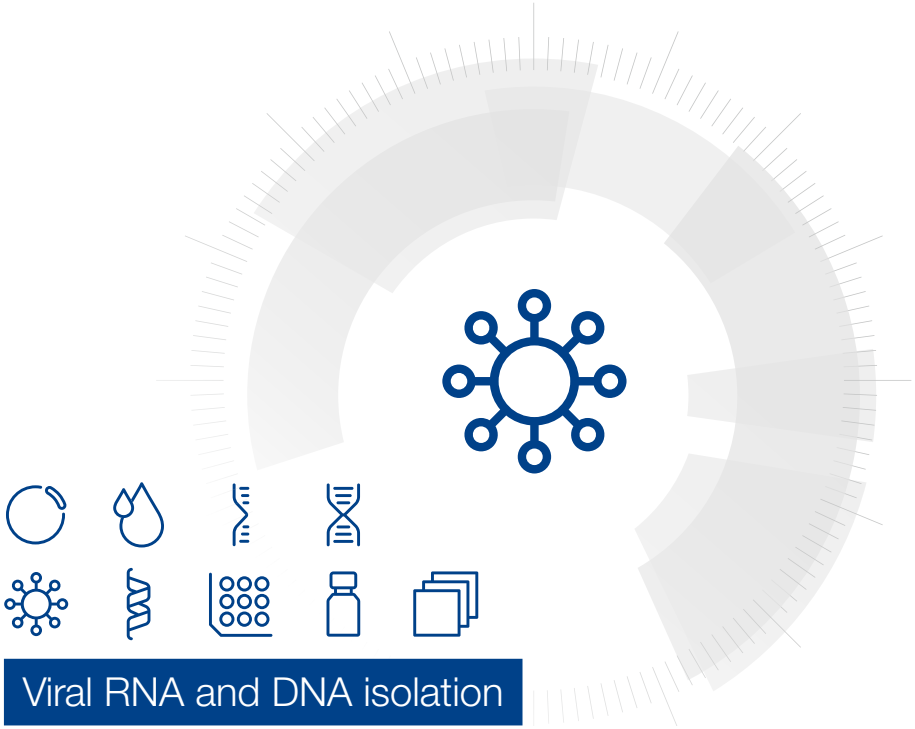
Bioanalysis

MACHEREY-NAGEL

User manual



Viral RNA and DNA isolation

- NucleoSpin® 96 Virus
- NucleoSpin® 96 Virus Core Kit

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Virus	
	2 × 96 preps	**4 × 96 preps**
REF	**740691.2**	**740691.4**
Lysis Buffer RAV1[1]	3 × 40 mL	6 × 40 mL
Wash Buffer RAW	2 × 75 mL	300 mL
Wash Buffer RAV3 (Concentrate)[1]	100 mL	2 × 100 mL
RNase-free H_2O	125 mL	125 mL
Elution Buffer RE[2]	125 mL	125 mL
Carrier RNA (lyophilized)[1]	3 × 1 mg	6 × 1 mg
Proteinase K (lyophilized)[1]	2 × 50 mg	3 × 75 mg
Proteinase Buffer PB	8 mL	15 mL
NucleoSpin® Virus Binding Plates (blue rings)	2	4
MN Wash Plates[4]	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
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[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

[4] For use with vacuum or positive pressure only

Kit contents continued

	NucleoSpin® 96 Virus Core Kit
	4 × 96 preps
REF	740452.4
Lysis Buffer RAV1[1]	6 × 40 mL
Wash Buffer RAW	300 mL
Wash Buffer RAV3 (Concentrate)[1]	2 × 100 mL
RNase-free H_2O	125 mL
Elution Buffer RE[2]	125 mL
Carrier RNA (lyophilized)[1]	6 × 1 mg
NucleoSpin® Virus Binding Plates (blue rings)	4
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1.2 Reagents and to be supplied by user

Reagents

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

For more detailed information regarding special hardware required for centrifuge or vacuum, positive pressure processing, please see section 2.3.

