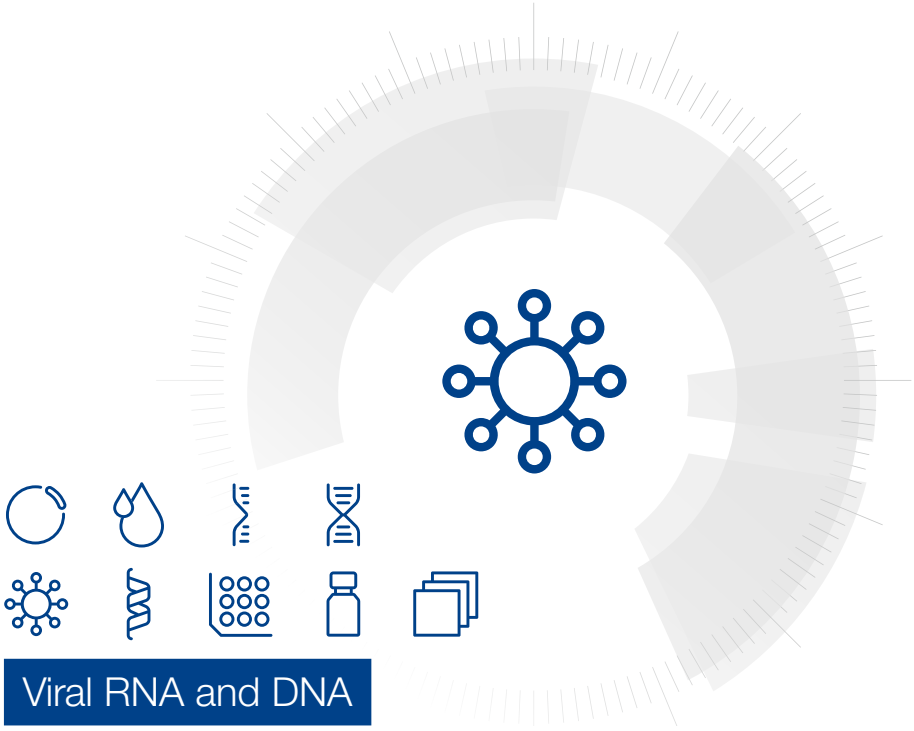
Bioanalysis

MACHEREY-NAGEL

User manual



Viral RNA and DNA

■ NucleoSpin® Virus

February 2023 / Rev. 05

Viral RNA and DNA isolation

Protocol at a glance (Rev. 05)

NucleoSpin® Virus

	200 µL sample	400 µL sample
1 Lyse viruses	5 µL Pro. K 200 µL sample 200 µL VL Mix 5.6 µL Carrier RNA Mix RT, 3 min Quick spin	10 µL Pro. K 400 µL sample 400 µL VL Mix 11.2 µL Carrier RNA Mix RT, 3 min Quick spin
2 Adjust binding conditions	200 µL ethanol Mix RT, 5 min Quick spin	400 µL ethanol Mix RT, 5 min Quick spin
3 Bind viral RNA / DNA	Load sample (~ 610 µL) 4,000 x *g*, 3 min	Load sample stepwise 4,000 x *g*, 3 min
4 Wash and dry silica membrane	400 µL VW1 400 µL VW2 200 µL VW2	400 µL VW1 400 µL VW2 200 µL VW2
1st and 2nd wash	11,000 x *g*, 30 s	11,000 x *g*, 30 s
3rd wash	20,000 x *g*, 5 min	20,000 x *g*, 5 min
dry	56 °C, 5 min with open lid	56 °C, 5 min with open lid
5 Elute RNA / DNA	30 µL RNase-free H_2O (70 °C) RT, 3 min 20,000 x *g*, 3 min	30 µL RNase-free H_2O (70 °C) RT, 3 min 20,000 x *g*, 3 min

