Viral DNA/RNA Isolation

User Manual

NucleoSpin® 96 Virus

NucleoSpin® 96 Virus Core Kit

March 2010 / Rev. 03

MACHEREY-NAGEL

Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® 96 Virus	
	2 x 96 preps	**4 x 96 preps**
Cat. No.	**740691.2**	**740691.4**
Lysis Buffer RAV1[1]	3 x 40 ml	6 x 40 ml
Wash Buffer RAW	150 ml	2 x 150 ml
Wash Buffer RAV3 (Concentrate)[1]	100 ml	200 ml
RNase-free H_2O	65 ml	125 ml
Elution Buffer RE[2]	75 ml	125 ml
Carrier RNA (lyophilized)[1]	3 x 1 mg	6 x 1 mg
Proteinase K (lyophilized)[1]	2 x 50 mg	3 x 75 mg
Proteinase Buffer PB	8 ml	15 ml
NucleoSpin® Virus Binding Plates (blue rings)	2	4
Round-well Block with Cap Strips	2	4
Cap Strips	24	48
MN Square-well Blocks	6	12
Rack of Tube Strips[3]	2	4
Self-adhering PE Foil	10	20
User Manual	1	1

[1] For preparation of working solutions and storage conditions see section 3.

[2] Elution Buffer RE: 5 mM Tris/HCl, pH 8.5

[3] Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

1.1 Kit contents *continued*

	NucleoSpin® 96 Virus Core Kit
	4 x 96 preps
Cat. No.	**740452.4**
Lysis Buffer RAV1[1]	6 x 40 ml
Wash Buffer RAW	2 x 150 ml
Wash Buffer RAV3 (Concentrate)[1]	200 ml
RNase-free H_2O	125 ml
Elution Buffer RE[2]	125 ml
Carrier RNA (lyophilized)[1]	6 x 1 mg
NucleoSpin® Virus Binding Plates (blue rings)	4
User Manual	1

Additional material required (see section 2.4).

1.2 Reagent to be supplied by user

- 96 – 100% ethanol (for preparation of working solutions; see section 3)

[1] For preparation of working solutions and storage conditions see section 3.

[2] Elution Buffer RE: 5 mM Tris/HCl, pH 8.5

2 Product description

2.1 The basic principle

The **NucleoSpin® 96 Virus** kit is designed for the simultaneous purification of viral RNA and DNA. The kit combines the selectivity of well established silica membrane binding of nucleic acids with high-throughput 96-well format. With the **NucleoSpin® 96 Virus** method, RNA viruses are quickly and efficiently lysed by Lysis Buffer RAV1 which is a highly concentrated GITC solution. Compared to RNA viruses, DNA viruses (e.g., HBV) are usually more difficult to isolate and require a digestion of samples with Proteinase K which is provided in the kit. Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the NucleoSpin® Virus Binding Plate. Carrier RNA included in Lysis Buffer RAV1 improves binding and recovery of low concentrated viral RNA/DNA. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in washing steps with ethanolic Wash Buffer RAW and Buffer RAV3. The purified viral nucleic acids can be eluted in low salt buffer or water and are ready-to-use in subsequent downstream applications like RT-PCR or PCR.

Choice of NucleoSpin® Virus kits

The **NucleoSpin® 96 Virus** kit allows the purification of up to 96 samples. The kit is primarily designed for centrifugation use; vacuum use is also possible. Use of the kit on liquid handling instruments (mainly vacuum) allows more variation and higher flexibility in the consumables used for lysis, washing, and elution. MACHEREY-NAGEL takes this into account by introducing the **NucleoSpin® 96 Virus Core Kit**, which is primarily recommended for manual or automated vacuum use. Core kits contain the core items like binding plates and buffers but no accessories like plastics or enzymes. The core kits together with a large variety of suitable disposables ensure the highest degree of flexibility for the user. For lower or medium throughput the **NucleoSpin® 96 Virus** kit is also available in 8-well strip format (see ordering information).

Table 1: Kit selection guide

	Application	Kit recommendation
Manual use, centrifuge	Low-/medium throughput High throughput	NucleoSpin® 8 Virus[1] NucleoSpin® 96 Virus
Manual use, vacuum	Low-/medium throughput	NucleoSpin® 8 Virus[1] NucleoSpin® 8 Virus Core Kit
	High throughput	NucleoSpin® 96 Virus NucleoSpin® 96 Virus Core Kit
Automated use, vacuum or centrifuge	Low-/medium throughput High throughput	NucleoSpin® 8 Virus Core Kit[1] NucleoSpin® 96 Virus Core Kit

[1] Please refer to the NucleoSpin® 8 Virus User Manual. See section 6.2 for ordering information.

