Viral DNA/RNA Isolation

User manual

NucleoSpin® 8 Virus
NucleoSpin® 96 Virus
NucleoSpin® 96 Virus Core Kit

January 2008/ Rev. 02

MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoSpin® 8 Virus	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740643**	**740643.5**
Lysis Buffer RAV1*	2 x 40 ml	8 x 40 ml
Wash Buffer RAW	70 ml	2 x 150 ml
Wash Buffer RAV3 (Concentrate)	40 ml	200 ml
H_2O, RNase-free	30 ml	125 ml
Elution Buffer RE**	25 ml	125 ml
Carrier RNA* (lyophilized)	2 x 1 mg	8 x 1 mg
Proteinase K* (lyophilized)	50 mg	3 x 75 mg
Proteinase Buffer PB	8 ml	15 ml
NucleoSpin® Virus Binding Strips (blue rings)	12	60
Tube Strips with Cap Strips ***	4	15
MN Square-well Blocks	6	20
Self-adhering PE Foil	10	50
User Manual	1	1

* For preparation of working solutions see section 3.
** Composition of Elution Buffer RE: 5 mM Tris/HCl, pH 8.5
*** Set of 1 rack with 12 8-well Tube Strips and 12 Cap Strips

1 Kit contents *continued*

	NucleoSpin® 96 Virus	
	2 x 96 preps	**4 x 96 preps**
Cat.No.	**740691.2**	**740691.4**
Lysis Buffer RAV1*	3 x 40 ml	6 x 40 ml
Wash Buffer RAW	150 ml	2 x 150 ml
Wash Buffer RAV3* (Concentrate)	100 ml	200 ml
H_2O, RNase-free	65 ml	125 ml
Elution Buffer RE**	75 ml	125 ml
Carrier RNA* (lyophilized)	3 x 1 mg	6 x 1 mg
Proteinase K* (lyophilized)	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 Block	6	12
Tube Strips with Cap Strips***	2	4
Self-adhering PE Foil	10	20
User Manual	1	1

* For preparation of working solutions see section 3.
** Composition of Elution Buffer RE: 5 mM Tris/HCl, pH 8.5
*** Set of 1 rack with 12 8-well Tube Strips and 12 Cap Strips

1 Kit contents *continued*

	NucleoSpin® 8/96 Virus Core Kit	
	NucleoSpin® 8 Virus Core Kit	**NucleoSpin® 96 Virus Core Kit**
	48 x 8 preps	**4 x 96 preps**
Cat.No.	**740451.4**	**740452.4**
Lysis Buffer RAV1*	6 x 40 ml	6 x 40 ml
Wash Buffer RAW	2 x 150 ml	2 x 150 ml
Wash Buffer RAV3* (Concentrate)	200 ml	200 ml
H_2O, RNase-free	125 ml	125 ml
Elution Buffer RE**	125 ml	125 ml
Carrier RNA* (lyophilized)	6 x 1 mg	6 x 1 mg
NucleoSpin® Virus Binding Plates (blue rings)	/	4
NucleoSpin® Virus Binding Strips (blue rings)	48	/
User Manual	1	1

* For preparation of working solutions see section 3.
** Composition of Elution Buffer RE: 5 mM Tris/HCl, pH 8.5

1 Kit contents *continued*

Accessories for **NucleoSpin® 8/96 Virus Core Kit** (not supplied with the kits):

	NucleoSpin® 8/96 Virus Core Kit	
Cat. No.	**Pack of**	**Ordering information**
Round-well Block with Cap Strips	4	740475
MN Square-well Block	4	740476
Square-well Block	4	740481
Tube Strips with Cap Strips*	4	740477
Cap Strips	48	740478
Self-adhering PE Foil	50	740676
MN Wash Plate	4	740479
Proteinase K	100 mg	740506

* Set of 1 rack with 12 8-well Tube Strips and 12 Cap Strips

2 Product description

2.1 The basic principle

The **NucleoSpin® 8/96 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 together with the variable and highthroughput of the 8-well- and 96-well formats. With the **NucleoSpin® 8/96 Virus** method, RNA viruses are quickly and efficiently lysed by Lysis Buffer RAV1 which is a highly concentrated solution of GITC. DNA viruses (e.g. HBV) are usually more difficult to isolate and require a digestion of samples with Proteinase K which is enclosed in the kit. Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the NucleoSpin® Virus Binding Strips or Plate. Carrier RNA included in Lysis Buffer RAV1 improves binding and recovery of the 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 buffers RAW and 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.