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1 Components

1.1 Kit contents

	NucleoSpin® Virus		
REF	**10 preps 740983.10**	**50 preps 740983.50**	**250 preps 740983.250**
Lysis Buffer VL	13 mL	25 mL	125 mL
Wash Buffer VW1	6 mL	30 mL	125 mL
Wash Buffer VW2 (Concentrate)*	6 mL	12 mL	50 mL
RNase-free H_2O	13 mL	13 mL	13 mL
Carrier RNA (lyophilized)	300 µg	2 × 300 µg	3 × 1 mg
Liquid Proteinase K	120 µL	600 µL	2 × 1.5 mL
NucleoSpin® Virus Columns (light red rings, plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	30	150	750
Collection Tubes (1.5 mL) for lysis and elution	20	100	500
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* For preparation of working solutions and storage conditions see section 3.

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (to prepare Wash Buffer VW2 and to adjust RNA / DNA binding conditions); non-denaturated ethanol is recommended

Consumables

- Disposable pipette tips (aerosol barrier pipette tips are recommended to avoid cross-contamination)

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating block for 56 °C incubation
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended to read the detailed protocol sections of this user manual if using the **NucleoSpin® Virus** kit for the first time. However, experienced users may refer to the Protocol-at-a-glance. 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***.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Virus** method, RNA and DNA viruses are lysed quickly and efficiently by Lysis Buffer VL which is a highly concentrated solution of chaotropic ions. DNA viruses (e.g., HBV) are usually more difficult to lyse and require a digestion with Proteinase K which is supplied in the kit. Lysis buffer and ethanol create appropriate binding conditions of nucleic acids to the NucleoSpin® Virus Columns. Carrier RNA improves binding and recovery of low-concentrated viral nucleic acids. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in simple wash steps with alcoholic buffers VW1 and VW2. The nucleic acids are eluted in water and are ready-for-use in subsequent reactions.

2.2 Kit specifications

NucleoSpin® Virus kit is designed for the rapid preparation of highly pure viral nucleic acids (e.g., HCV, HIV, CMV) from biological fluids, such as plasma, and serum. Whole blood samples cannot be used.

- No cross-contamination due to closed systems.
- The **NucleoSpin® Virus** kit is suited to process 200 µL plasma / serum or 400 µL plasma / serum.
- The NucleoSpin® Virus Column allows a small elution volume (30 µL) for highly concentrated viral nucleic acids.
- The prepared nucleic acids are suitable for applications, such as automated fluorescent DNA sequencing, RT-PCR, PCR, or any kind of enzymatic reaction.
- The detection limit of viruses depends on the individual detection procedures, such as, in-house nested (RT-) PCR or qRT-PCR. We highly recommend using standards as well as positive and negative controls to monitor the purification, amplification, and detection processes.
- **Carrier RNA** (poly(-A) RNA: poly(A) potassium salt, prepared from ADP with polynucleotide phosphorylase) is included for optimal performance.
- **Liquid Proteinase K** is included to facilitate adequate lysis of protein in the samples.
- For research use only.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Virus
Technology	Silica-membrane technology
Format	Mini spin columns
Sample material	200 µL serum, plasma, cell-free biological fluids (400 µL with two loading steps)
Fragment size	approx. 100 bp–50 kb
Elution volume	30 µL
Preparation time	50 min/6 preps

2.3 Remarks regarding sample quality and preparation

Biological fluids or semi-fluid samples, such as plasma and serum, can be processed with **NucleoSpin® Virus** kit. For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and non-viscous sample before loading onto the **NucleoSpin® Virus** Columns. Therefore, check all samples (especially old or frozen ones) for precipitates. Avoid clearing samples by centrifugation / filtration before the VL-lysis step, because viruses may be associated with particles or aggregates.