2.2 Kit specifications

- **NucleoSpin® 96 Virus** allows the parallel purification of viral DNA and RNA from 100 – 150 µl plasma, serum, or other cell-free biological fluids. Samples can either be fresh or frozen. Furthermore, particle-free supernatants of tissue suspensions, supernatants of stool samples, swab material, or diluted blood samples may also be processed. For detailed information on sample pre-treatment please refer to section 2.6.

- The purified nucleic acids are suitable for applications like real-time PCR/RT-PCR, PCR, or any kind of enzymatic manipulation. The detection limit for certain viruses depends on individual procedures, for example in-house nested (RT-) PCR. Use of internal extraction control samples as well as positive and negative amplification controls in order to monitor the purification, amplification and detection processes is highly recommended.

- **NucleoSpin® 96 Virus Core Kit** is primarily designed for vacuum use (for manual use or automated use on robotic platforms). Processing under vacuum allows easy automation on common liquid handling instruments. For more information about the automation process and the availability of ready-to-run scripts for certain platforms please refer to section 2.5 and contact your local distributor or MN directly.

Table 2: Kit specifications at a glance

Parameter	NucleoSpin® 96 Virus (Core Kit)
Sample size	100 – 150 µl[1]
Recovery rate	> 90%
Anlysis limit	30 – 60 cp/ml
Elution volume	70 – 100 µl
Binding capacity	40 µg
Preparation time	60 min
Column type	96-well plate

[1] Lysis must be done in MN Square-well Blocks if sample size is 150 µl. Additional MN Square-well Blocks may be necessary (see ordering information).

2.3 Required hardware

Centrifugation

For centrifugation a microtiterplate centrifuge which is able to accommodate the NucleoSpin® Virus Binding Plate stacked on a Round- or Square-well Block and reaches accelerations of 5,600 - 6,000 x *g* is required (bucket height: 85 mm).

Vacuum processing

Although the **NucleoSpin® 96 Virus** kit is designed primarily for processing under centrifugation, processing under vacuum is also possible. The dead volume for the elution step is higher in comparison to centrifuge based elution. In order to achieve highly concentrated eluates and to avoid contamination, it is recommended performing the elution step by centrifugation. Consumables for vacuum processing differ from the consumables required for centrifugation. Therefore, for vacuum processing, we recommend using the **NucleoSpin® 96 Virus Core Kit**. For manual processing under vacuum a NucleoVac 96 Vacuum Manifold (see ordering information) is required for **NucleoSpin® 96 Virus Core Kit**.

2.4 Recommended accessories for use of the NucleoSpin® 96 Virus Core Kit

The **NucleoSpin® 96 Virus Core Kit** provides the buffers, Carrier RNA, and NucleoSpin® Virus Binding Plates. Accessory plates (e.g., lysis plates, elution plates, and Proteinase K) are not provided with the core kits. The user can individually select additional consumables from a variety of suitable accessory plates according to his requirements for highest flexibility.

For use of the **NucleoSpin® 96 Virus Core Kit** follow the standard protocol (see section 5).

Recommended accessories for use of the **NucleoSpin® 96 Virus Core Kits** are available from MACHEREY-NAGEL (see ordering information):

Protocol step	Suitable consumables, not supplied with the core kits - ⓘ	Remarks
1. Lysis	Round-well Block with 12 Cap Strips or Rack of Tube Strips with 12 Cap Strips or MN Square-well Block Square-well Block	Round-well Blocks and Tube Strips can be closed with Cap Strips. Square-well Blocks cannot be closed with Cap Strips. Closing with a Self-adhering PE Foil is not recommended (no tight sealing when mixing). Repeated pipetting up and down is recommended for mixing samples with Buffer RAV1.
	Proteinase K	For certain samples and for viral DNA isolation use of Proteinase K is required.
2. Adjustment of binding conditions	Cap Strips	When using Round-well Block or Tube Strips for lysis, new Cap Strips are required for closure of wells.
3. Transfer of samples	MN Square-well Block	Can be used for waste collection if required.
4. Binding of nucleic acids to membrane	MN Wash Plate	MN Wash Plate minimizes the risk of cross contamination. **(used for vacuum processing only)**
5. Wash silica membrane[1]	MN Square-well Block	Can be used for waste collection if required.
6. Elution	Rack of Tubes Strips with 12 Cap Strips or Round-well Block with 12 Cap Strips	Round-well Blocks and Tube Strips can be closed with Cap Strips.