About this User Manual

The **NucleoSpin® 8/96 Virus** kits allow for the purification of multiples of 8 (**NucleoSpin® 8 Virus**) or 96 samples (**NucleoSpin® 96 Virus**). Both kits are primarily designed for manual use in a centrifuge. A vacuum use of this kits allow for more variation and higher flexibility in the consumables used for lysis, washing and elution. MN takes this into account by introducing the **NucleoSpin® 8/96 Virus Core Kits**, which are primarily recommended for manual or automated vacuum use. The Core kits together with a large variety of suitable disposables ensure the highest degree of flexibility for the user.

Kit selection guide:

	Application	Kit recommendation
manual use, centrifuge	low-/medium throughput high throughput	**NucleoSpin® 8 Virus** **NucleoSpin® 96 Virus**
manual use, vacuum	low-/medium throughput high throughput	**NucleoSpin® 8 Virus** **NucleoSpin® 8 Virus Core Kit** **NucleoSpin® 96 Virus** **NucleoSpin® 96 Virus Core Kit**
automated use, vacuum or centrifuge	low-/medium throughput high throughput	**NucleoSpin® 8 Virus Core Kit** **NucleoSpin® 96 Virus Core Kit**

2.2 Kit specifications

- **NucleoSpin® 8/96 Virus** kits allow for the parallel purification of viral DNA and RNA from 100 - 150 µl plasma, serum, or other cell-free biological fluids. Samples can be either 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 2.4.

- The prepared 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, e.g. 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® 8/96 Virus** kits allow for the parallel purification of samples in multiples of 8 (**NucleoSpin® 8 Virus**) or 96 samples (**NucleoSpin® 96 Virus**).

- **NucleoSpin® 8/96 Virus** kits can be processed by centrifugation or under vacuum. Processing under vacuum allows for 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 2.6 and contact your local distributor or MN directly.

- **NucleoSpin® 8/96 Virus Core Kits** are primarily designed for vacuum use (for manual use or automated use on robotic platforms).

Kit specifications at a glance	
	NucleoSpin® 8/96 Virus
Sample size	100 -150 µl
Recovery rates	> 90%
Analysis limit	30-60 cp/ml
Elution volume	70-100 µl
Binding capacity	40 µg
Time/ 6 strips or 1 plate	60 min
Column type	8-well strip or 96-well plate

2.3 Required hardware

Centrifugation

For centrifugation, a microtiterplate centrifuge which is able to accommodate the NucleoSpin® Virus Binding Strips/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), e.g. Hermle Z 513/Z 513 K, Jouan KR4i, Heraeus Kendro Multifuge 3/3-R, Beckman Coulter Allegra 25R, Hettich Rotanta 460 series, Sigma 4-15/4K15/6-15/6K15. For processing the 8-well strips of the **NucleoSpin® 8 Virus** kit the Starter Set C (see ordering information, containing Column Holders C, MN Square-well Blocks, Tube Strips) is required, too. For detailed information refer to the Starter Set C manual.

Vacuum processing

Although the **NucleoSpin® 8/96 Virus** kit is designed primarily for processing under centrifugation, processing under vacuum is also possible. It should be considered that the dead volume for the elution step is higher in comparison to centrifuge based elution. In order to achieve high concentrated eluates and to avoid contamination, it is recommended to perform the elution step by centrifugation. Consumables for vacuum processing differ from the consumables required for centrifugation. Therefore, for vacuum processing, we recommend to use the **NucleoSpin® 8/96 Virus Core Kits**. For manual processing under vacuum a NucleoVac 96 Vacuum Manifold (Cat.No. 740681) is required for both **NucleoSpin® 8 Virus (Core Kit)** and **NucleoSpin® 96 Virus (Core Kit)**. If the **NucleoSpin® 8 Virus (Core Kit)** kit is used with vacuum in addition to NucleoVac 96 Vacuum Manifold, the Starter Set A (containing Column Holders A and NucleoSpin® Dummy Strips, see ordering information) is required.

2.4 Sample material

Liquid samples

Biological fluids or semi-fluid samples can be processed, e.g. serum, urine or BAL. For successful nucleic acid purification it is important to obtain a homogeneous, clear, and non-viscous sample before loading onto the NucleoSpin® Virus Binding Strips or Plate. Therefore, check all samples (especially old or frozen ones) for the presence of precipitates. Precipitates can be removed following addition of Lysis Buffer RAV1 and lysis incubation by centrifugation. Avoid clearing samples by centrifugation/filtration before the 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. However, RNA is sensitive and prolonged incubation may cause decreased yields.