For recommended accessories for use of the flexible **NucleoSpin® 96 Virus Core Kit** (reduced kit composition; REF 740452.4), please see section 2.4.

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® 96 Virus** or **NucleoSpin® 96 Virus Core** 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.

[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® 8 / 96 Virus kits

The **NucleoSpin® 96 Virus** kit allows the purification of up to 96 samples. The kit is primarily designed for centrifugation use; vacuum or positive pressure 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 or positive pressure 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* NucleoSpin® 96 Virus
Manual use, vacuum or positive pressure	Low / medium throughput	NucleoSpin® 8 Virus* NucleoSpin® 8 Virus Core Kit
	High throughput	NucleoSpin® 96 Virus NucleoSpin® 96 Virus Core Kit
Automated use, vacuum, positive pressure or centrifuge	Low / medium throughput High throughput	NucleoSpin® 8 Virus Core Kit* NucleoSpin® 96 Virus Core Kit

* 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 or positive pressure use (for manual use or automated use on robotic platforms). Processing under vacuum or positive pressure 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)
Technology	Silica membrane technology
Format	96-well plates
Processing	Manual or automated, vacuum, positive pressure or centrifugation
Sample volume	100 – 150 µL*
Typical recovery	> 90 %
Analysis limit	30 – 60 cp/mL
Elution volume	70 – 100 µL
Preparation time	60 min/plate
Binding capacity	40 µg
Use	For research use only

* 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

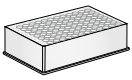
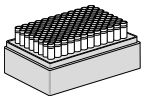
Although the **NucleoSpin® 96 Virus** kit is designed primarily for processing under centrifugation, processing under vacuum is also possible. The dead volume for the elution step is higher in comparison to centrifuge based elution. In order to achieve highly concentrated eluates and to avoid contamination, 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.**

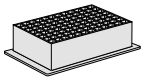
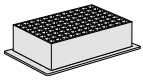
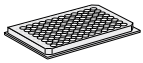
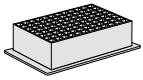
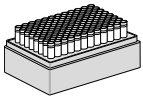
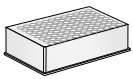
2.4 Recommended accessories for use of the NucleoSpin® 96 Virus Core Kit

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. Lyse samples	Round-well Block with 12 Cap Strips		Round-well Blocks and Tube Strips can be closed with Cap Strips.
	or		
	Rack of Tube Strips with 12 Cap Strips	 	

Protocol step	Suitable consumables, not supplied with the core kits		Remarks
	or MN Square-well Block Square-well Block Proteinase K		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. For certain samples and for viral DNA isolation use of Proteinase K is required.
2. Adjust 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 samples	MN Square-well Block		Can be used for waste collection if required.
4. Bind nucleic acids to the membrane	MN Wash Plate		MN Wash Plate minimizes the risk of cross contamination. **(used for vacuum processing only)**
7. Wash silica membrane*	MN Square-well Block		Can be used for waste collection if required.
8. Elute DNA	Rack of Tubes Strips with Cap Strips or Round-well Block	  	Round-well Blocks and Tube Strips can be closed with Cap Strips

* 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 semifluid 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*.

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 15 – 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.

* 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!

CAUTION: Buffer RAV1 contains guanidinium thiocyanate and Buffer RW 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.

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. Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardly be visible in the vial.

  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 15 – 25 °C for 1 – 2 weeks. Storage at 15 – 25 °C prevents salt precipitation and avoids preheating 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 × 96 preps	**4 × 96 preps**
REF	**740691.2**	**740691.4**
Wash Buffer RAV3 (Concentrate)	100 mL Add 400 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle
Proteinase K (lyophilized)	2 × 50 mg Add 2.5 mL Proteinase Buffer to each vial	3 × 75 mg Add 3.5 mL Proteinase Buffer to each vial
Carrier RNA (lyophilized)	3 × 1 mg Transfer each vial to one bottle of 40 mL Buffer RAV1	6 × 1 mg Transfer each vial to one bottle of 40 mL Buffer RAV1

	NucleoSpin® 96 Virus Core Kit
	4 × 96 preps
REF	**740452.4**
Wash Buffer RAV3 (Concentrate)	2 × 100 mL Add 400 mL ethanol to each bottle
Carrier RNA (lyophilized)	6 × 1 mg Transfer each vial to one bottle of 40 mL Buffer RAV1

4 Safety instructions

When working with the **NucleoSpin® 96 Virus** or **NucleoSpin® 96 Virus Core Kit** wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at *www.mn-net.com/msds*).