[1] Use of MN Square well Block is optional. For waste collection the waste tray of the NucleoVac Vacuum Manifold can be used.

2.5 Automated processing on robotic platforms

For automated use we recommend using the **NucleoSpin® 96 Virus Core Kit** which can be automated on many common laboratory workstations. For a protocol which can be used as a guideline to create robotic script see section 5.2. For the availability of scripts and general considerations about adapting **NucleoSpin® 96 Virus Core Kit** on a certain workstation please contact MACHEREY-NAGEL.

For vacuum processing the use of the disposable MN Wash Plate inside the vacuum manifold is recommended. Use of the MN Wash Plate reduces the risk of cross-contamination caused by spraying of solutions during vacuum filtration steps. Visit MN at *www.mn-net.com* or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol.

2.6 Sample material

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® Virus Binding Plate. Therefore, check all samples (especially old or frozen ones) for presence of precipitates. Precipitates can be removed after addition of Lysis Buffer RAV1 and lysis incubation by centrifugation. Avoid clearing samples by centrifugation/filtration before the Buffer RAV1-lysis step, 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.

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.

Sample volume

The **NucleoSpin® 96 Virus** and **NucleoSpin® 96 Virus Core Kits** are specified for a sample volume of 100 µl. If necessary, the sample volume can be increased to 150 µl. For sample volumes of 150 µl the volumes of Lysis Buffer RAV1 and ethanol have to be increased to 600 µl each. Depending on the size of pipetting tips, the total lysate volume of 1300 µl may be loaded in two steps onto the NucleoSpin® Virus Binding Plate. The buffers supplied with the kit are sufficient for processing a sample volume of 150 µl[1].

Proteinase K treatment

Addition of Proteinase K solution 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.

2.7 Carrier RNA

The **NucleoSpin® 96 Virus** kits include Carrier RNA that 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® 96 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.

[1] Lysis must be done in MN Square-well Blocks if sample size is 150 µl. Additional MN Square-well Blocks may be necessary (see ordering information).

2.8 Elution procedures

Recovery of viral RNA or DNA from the membrane depends on the elution volume. Elution volumes of 75 – 200 µl are possible, with an optimum of 100 – 125 µl dispensed volume. The dead volume of the membrane is approx. 45 µl and the recovered elution buffer can thus easily be estimated.

Highly concentrated eluates: When using a minimal elution volume (75 – 100 µl), about 70 – 80% of bound nucleic acids can be eluted, resulting in highly concentrated RNA/DNA. Alternatively, elution can be done in two steps with, for example 75 µl each, resulting in a higher elution efficiency but with a lower concentrated eluate.

Preheated elution buffer (70°C): Use preheated elution buffer to increase overall yield. Optionally, following addition of preheated elution buffer incubate the NucleoSpin® Virus Binding Plate for 3 min at 60 – 70°C before elution.

3 Storage conditions and preparation of working solutions

Attention:

Buffers RAV1 and RAW contain guanidinium salts! Wear gloves and goggles!

Before starting any **NucleoSpin® 96 Virus (Core Kit)** protocol prepare the following:

- **Wash Buffer RAV3**: Add indicated volume of 96 – 100% ethanol to the **Wash Buffer RAV3 Concentrate**. Mark the label of the bottle to indicate that ethanol was added.

- Before first use of the kit, add the indicated volume of **Proteinase Buffer PB** to dissolve lyophilized Proteinase K. Proteinase K solution is stable at -20°C for 6 months. Dividing the solution into aliquots is recommended.

- Before use, add 1 ml Lysis Buffer RAV1 to the **Carrier RNA** tube. Dissolve the RNA and transfer it back to the Buffer RAV1 bottle. Mark the label of the bottle to indicate that Carrier RNA was added.

  Carrier RNA has a limited shelf-life in Buffer RAV1. For this reason the **NucleoSpin® 96 Virus (Core Kit)** kit contains several vials of lyophilized Carrier RNA which should be used successively as required.