Solid samples (Tissue samples, stool samples)

Prepare a 10% (w/v) suspension of tissue in e.g. PBS buffer 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 suitable buffer (e.g. PBS buffer) 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 Strip or Plate. The amount of PBS buffer added to a blood sample 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® 8/96 Virus** and **NucleoSpin® 8/96 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 volumes of Lysis Buffer RAV1 and ethanol have to be increased to 600 µl. Depending on the size of pipetting tips, the total lysate volume of 1300 µl may be loaded in two steps to the NucleoSpin® Virus Binding Strips/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 room temperature (20-25°C) will be sufficient. For isolation of viral RNA from viscous samples, e.g. 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.

2.5 Carrier RNA

The **NucleoSpin® 8/96 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 the eluates of the **NucleoSpin® 8/96 Virus** kit contain both viral nucleic acids and Carrier RNA with amounts of Carrier RNA that exceeds the amount of viral nucleic acids by far. Therefore it is not possible to quantify the nucleic acids isolated with the kit by photometric or fluorometric methods. 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.

2.6 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, e.g. 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 pre-warmed elution buffer incubate the NucleoSpin® Virus Binding Strip/Plate for 3 min at 60-70°C before elution.

2.7 Automation

For automated use we recommend using the **NucleoSpin® 8/96 Virus Core Kits** 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® 8/96 Virus Core Kits** on a certain workstation please contact MN.

For vacuum processing the use of the disposable MN Wash Plate inside the vacuum manifold is recommended. Use of MN Wash Plate reduces risk of contamination caused by spraying of solutions during vacuum filtration steps. Visit MN on the internet 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.

3 Storage conditions and preparation of working solutions

Attention:

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

- Carrier RNA has a limited shelf-life in Buffer RAV1. For this reason the **NucleoSpin® 8/96 Virus** kit contains several vials of lyophilized Carrier RNA which should be used successively as required. For the stability and storage of Carrier RNA refer to section 3.1.

Before starting any **NucleoSpin® 8/96 Virus** protocol prepare the following:

- Add indicated volume of 96-100% ethanol to the Wash Buffer RAV3 (Concentrate).

- 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 +4°C for up to 6 months. Dividing the solution into small aliquots and storage at –20°C is recommended if the solution will not be used up during this period.

- Before use, add 1 ml Lysis Buffer RAV1 to the complete content of the Carrier RNA tube. Dissolve the RNA and transfer it back to the RAV1 bottle.

	NucleoSpin® 8 Virus	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740643**	**740643.5**
Buffer RAV3 (Concentrate)	40 ml add 160 ml ethanol	200 ml add 800 ml ethanol
Proteinase K (lyophilized)	50 mg add 2.5 ml Proteinase Buffer	3 x 75 mg add to each vial 3.5 ml Proteinase Buffer
Carrier RNA (lyophilized)	1 mg transfer to 40 ml Buffer RAV1	8 x 1 mg transfer each vial to one bottle of 40 ml Buffer RAV1

	NucleoSpin® 96 Virus	
	2 x 96 preps	**4 x 96 preps**
Cat. No.	**740691.2**	**740691.4**
Buffer RAV3 (Concentrate)	100 ml add 400 ml ethanol	200 ml add 800 ml ethanol
Proteinase K (lyophilized)	2 x 50 mg dissolve in 2.5 ml Proteinase Buffer each	3 x 75 mg dissolve in 3.5 ml Proteinase Buffer each
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® 8/96 Virus Core Kit	
	NucleoSpin® 8 Virus Core Kit	**NucleoSpin® 96 Virus Core Kit**
	48 x 8 preps	**4 x 96 preps**
Cat. No.	**740451.4**	**740452.4**
Buffer RAV3 (Concentrate)	200 ml add 800 ml ethanol	200 ml add 800 ml ethanol
Carrier RNA (lyophilized)	6 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

3.1 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-warming 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 pre-warmed at 40-60°C for a maximum of 5 min in order to re-dissolve precipitated salts.

Attention:

Frequent warming, 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 !

