

Hazard phrases

H 226	Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i>
H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 412	Harmful to aquatic life with long lasting effects. <i>Schädlich für Wasserorganismen, mit langfristiger Wirkung.</i>
EUH031	Contact with acids liberates toxic gas. <i>Entwickelt bei Berührung mit Säure giftige Gase.</i>

Precaution phrases

P 210	Keep away from heat / sparks / open flames / hot surfaces – No smoking. <i>Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten.</i>
P 233	Keep container tightly closed <i>Behälter dicht verschlossen halten.</i>
P 260	Do not breathe vapours. <i>Dampf nicht einatmen.</i>
P 273	Avoid release to the environment. <i>Freisetzung in die Umwelt vermeiden.</i>
P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell. <i>BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.</i>
P 330	Rinse mouth. <i>Mund ausspülen.</i>
P 403+235	Store in a well ventilated place. Keep cool. <i>Kühl an einem gut belüfteten Ort aufbewahren.</i>

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (www.mn-net.com).

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 H₂O to 70 °C.

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 sample
stepwise**



**8,000 x g,
1 min**

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

4 Wash and dry silica membrane

1st wash

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

This washing step removes contaminants and PCR inhibitors.

+ 500 µL RAW
8,000 x g,
1 min

2nd wash

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



+ 600 µL RAV3
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

Optional: 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 H₂O (preheated to 70 °C)** and incubate for **1–2 min**. Centrifuge for **1 min** at **11,000 x g**.



50 µL
RNase-free H₂O
(70 °C)

RT,
1–2 min

To elute viral DNA which was prepared according to the support protocol 4.2, we recommend using Buffer RE, preheated to 70 °C (also see section 2.4).



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 H₂O to 70 °C.

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.*



150 µL sample

+ 600 µL RAV1

+ 20 µL Prot. K

**70 °C,
5 min**

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 H₂O to 70 °C.

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).

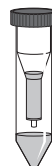
+ 4 mL EtOH

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 flow-through 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 flow-through 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 flow-through 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

1st wash

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

This washing step removes contaminants and PCR inhibitors.

2nd wash

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

3rd wash

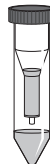
Place the NucleoSpin® RNA Virus F Column in a new Collection Tube (50 mL) and add **2 mL Buffer RAV3**. Centrifuge for **10 min** at **3,000 x g** to remove ethanolic Buffer RAV3 completely.

Optional: 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

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 H₂O (preheated to 70 °C)** and incubate for **1–2 min** at **room temperature**. Centrifuge for **3 min** at **3,000 x g**.

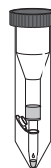
+ 5 mL RAW
3,000 x g,
3 min



+ 8 mL RAV3
3,000 x g,
3 min



+ 2 mL RAV3
3,000 x g,
10 min



50–100 µL
RNase-free
H₂O (70 °C)

RT,
1–2 min



3,000 x g,
3 min

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 H₂O to 70 °C.

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	<p><i>Problems with Carrier RNA</i></p> <ul style="list-style-type: none"> Carrier RNA not added. See remarks concerning storage of Buffer RAV1 with Carrier RNA (section 3).
	<p><i>Proteinase K digestion may be necessary</i></p> <ul style="list-style-type: none"> Use and compare protocols with and without Proteinase K digestion or prolong incubation time to 10 min. <p><i>Viral nucleic acids degraded</i></p> <ul style="list-style-type: none"> 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	<p><i>Reduced sensitivity</i></p> <ul style="list-style-type: none"> 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.
	<p><i>Ethanol carry-over</i></p> <ul style="list-style-type: none"> Prolong centrifugation steps in order to remove Buffer RAV3 completely.
General problems	<p><i>Clogged membrane</i></p> <ul style="list-style-type: none"> 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

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

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

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

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

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

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

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

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

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

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