  Storage of Carrier RNA in Buffer RAV1

  Buffer RAV1 with Carrier RNA can be stored at room temperature for 1 – 2 weeks. Storage at room temperature prevents salt precipitation and avoids pre-heating of the buffer solution!

  For storage for up to 4 weeks storage of Buffer RAV1 with added Carrier RNA at 4°C is recommended. For long time storage Buffer RAV1 with added Carrier RNA can be stored in aliquots at -20°C. 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 dissolve precipitated salts.

  Attention:

  Frequent heating, temperatures >80°C, and extended heat incubation will lead to the degradation of the Carrier RNA and to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular if low-titer samples are used. Do not heat-up Buffer RAV1 containing Carrier RNA more than 4 times!

	NucleoSpin® 96 Virus	
	2 x 96 preps	**4 x 96 preps**
Cat. No.	**740691.2**	**740691.4**
Wash Buffer RAV3 (Concentrate)	100 ml Add 400 ml ethanol	200 ml Add 800 ml ethanol
Proteinase K (lyophilized)	2 x 50 mg Add 2.5 ml Proteinase Buffer to each vial	3 x 75 mg Add 3.5 ml Proteinase Buffer to each vial
Carrier RNA (lyophilized)	3 x 1 mg Transfer each vial to one bottle of 40 ml Buffer RAV1	6 x 1 mg Transfer each vial to one bottle of 40 ml Buffer RAV1

	NucleoSpin® 96 Virus Core Kit
	4 x 96 preps
Cat. No.	**740452.4**
Wash Buffer RAV3 (Concentrate)	200 ml Add 800 ml ethanol
Carrier RNA (lyophilized)	6 x 1 mg Transfer each vial to one bottle of 40 ml Buffer RAV1

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 96 Virus** and **NucleoSpin® 96 Virus Core Kit** contain hazardous contents.

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

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
RAV1	Guanidinium thiocyanate	✖ Xn[1]	Harmful by inhalation, in contact with skin, and if swallowed	R 20/21/22	S 13
RAW	Guanidinium hydrochloride + ethanol <50%	✖ Xn[1]	Flammable - Harmful by if swallowed - Irritating to eyes and skin	R 10-22-36/38	S 7-16
Proteinase K	Proteinase K, lyophilized	✖ Xn Xi[1]	Irritating to eyes, respiratory system and skin - May cause sensitization by inhalation	R 36/37/38-42	S 22-24-26-36/37

Risk phrases

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

Safety phrases

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

[1] Hazard labeling not neccessary 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.

5 Protocols

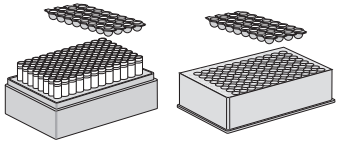
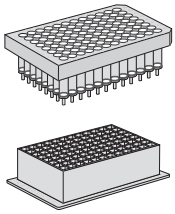
5.1 NucleoSpin® 96 Virus – centrifuge processing

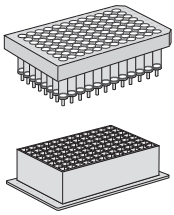
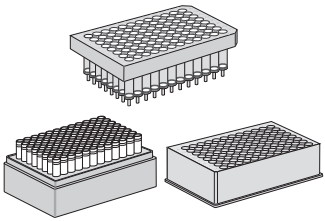
- For detailed information on each step see page 18.
- For use of the **NucleoSpin® 96 Virus Core Kit,** refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer RAV1, Buffer RAV3, and Proteinase K were prepared according to section 3.
- Set incubator or oven to 25 – 70°C.
- Preheat Elution Buffer RE or water to 70°C.

Protocol-at-a-glance

1	Lysis	**100 µl sample** **400 µl Buffer RAV1** **(20 µl Proteinase K)** **Mix** **25 – 70°C** **10 min**	 Rack of Tube Strips or Round-well Block with Cap Strips
2	Adjust binding conditions	**400 µl ethanol (96 – 100%)** **Mix**	
3	Load samples	**Transfer samples to NucleoSpin® Virus Binding Plate**	 NucleoSpin® Virus Binding Plate on MN Square-well Block
4	Bind viral nucleic acids to silica membrane	**5,600 x *g*** **2 min**	

5	Wash silica membrane	**500 µl RAW** **5,600 x *g* 2 min** **700 µl RAV3** **5,600 x *g* 2 min** **700 µl RAV3** **5,600 x *g* 15 min**	 NucleoSpin® Virus Binding Plate on MN Square-well Block
6	Elution	**100 µl RE (70°C)** **5,600 x *g* 2 min** ***Optional: Repeat elution step once.***	 NucleoSpin® Virus Binding Plate on Rack of Tube Strips or Round-well Block

Detailed protocol

This standard protocol is recommended for purification of viral RNA from, for example HCV or HIV. DNA viruses such as CMV can also be isolated but lysis including Proteinase K digestion is recommended.