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 8/96 Virus** and **NucleoSpin® 8/96 Virus Core Kits** 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	Guanidine thiocyanate	 Xn*	Harmful by inhalation, in contact with skin and if swallowed.	R 20/21/22	S 13
RAW	Guanidine hydrochloride + ethanol < 50%	 Xn*	Flammable. Harmful if swallowed. Irritating to eyes and skin.	R 10-22-36/38	S 7-16
Proteinase K	Proteinase K, lyophilized	 Xn Xi*	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 the 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

Safety Phrases

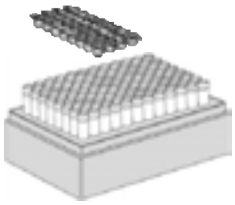
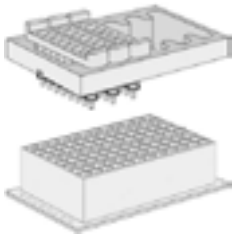
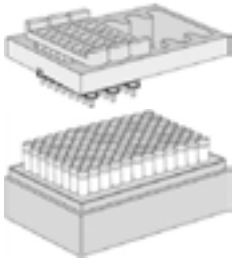
S 7	Keep container tightly closed
S 13	Keep away from food, drink and animal feedstuffs
S 16	Keep away from sources of ignition – No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 36/37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

* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. Nr. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1)

5 General Procedure

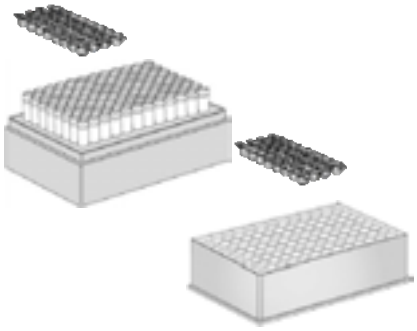
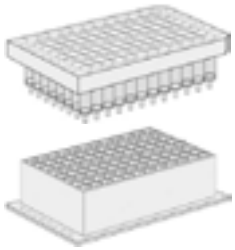
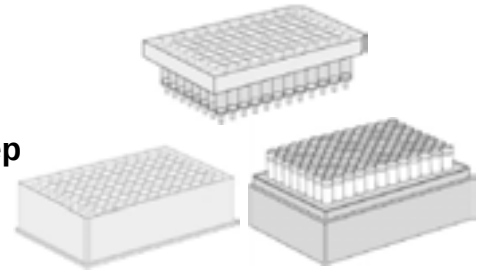
NucleoSpin® 8 Virus centrifuge processing

For detailed information on each step see section 5.1.

1 Lysis	**100 µl sample** **400 µl Buffer RAV 1** **(20 µl Proteinase K)** **mix** **25°C – 70°C, 10 min**	 Tube Strips with Cap Strips
2 Adjust DNA binding conditions	**400 µl ethanol** **mix**	
3 Load samples	**Transfer samples to NucleoSpin® Virus Binding Strips**	 Column Holder C with NucleoSpin® Virus Binding Strips on MN Square-well Block
4 Bind DNA 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*, 5 min** **700 µl RAV3** **5,600 x *g*, 15 min**	
6 Elution	**100 µl RE, 70°C** **5,600 x g, 2 min** **Optional: repeat elution step once**	 Column Holder C with NucleoSpin® Virus Binding Strips on Tube Strips

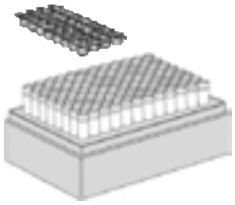
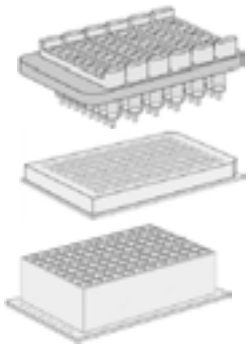
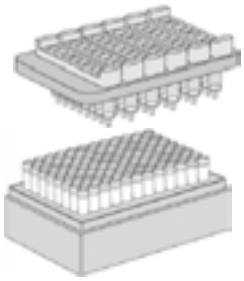
NucleoSpin® 96 Virus centrifuge processing

For detailed information on each step see section 5.1.

