# Viral RNA isolation

## User manual

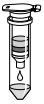
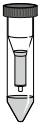
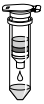
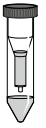
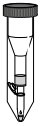
NucleoSpin® RNA Virus
NucleoSpin® RNA Virus F

July 2014 / Rev. 11

MACHEREY-NAGEL
www.mn-net.com

# Viral RNA isolation

## Protocol-at-a-glance (Rev. 11)

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

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# 1 Components

## 1.1 Kit contents

| | NucleoSpin® RNA Virus | | |
|---|---|---|---|
| | **10 preps** | **50 preps** | **250 preps** |
| **REF** | **740956.10** | **740956.50** | **740956.250** |
| Lysis Buffer RAV1 | 10 mL | 35 mL | 5 x 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 x 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

## 1.1 Kit contents *continued*

| | NucleoSpin® RNA Virus F |
|---|---|
| | **25 preps** |
| **REF** | **740958** |
| Lysis Buffer RAV1 | 2 x 120 mL |
| Wash Buffer RAW | 150 mL |
| Wash Buffer RAV3 (Concentrate)* | 3 x 25 mL |
| RNase-free $H_2O$ | 13 mL |
| Elution Buffer RE** | 13 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 |
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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, 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 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 |

## 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 $H_2O$ (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 (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 | | |
|---|---|---|---|
| | **10 preps** | **50 preps** | **250 preps** |
| **REF** | **740956.10** | **740956.50** | **740956.250** |
| Wash Buffer RAV3 (Concentrate) | 6 mL<br>Add 24 mL ethanol | 12 mL<br>Add 48 mL ethanol | 50 mL<br>Add 200 mL ethanol |

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

# 4 Safety instructions

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.*

### GHS classification

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

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

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

### Hazard phrases

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

### Precaution phrases

| | |
|---|---|
| P 210 | Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.<br>*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.* |
| P 233 | Keep container tightly closed<br>*Behälter dicht verschlossen halten.* |
| P 260 | Do not breathe vapours.<br>*Dampf nicht einatmen.* |
| P 273 | Avoid release to the environment.<br>*Freisetzung in die Umwelt vermeiden.* |

**Precaution phrases**

| | |
|---|---|
| P 301+312 | IF SWALLOWED: Call a POISON CENTER/ doctor/…/if you feel unwell.<br>*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.* |
| P 330 | Rinse mouth.<br>*Mund ausspülen.* |
| P 403+235 | Store in a well ventilated place. Keep cool.<br>*Kühl an einem gut belüfteten Ort aufbewahren.* |

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 $H_2O$ 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.

**+ 500 µL RAW**

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

*This washing step removes contaminants and PCR inhibitors.*

**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**

*<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

**50 µL RNase-free $H_2O$ (70 °C)**

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***.

**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 $H_2O$ 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 $H_2O$ 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).

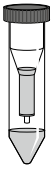
**+ 4 mL EtOH**

### 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

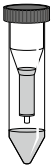
**1st wash**

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

**+ 5 mL RAW**

**3,000 x *g*, 3 min**

*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.

**+ 8 mL RAV3**

**3,000 x *g*, 3 min**

**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.

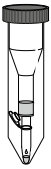
**+ 2 mL RAV3**

**3,000 x *g*, 10 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 (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 $H_2O$ (preheated to 70 °C)** and incubate for **1–2 min** at **room temperature**. Centrifuge for **3 min** at **3,000 x *g***.



**50–100 µL RNase-free $H_2O$ (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 $H_2O$ to 70 °C.

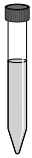
**1 Lysis of viruses**

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 | *Problems with Carrier RNA*<br>• Carrier RNA not added.<br>• See remarks concerning storage of Buffer RAV1 with Carrier RNA (section 3).<br><br>*Proteinase K digestion may be necessary*<br>• Use and compare protocols with and without Proteinase K digestion or prolong incubation time to 10 min.<br><br>*Viral nucleic acids degraded*<br>• Samples should be processed immediately. If necessary, add RNase inhibitor to the sample and ensure appropriate storage conditions up to the processing.<br>• 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*<br>• Change the volume of eluate added to the PCR/RT-PCR.<br>• 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.<br><br>*Ethanol carry-over*<br>• Prolong centrifugation steps in order to remove Buffer RAV3 completely. |
| General problems | *Clogged membrane*<br>• 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.

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