Place the NucleoSpin® Virus Binding Plate on an MN Square-well Block. The use of a second plate placed on an MN Square-well Block avoids the need to balance the centrifuge.

Before starting the preparation:

- Check if Buffer RAV1, Buffer RAV3, and Proteinase K were prepared according to section 3.
- Set incubator or oven to 25 – 70°C.
- Preheat Elution Buffer RE or water to 70°C.

1 Lyse samples

Pipette **400 µl Buffer RAV1** into the wells of a Rack of Tube Strips or Round-well Block according to the number of samples. Dispense solution to the bottom of the wells.

If 150 µl sample are to be prepared[1], pipette 600 µl Buffer RAV1 into the wells.

We recommend using an electronic 8-channel pipetting device with extra long tips capable of holding more than 650 µl.

Add **100 µl sample** to each Buffer RAV1-filled well. Take care to dispense the sample directly into Buffer RAV1. Pipette mixture up and down several times. Do not moisten the rims.

Close Tube Strips or Round-well Block with Cap Strips. Incubate mixture for **10 min** at **room temperature (18 – 25°C)**.

*Optional: Add **20 µl Proteinase K** to each sample pre-mixed with Buffer RAV1. Close the lysis vessels with Cap Strips and incubate for **5 – 10 min** at **56 – 70°C**. Addition of Proteinase K is required for viral DNA extraction and may be useful for viral RNA extraction from some sample types. For details on incubation time and temperature see section 2.6.*

Spin down droplets (30 s; 1,500 x *g*) before opening the Cap Strips.

[1] Lysis must be done in MN Square-well Blocks if sample size is 150 µl. Additional MN Square-well Blocks may be necessary (see ordering information).

2 Adjust viral nucleic acid binding conditions

Remove Cap Strips and add **400 µl ethanol (96 – 100%)** to each lysate. Take care not to moisten the rims of the individual wells while dispensing. Close the individual wells with new Cap Strips (supplied). Invert 10 times and **mix** by shaking for 15 s. Spin down droplets (30 s; 1,500 x *g*) from the Cap Strips.

If 150 µl sample has been prepared, add 600 µl ethanol (96 – 100%) to each lysate.

3 Transfer samples to binding plates

Remove the first Cap Strip and transfer all of each sample into the wells of a NucleoSpin® Virus Binding Plate positioned on top of the MN Square-well Block. Do not moisten the rims of the individual wells while dispensing samples (moistened rims may cause cross-contamination during centrifugation). Seal NucleoSpin® Virus Binding Plates with Self-adhering PE Foil.

4 Bind viral nucleic acids to silica membrane

Place the MN Square-well Blocks with Binding Plate onto the centrifuge carrier and insert it into the rotor buckets. Centrifuge at **5,600 - 6,000 x *g*** for **2 min**.

Typically, samples will pass through the columns within ≤ 1 min.

Optional: If 150 µl sample has been prepared, load it in successive steps onto the NucleoSpin® Virus Binding Plate as described in step 3. In this case use a new MN Square-well Block for the washing steps as the maximum volume of the MN Square-well Block may be exceeded (additional MN Square-well Blocks are not included in the kit, see ordering information).

5 Wash silica membrane

1st wash

Remove Self-adhering PE Foil and add **500 µl Buffer RAW** to each well of the NucleoSpin® Virus Binding Plate. Seal the NucleoSpin® Virus Binding Plates with new Self-adhering PE Foil. Centrifuge at **5,600 – 6,000 x *g*** for **1 – 2 min**.

Remove Self-adhering PE Foil and place NucleoSpin® Virus Binding Plate onto a new MN Square-well Block.

2nd wash

Add **700 µl Buffer RAV3** to each well of the NucleoSpin® Virus Binding Plate. Seal with new Self-adhering PE Foil. Centrifuge at **5,600 – 6,000 x *g*** for **1 – 2 min**.