1 Lysis	**100 µl sample** **400 µl Buffer RAV 1** **(20 µl Proteinase K)** **mix** **25°C – 70°C, 10 min**	 Tube Strips **or** Round-well Block with Cap Strips
2 Adjust DNA binding conditions	**400 µl ethanol** **mix**	
3 Load samples	**Transfer samples to NucleoSpin® Virus Binding Strips**	 NucleoSpin® Virus Binding Plate on MN Square-well Block
4 Bind DNA 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*, 5 min** **700 µl RAV3** **5,600 x *g*, 15 min**	
6 Elution	**100 µl RE, 70°C** **5,600 x *g*, 2 min** **Optional: repeat elution step once**	 NucleoSpin® Virus Binding Strips on Round-well Block **or** Tube Strips

NucleoSpin® 8 Virus vacuum processing

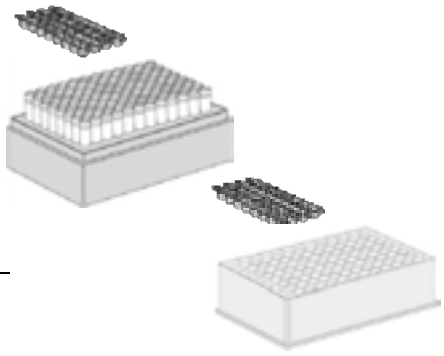
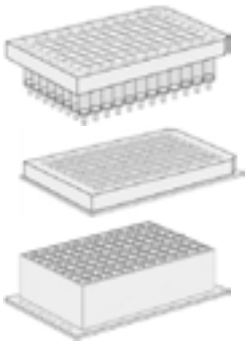
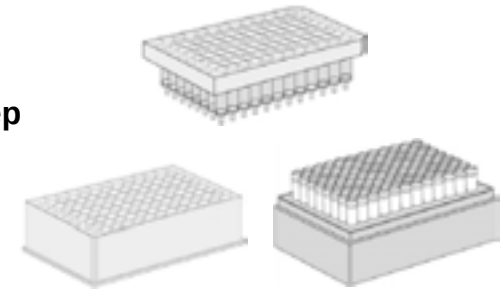
For detailed information on each step see section 5.2.

1 Lysis	**100 µl sample** **400 µl Buffer RAV 1** **(20 µl Proteinase K)** **mix** **25°C – 70°C, 10 min**	 Tube Strips with Cap Strips
2 Adjust DNA binding conditions	**400 µl ethanol** **mix**	
3 Load samples	**Transfer samples to NucleoSpin® Virus Binding Strips**	 Column Holder A with NucleoSpin® Virus Binding Strips and MN Wash Plate on MN Square-well Block (optional)
4 Bind DNA to silica membrane	**-0.2 bar*, 5 min**	
5 Wash silica membrane	**500 µl RAW** **-0.2 bar*, 5 min** **700 µl RAV3** **-0.2 bar*, 5 min** **700 µl RAV3** **-0.2 bar*, 5 min**	
Dry silica membrane	**Remove MN Wash Plate** **-0.6 bar, 15 min**	
6 Elution	**100 µl RE, 70°C** **-0.4 bar*, 2 min** **Optional: repeat elution step once** **Elution under centrifugation is recommended**	 Column Holder A with NucleoSpin® Virus Binding Strips on Tube Strips

* 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.

NucleoSpin® 96 Virus vacuum processing

For detailed information on each step see section 5.2.

1 Lysis	**100 µl sample** **400 µl Buffer RAV 1** **(20 µl Proteinase K)** **mix** **25°C – 70°C, 10 min**	 Tube Strips **or** Round-well Block with Cap Strips
2 Adjust DNA binding conditions	**400 µl ethanol** **mix**	
3 Load samples	**Transfer samples to NucleoSpin® Virus Binding Plate**	 NucleoSpin® Virus Binding Plate on MN Square-well Block (optional)
4 Bind DNA to silica membrane	**-0.2 bar*, 5 min**	
5 Wash silica membrane	**500 µl RAW** **-0.2 bar*, 5 min** **700 µl RAV3** **-0.2 bar*, 5 min** **700 µl RAV3** **-0.2 bar*, 5 min**	
Dry silica membrane	**Remove MN Wash Plate** **-0.6 bar*, 15 min**	
6 Elution	**100 µl RE, 70°C** **-0.4 bar*, 2 min** **Optional: repeat elution step once** **Elution under centrifugation is recommended**	 NucleoSpin® Virus Binding Strips on Round-well Block **or** Tube Strips

* 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.

5.1 NucleoSpin® 8/96 Virus Protocol – purification of viral RNA/DNA under centrifugation

This standard protocol is recommended for purification of viral RNA from, e.g. HCV or HIV. Certain DNA viruses such as CMV can also be isolated but for HBV and similar types Proteinase K digestion is recommended. Before starting the isolation procedure, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water.