Caution: Guanidin thiocyanate in Buffer RAV1 and guanidin thiocyanate in buffer RAW can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® 96 Virus** kit or the **Core Kit** has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 NucleoSpin® 96 Virus – centrifuge processing

- For hardware requirements, refer to section 2.3.
- For detailed information on each step see page 18.
- For use of the NucleoSpin® 96 Virus Core Kit (REF 740452.4), 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	**Lyse** samples	**100 µL sample** **400 µL Buffer RAV1** **(20 µL Proteinase K)** **Mix** **25 – 70 °C, 10 min**
2	**Adjust** binding conditions	**400 µL ethanol (96 – 100 %)** **Mix**
3	**Transfer** samples to NucleoSpin® Virus Binding Plate	
4	**Bind** viral RNA and DNA to silica membrane of the NucleoSpin® Virus Binding Plate	**5,600 – 6,000 x *g*, 2 min**

5	**Wash** silica membrane	**500 µL RAW** **5,600 – 6,000 x *g*, 2 min** **700 µL RAV3** **5,600 – 6,000 x *g*, 2 min** **700 µL RAV3** **5,600 x *g* 15 min**
6	**Elute** viral RNA and DNA	**100 µL RE (70 °C)** **5,600 – 6,000 x *g*, 2 min** *Optional: Repeat elution step once*

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.

- For hardware requirements, refer to section 2.3.
- For use of the NucleoSpin® 96 Virus Core Kit (REF 740452.4), 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.

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, 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 premixed 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.

* 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 and 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 hardware requirements refer to section 2.3.
- For detailed information on each step see page 24.
- For detailed information regarding the vacuum manifold setup, see page 23.
- For use of the **NucleoSpin® 96 Virus Core Kit** (REF 740452.4), 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	**Lyse** samples	**100 µL sample** **400 µL Buffer RAV1** **(20 µL Proteinase K)** **Mix** **25 – 70 °C, 10 min**
2	**Adjust** binding conditions	**400 µL ethanol (96 – 100 %)** **Mix**
3	**Transfer** samples to NucleoSpin® Virus Binding Plate	
4	**Bind** nucleic acid to NucleoSpin® Virus Binding Plate	**-0.2 bar*, 5 min**

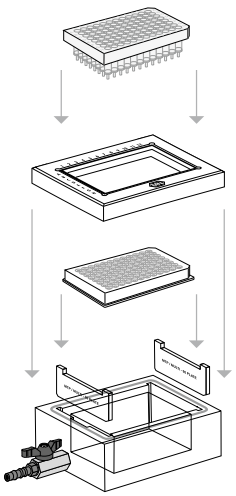
* Reduction of atmospheric pressure.

5	**Wash** and **dry** silica membrane	**500 µL RAW** **- 0.2 bar*** **5 min** **700 µL RAV3 – 0.2 bar*** **2 min** **700 µL RAV3 – 0.2 bar*** **5 min** **Remove MN Wash Plate** **-0.6 bar*** **15 min**
6	**Elute** viral RNA and DNA	**100 µL RE (70 °C)** **-0.4 bar*** **2 min** *Optional: Repeat elution step once* *Note: Elution under centrifugation is recommended.*

* Reduction of atmospheric pressure.

Setup of vacuum manifold:

Binding / Washing steps

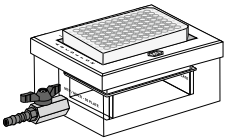


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

Step 3:
Place the manifold lid on top of the manifold base.