3rd wash

Repeat second wash step once. Prolong centrifugation to **15 min** in order to remove ethanol from residual Wash Buffer RAV3.

Alternatively, remove the adhesive foil and place the NucleoSpin® Virus Binding Plate into an incubator for 20 min at 37°C to evaporate residual ethanol.

Removal of ethanol by evaporation at 37°C is more effective than additional, prolonged centrifugation (15 min, 6,000 x g*).*

6 Elute viral RNA/DNA

Place the NucleoSpin® Virus Binding Plate onto the Rack of Tube Strips.

Dispense **75 – 100 µl RNase-free water** or **Buffer RE (preheated to 70°C)** to each well of the NucleoSpin® Virus Binding Plate. Pipette the buffer directly onto the membrane. Incubate at room temperature for 1 min. Seal with a new Self-adhesive PE Foil. Centrifuge at **5,600 – 6,000 x *g*** for **2 – 3 min**.

Tube Strips containing eluted RNA/DNA can be conveniently closed with Cap Strips for storage.

Yields will be 10 – 15% higher when eluting in 100 – 200 µl water. The concentration of nucleic acids in the complete eluate, however, will be lower. For RT-PCR/PCR a more concentrated eluate is favorable. If only viral DNA is processed, elution should be done with Elution Buffer RE optimized for elution and storage of DNA.

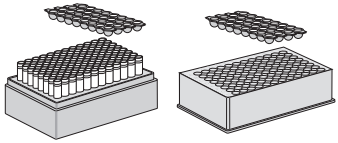
5.2 NucleoSpin® 96 Virus (Core Kit) – vacuum processing

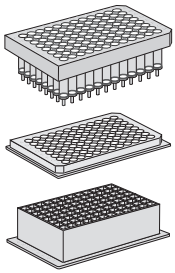
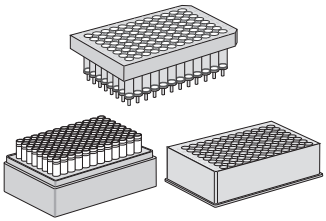
- For detailed information on each step see page 22.
- For use of the **NucleoSpin® 96 Virus Core Kit,** refer to section 2.4 regarding recommended accessories.
- For vacuum use of **NucleoSpin® 96 Virus,** refer to section 2.1 and 2.2 or contact MN technical support.

Before starting the preparation:

- Check if Buffer RAV1, Buffer RAV3, and Proteinase K were prepared according to section 3.
- Set incubator or oven to 25 – 70°C.
- Preheat Elution Buffer RE or water to 70°C.

Protocol-at-a-glance

1	Lysis	**100 µl sample** **400 µl Buffer RAV1** **(20 µl Proteinase K)** **Mix** **25 – 70°C** **10 min**	 Rack of Tube Strips or Round-well Block with Cap Strips
2	Adjust binding conditions	**400 µl ethanol (96 – 100%)** **Mix**	
3	Load samples	**Transfer samples to NucleoSpin® Virus Binding Plate**	

4	Bind nucleic acid to silica membrane	**-0.2 bar**[1] **5 min**	
5	Wash silica membrane	**500 µl RAW** **-0.2 bar**[1] **5 min** **700 µl RAV3** **-0.2 bar**[1] **2 min** **700 µl RAV3** **-0.2 bar**[1] **5 min**	 NucleoSpin® Virus Binding Plate and MN Wash Plate on MN Square-well Block *(optional)*
	Dry silica membrane	**Remove MN Wash Plate** **-0.6 bar**[1] **15 min**	
6	Elution	**100 µl RE (70°C)** **-0.4 bar**[1] **2 min** ***Optional:*** ***Repeat elution step once.*** *<u>Note</u>:* *Elution under centrifugation is recommended.*	 NucleoSpin® Virus Binding Plate on Rack of Tube Strips <u>or</u> Round-well Block

[1] Reduction of atmospheric pressure. Depending on sample viscosity extension of filtration time or increase of vacuum (e.g., -0.2 to -0.4 bar) may be required.

Detailed protocol

Whereas the use of a centrifuge for the processing of the **NucleoSpin® 96 Virus** kit determines most of the consumables to be used (Tube Strips, MN Square-well Blocks, etc.) the vacuum use of the kit allows for more variation and higher flexibility.