NucleoSpin® 8 Virus:

The use of NucleoSpin® Virus Binding Strips in a Column Holder C allows the isolation of up to n x 8 samples (n = 1-6). Insert as many of the NucleoSpin® Virus Binding Strips as required into the same positions of each one of the two reusable column holders and place column holders onto the MN Square-well Blocks. Label the column holders or 8-well strips for later identification.

Always use 2 Column Holders C containing identical numbers of NucleoSpin® Virus Binding Strips for centrifugation. This avoids the need to balance the centrifuge, and allows multiples of 16 samples to be processed in parallel. We recommend inserting the NucleoSpin® Virus Binding Strips around the center of the column holder.

NucleoSpin® 96 Virus:

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

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1 Lyse samples

Pipette 400 µl Buffer RAV1 into the wells of a rack with 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. A good choice is the Matrix Impact² multichannel pipettor with 102-mm-long 1,250 µl tips (Matrix # 8245).

Add 100 µl sample to each 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 (20-25°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

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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 2.4

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

2 Adjust 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 x and mix by shaking for 15 sec. Spin down droplets (30 sec; 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 strips/plates

Remove the first Cap Strip and transfer all of each sample into the wells of a NucleoSpin® Virus Binding Strip/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 Strips/Plates with a Self-adhering PE Foil.

4 Bind viral nucleic acids to silica membrane

Place the MN Square-well Blocks with Binding Strips/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.

Optionally, if 150 µl sample has been prepared, load it in successive steps onto the NucleoSpin® Virus Binding Strips/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 Strips/Plate. Seal the NucleoSpin® Virus Binding Strips/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 Strips/Plate onto a new MN Square-well Block.

2nd wash

Add 700 µl Buffer RAV3 to each well of the NucleoSpin® Virus Binding Strips/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 evaporate ethanol from residual Wash Buffer RAV3.

Alternatively, remove the adhesive foil and place the NucleoSpin® Virus Binding Strips/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 Elution

Place the NucleoSpin® Virus Binding Strips/Plate onto the elution-rack with Tube Strips.

Dispense 75-100 µl RNase-free water (preheated to 70°C) to each well of the NucleoSpin® Virus Binding Strips/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 much 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.

For processing under vacuum see section 5.2.

5.2 NucleoSpin® 8/96 Virus Core Kits – purification of viral RNA/DNA under vacuum

Whereas the use of a centrifuge for the processing of the **NucleoSpin® 8/96 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.

MACHEREY-NAGEL takes this demand into account by the introduction of the **NucleoSpin® 8/96 Virus Core Kits**. The Core kits contain all buffers and reagents required for the purification of 48x8 samples (**NucleoSpin® 8 Virus Core Kit**) or 4x96 samples, respectively (**NucleoSpin® 96 Virus Core Kit**). The Core kit is supplemented by a large variety of suitable disposables, especially for lysis and elution. Here, depending on individual needs or affinities the user can choose from Tube Strips, Square- and Round-well Blocks, etc. The various options and requirements are outlined below.

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 Strips or Binding Plate. This very assistant tool is thus recommended for use with the Core kit.

When using the **NucleoSpin® 8/96 Virus Core Kit** under vacuum the NucleoVac 96 vacuum manifold is required (see ordering information). For use of the **NucleoSpin® 8 Virus Core Kit** in addition the Starter Set A is required which contains the Column Holder A and the NucleoSpin® Dummy Strips to close/seal unused rows of the column holder when applying vacuum.

NucleoSpin® 8 Virus:

The use of NucleoSpin® Virus Binding Strips in a Column Holder A allows the isolation of up to n x 8 samples (n = 1-6). Insert as many of the NucleoSpin® Virus Binding Strips as required into the reusable column holder. Close unused openings of the column holder with NucleoSpin® Dummy Strips and place column holders containing NucleoSpin® Virus Binding Strips and NucleoSpin® Dummy Strips onto NucleoVac Vacuum Manifold. If a final elution step by centrifugation is preferred (recommended), either a Column Holder C or a Support Frame (Support Frame for centrifugation of Column Holder A, see ordering information) are required.

NucleoSpin® 96 Virus:

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.

General protocol for processing NucleoSpin® 8/96 Virus Core Kit under vacuum

Please 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 5.3.