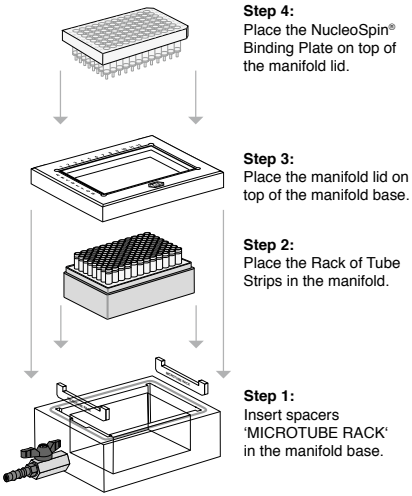
Step 2:
Place the MN Wash Plate in the manifold.

Step 1:
Insert spacers 'MTP/MULTI-96 PLATE' and waste container in the manifold base.



Final setup

Elution step

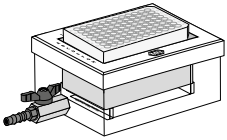


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the Rack of Tube Strips in the manifold.

Step 1:
Insert spacers 'MICROTUBE RACK' in the manifold base.



Final setup

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 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.

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).

- For hardware requirements refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 23.
- For use of the **NucleoSpin® 96 Virus Core Kit** (REF 740452.4), 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.

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, 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.

* '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).

Pipette mixture up and down several times. Do not moisten the rims.

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

*Optional: Add **20 µL Proteinase K** (20 mg/mL) to each sample premixed with Buffer RAV1. Close the lysis vessels 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 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 not to moisten the rims of the wells.

4 Bind viral nucleic acids to silica membrane

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

5 Wash and dry 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*)** until all buffer has passed through the wells of the NucleoSpin® Virus Binding Plate **(2 – 5 min)**. Release the vacuum.

* Reduction of atmospheric pressure.

2nd wash

Add 700 µL Buffer RAV3 to each well of the NucleoSpin® Virus Binding Plate. Apply vacuum **(- 0.2 to - 0.4 bar*)** 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*)** 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. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Optional: If necessary move the NucleoSpin® Virus Binding plate to a clean paper sheet (included with the MN Wash Plate) to remove residual wash buffer

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

6 Elute viral RNA and 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*** 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.

* Reduction of atmospheric pressure.

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	REF	Pack of
NucleoSpin® 96 Virus	740691.2 740691.4	2 × 96 preps 4 × 96 preps
NucleoSpin® 96 Virus Core Kit	740452.4	4 × 96 preps
NucleoSpin® 8 Virus	740643 740643.5	12 × 8 preps 60 × 8 preps
NucleoSpin® 8 Virus Core Kit	740451.4	48 × 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
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

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

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

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

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

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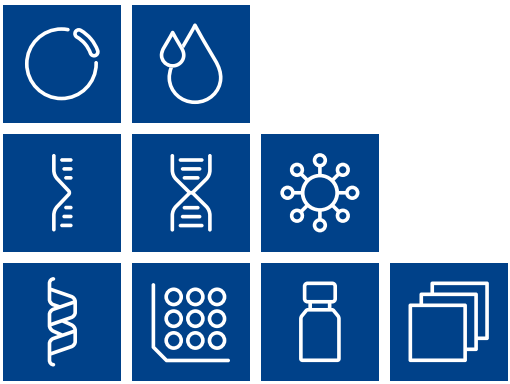
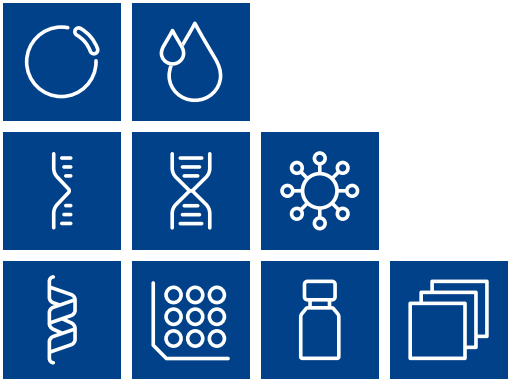


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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