Especially when processing a large number of samples under vacuum cross-contamination is a major concern due to spraying of liquids or aerosol formation. The use of the MN Wash Plate[1] prevents the contamination by droplets at the outlets of the individual wells of the NucleoSpin® Binding Plate. This very assistant tool is thus recommended for vacuum processing.

When using the **NucleoSpin® 96 Virus** and the **Core Kit** under vacuum the NucleoVac 96 Vacuum Manifold is required (see ordering information). Place NucleoSpin® 96 Virus Binding Plate on NucleoVac Vacuum Manifold. If processing less than 96 samples, seal unused wells with a Self-adhering PE Foil in order to ensure proper vacuum during the filtration steps.

Note: Reagents or consumables not included in the core kit are highlighted by the ⓘ symbol. A list of recommended accessories can be found in section 2.4.

This standard protocol is recommended for purification of viral RNA from for example HCV or HIV. DNA viruses such as CMV can also be isolated but lysis including Proteinase K digestion is recommended (not included in the core kit).

Before starting the preparation:

- Check if Buffer RAV1, Buffer RAV3, and Proteinase K were prepared according to section 3.
- Set incubator or oven to 25 – 70°C.
- Preheat Elution Buffer RE or water to 70°C

[1] The MN Wash Plate is not part of the kits. Please order separately (see ordering information).

1 Lyse samples

Pipette **400 µl Buffer RAV1** into the wells of a suitable vessel ⓘ used for lysis. Dispense solution to the bottom of the wells.

If 150 µl sample are to be prepared[1], pipette 600 µl Buffer RAV1 into the wells.

We recommend using an electronic 8-channel pipetting device with extra long tips capable of holding more than 650 µl.

Add **100 µl sample** to each Buffer RAV1-filled well. Take care to dispense the samples directly into Buffer RAV1. Pipette mixture up and down several times. Do not moisten the rims.

Close the wells ⓘ and incubate the mixture for **10 min** at **room temperature (18 – 25°C)**.

*Optional: Add **20 µl Proteinase K** ⓘ (20 mg/ml) to each sample pre-mixed with Buffer RAV1. Close the lysis vessels and incubate for 5 – 10 min at 56°C – 70°C. Addition of Proteinase K is required for viral DNA extraction and may be useful for viral RNA extraction from some sample types. For details on incubation time and temperature please also refer to section 2.6.*

Spin briefly (30 s, 1,500 x *g*) to collect any sample from the cover of the wells if required before opening the lysis vessels.

2 Adjust binding conditions

Remove the cover of the wells and add **400 µl ethanol (96 – 100 %)** to each sample. Take care not to moisten the rims of the individual wells while dispensing. Close the wells with a new cover ⓘ, invert 10 x, and **mix** by shaking for 15 s. Spin briefly (30 s, 1,500 x *g*) to collect any sample from the cover of the wells.

If 150 µl sample has been prepared, add 600 µl ethanol (96 – 100%) to each lysate.

3 Transfer samples to binding plate

Place waste tray into vacuum manifold base. Other plates for waste collection can also be used ⓘ. Insert spacers labeled “MTP/Multi-96 plate” notched side up and rest the MN Wash Plate ⓘ on them. Close manifold and place NucleoSpin® Virus Binding Plate on top of the manifold.

Transfer samples to the wells of the binding plate and be careful no to moisten the rims of the wells.

[1] Lysis must be done in MN Square-well Blocks if sample size is 150 µl. Additional MN Square-well Blocks may be necessary (see ordering information).

4 Bind viral nucleic acids to silica membrane

Apply vacuum of **-0.2 to -0.4 bar**[1] (reduction of atmospheric pressure) to allow samples to pass through the membrane (2 – 5 min). Flow-through rate should be about 1 – 2 drops per second. Adjust vacuum strength accordingly.

5 Wash silica membrane

1st wash

Add **500 µl Buffer RAW** to each well of the NucleoSpin® Virus Binding Plate. Apply vacuum **(-0.2 to -0.4 bar[1])** until all buffer has passed through the wells of the NucleoSpin® Virus Binding Plate (2–5 min). Release the vacuum.

2nd wash

Add **700 µl Buffer RAV3** to each well of the NucleoSpin® Virus Binding Plate. Apply vacuum **(-0.2 to -0.4 bar[1])** until all buffer has passed through the wells of the NucleoSpin® Virus Binding Plate (2 – 5 min). Release the vacuum.