This standard protocol is recommended for purification of viral RNA from e.g. HCV or HIV. Certain DNA viruses such as CMV can also be isolated. For HBV and similar types Proteinase K digestion is recommended (not included in the Core kits). Before starting the isolation procedure, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water.

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. A good choice is the Matrix Impact^2 multichannel pipettor with 102-mm-long 1,250 µl tips (Matrix # 8245).

Add 100 µl sample to each 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 (20-25°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 2.4.

Spin briefly (30 sec, 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 10x and mix by shaking for 15 sec. Spin briefly (30 sec, 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 strips/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 Strips/Plate on top of the manifold. When using the NucleoSpin® 8 Virus Core Kit, Column Holder A has to be used to fix the 8-well strips on the manifold. Close unused openings of the column holder with NucleoSpin® Dummy Strips.

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

4 Bind DNA to silica membrane

Apply vacuum of -200 to -400 mbar (reduction of atmospheric pressure) to allow samples to pass through the membrane. 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 Strips/Plate and apply vacuum of -200 to -400 mbar (reduction of atmospheric pressure).

2nd wash

Add 700 µl Buffer RAV3 to each well of the NucleoSpin® Virus Binding Strips/Plate. Apply vacuum of -200 to -400 mbar (reduction of atmospheric pressure).

3rd wash

Add 700 µl Buffer RAV3 to each well of the NucleoSpin® Virus Binding Strips/Plate. Apply vacuum of -200 to -400 mbar (reduction of atmospheric pressure).

Remove MN Wash Plate and waste tray or other plates used for waste collection.

Reassemble the vacuum manifold and dry the membrane by applying maximum vacuum (e.g. -600 mbar) for 15 minutes.

6 Elution

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 Strips/Plate into manifold top. Dispense 100 µl RNase-free water (preheated to 70°C) to each well of the strips or plate. Pipette water directly onto the membrane. Incubate at room temperature for 2-3 min and apply vacuum of -400 mbar (reduction of atmospheric pressure) 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.

Optionally 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. For elution under centrifugation either a Column Holder C or a Support Frame (Support Frame for centrifugation of Column Holder A, see ordering information) are required.

For use of **NucleoSpin® 8/96 Core Kit** in a centrifuge see section 5.1.

5.3 Recommended accessories for use of the NucleoSpin® 8/96 Virus Core Kit under vacuum

Protocol step	Suitable consumables, not supplied with the Core kits - ⓘ	Remarks
1. Lysis	Round-well Block with 12 Cap Strips Tube Strips with 12 Cap Strips	Round-well Blocks and Tube Strips can be closed with Cap Strips.
	MN Square-well Block Square-well Block	Square-well Blocks can not be closed by 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.
5. Wash silica membrane*	MN Square-well Block	Can be used for waste collection if required.
6. Elution	Tubes Strips with 12 Cap Strips Round-well Block with 12 Cap Strips	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.

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 3.1). *Proteinase K digestion* • For certain sample types and for viral DNA isolation use of Proteinase K is required for 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 previous experiments *Ethanol carryover* • Extend centrifugation steps 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 or Strips.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Virus	740643	12 x 8 preps
NucleoSpin® 8 Virus	740643.5	60 x 8 preps
NucleoSpin® 8 Virus Core Kit	740451.4	48 x 8 preps
NucleoSpin® 96 Virus	740691.2	2 x 96 preps
NucleoSpin® 96 Virus	740691.4	4 x 96 preps
NucleoSpin® 96 Virus Core Kit	740452.4	4 x 96 preps
Proteinase K	740506	100 mg
MN Square-well Block	740476	4
Square-well Block	740481	4
Round-well Block with Cap Strips	740475	4
Tube Strips* with Cap Strips	740477	4
Cap Strips	740478	48
MN Wash Plates	740479	4
Self-adhering PE Foil	740676	50
MN Frame	740680	1
Starter Set A	740682	1
Starter Set C	740684	1
Vacuum Regulator	740641	1
NucleoVac 96 Vacuum Manifold	740681	1
Support Frame for Column Holder A (for centrifugation only)	740480	1

* Set of 1 rack, 12 strips with 8 tubes each

6.3 Product use restriction / warranty

NucleoSpin® 8/96 Virus (Core) kit components were developed, designed and sold for research purposes only. They are suitable *for in vitro uses only*. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather in the responsibility of the user to verify the use of the **NucleoSpin® 8/96 Virus (Core)** kits 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 guarantees 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.

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