3rd wash

Add **700 µl Buffer RAV3** to each well of the NucleoSpin® Virus Binding Plate. Apply vacuum **(-0.2 to -0.4 bar[1])** until all buffer has passed through the wells of the NucleoSpin® Virus Binding Plate (2 – 5 min). Release the vacuum.

Remove MN Wash Plate

After the final washing step close the valve, release the vacuum and remove the NucleoSpin® Virus Binding Plate. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry silica membrane

Reassemble the vacuum manifold and dry the membrane by applying maximum vacuum (e.g., **-0.6 bar**[1]) for **15 minutes**.

[1] Reduction of atmospheric pressure. Depending on sample viscosity extension of filtration time or increase of vacuum (e.g., -0.2 to -0.4 bar) may be required.

6 Elute viral RNA/DNA

Place a suitable vessel used for elution ⓘ on appropriate spacers (e.g., "Microtube rack") into the manifold base. Close manifold and insert NucleoSpin® Virus Binding Plate onto manifold top. Dispense **100 µl RNase-free water** or **Buffer RE (preheated to 70°C)** to each well of the plate. Pipette water directly onto the membrane. Incubate at room temperature for **2 – 3 min** and apply vacuum of **-0.4 bar**[1] until all of the samples have passed.

If only viral DNA is processed, elution should be done with Elution Buffer RE optimized for elution and storage of DNA.

Optional: Repeat elution step once (incubation not required).

Note: Elution by vacuum may cause cross-contamination due to aerosol formation and spraying of droplets. If possible, it is thus recommended to use centrifugation for the elution step.

[1] Reduction of atmospheric pressure. Depending on sample viscosity extension of filtration time or increase of vacuum (e.g., -0.2 to -0.4 bar) may be required.

6 Appendix

6.1 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 2.7). *Proteinase K digestion* • For certain sample types and for viral DNA isolation use of Proteinase K is required for the sample lysis step. Compare protocols with and without Proteinase K digestion. *Viral nucleic acids degraded* • Samples should be processed immediately. If necessary, add RNase inhibitor to the sample. Create a nuclease-free environment and ensure that no nucleases are present. Use suitable tips and buffer reservoirs. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer RAV1 and elution buffer.
Problems with subsequent detection	*Reduced sensitivity* • Carrier RNA may interfere with the PCR/RT-PCR system used. Change the volume of eluted viral DNA/RNA added to the PCR/RT-PCR. Use diluted eluates in order to exclude inhibition. Reduce Carrier RNA concentration in Buffer RAV1. Optimal concentration may require some preliminary experiments. *Ethanol carry-over* • Extend centrifugation times in order to remove Buffer RAV3 completely. *PCR inhibition* • Add an additional wash step with 96% ethanol following the last wash with Buffer RAV3.
General problems	*Clogged membrane* • Centrifuge sample lysate before the addition of ethanol and subsequent loading onto NucleoSpin® Virus Binding Plate.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 96 Virus	740691.2 740691.4	2 x 96 preps 4 x 96 preps
NucleoSpin® 96 Virus Core Kit	740452.4	4 x 96 preps
NucleoSpin® 8 Virus	740643 740643.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 8 Virus Core Kit	740451.4	48 x 8 preps
Proteinase K	740506	100 mg
MN Square-well Block	740476	4
Square-well Block	740481	4
Round-well Block with Cap Strips (set consists of 1 Round-well Block and12 Cap Strips)	740475	4
Rack of Tube Strips with Cap Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477	4
Cap Strips	740478	48
MN Wash Plates	740479	4
Self-adhering PE Foil	740676	50
MN Frame (for optimized handling of 96-well plates with vacuum manifold on BioRobot® 9600, 9604, and 3000 (Qiagen), MultiPROBE® II (PerkinElmer), Biomek® 2000, and FX (Beckman Coulter)	740680	1
Starter Set A (for use of 8-well strips on the NucleoVac 96 and automation platforms)	740682	1

Product	Cat. No.	Pack of
Starter Set C (for use of 8-well strips under centrifugation)	740684	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Support Frame for Column Holder A	740480	1

6.3 Product use restriction / warranty

NucleoSpin® 96 Virus (Core Kit) components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY.** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoSpin® 96 Virus (Core Kit)** for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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 an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon

warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

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

Last updated: 12/2006, Rev. 02