2.4 Remarks regarding elution

- Pure nucleic acids are finally eluted under low ionic strength conditions with RNase-free H_2O.
- Elution can be performed in a single step with water as indicated in the protocol, obtaining at least 80 % of the bound nucleic acids. To improve sensitivity, this eluate can be used in a second elution step increasing the efficiency of elution and concentration of viral nucleic acids slightly. Alternatively, a second elution step can be performed with an additional volume of water releasing practically all bound nucleic acids but resulting in a lower concentrated, combined eluate.
- A high RNA / DNA concentration in the elution fraction is of highest importance and desirable for all typical downstream applications. This is of particular interest if the total volume of a reaction mixture is limited as this in turn limits the possible amount of added DNA / RNA. Due to a high default elution volume, classical RNA / DNA purification kits often result in weakly concentrated RNA / DNA, if only small samples are processed. Such RNA / DNA often even requires a subsequent concentration before it can be used for typical downstream applications.
- In contrast to classical kits, **NucleoSpin® Virus** allows an efficient elution in a small volume which results in highly concentrated RNA / DNA. An elution volume of 30 µL is recommended by default.

2.5 Remarks regarding quality control

In accordance with MACHEREY-NAGEL's Quality Managment System, each component of **NucleoSpin® Virus** kits is tested against predetermined specifications to ensure consistent product quality.

3 Storage conditions and preparation of working solutions

Attention: Buffers VL and VW1 contain guanidine salts! Wear gloves and goggles!

- Check all components for damages after receiving the kit. If kit contents like buffer bottles or blisters packages are damaged, contact MACHEREY-NAGEL. Do not use damaged kit components.
- Upon arrival, the **NucleoSpin® Virus** kit should be stored at room temperature (15 – 25 °C). It is NOT required to open the kit on delivery and remove individual components for separate storage.
- After first time use, it is recommended to store Liquid Proteinase K at 4 °C or -20 °C.
- Use RNase-free equipment.

Before starting any **NucleoSpin® Virus** protocol, prepare the following:

- **Carrier RNA** (300 µg or 1 mg) is delivered in lyophilized form. Dissolve Carrier RNA in RNase-free water to obtain a stock solution (1 µg/µL). Store Carrier RNA stock solution at -20 °C. 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.
- **Wash Buffer VW2**: Add the indicated volume (see on the bottle or table below) of ethanol (96 – 100 %; non-denatured ethanol is recommended) to **Wash Buffer VW2 Concentrate**. Mark the label of the bottle to indicate that the ethanol is added. Store **Wash Buffer VW2** at room temperature.
- **Liquid Proteinase K** is ready to use. After fist time use, store liquid Proteinase K at 4 °C or -20 °C.

	NucleoSpin® Virus		
REF	**10 preps 740983.10**	**50 preps 740983.50**	**250 preps 740983.250**
Wash Buffer VW2 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol to each bottle	50 mL Add 200 mL ethanol
Carrier RNA	300 µg Add 300 µL RNase-free H_20	300 µg Add 300 µL RNase-free H_20	3 × 1 mg Add 1 mL RNase-free H_20 in each vial

4 Safety instructions

When working with the **NucleoSpin® Virus** 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: Guanidine hydrochloride in buffer VL and buffer VW1, 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® Virus** kit has not been tested for residualinfectious 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 Viral RNA and DNA purification from 200 µL serum, plasma, or cell-free biological fluids

Before starting the preparation:

- Check if Wash Buffer VW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (stock solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free H_2O (for elution) to 70 °C.

1 Lyse viruses

Provide **5 µL Liquid Proteinase K** in a Collection Tube (1.5 mL, provided).

5 µL Proteinase K

Proteinase K may be pipetted into the inside of the lid or onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube or lid!

+200 µL sample

Add **200 µL sample** to the tube and mix moderately.

+200 µL VL

Add **200 µL Lysis Buffer VL** to the tube.

Mix

Mix the tube content moderately by vortexing (10 – 15 s).

~ 1 s, ~ 2,000 x *g*

If necessary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only).

+5.6 µL Carrier RNA

Add 5.6 µL Carrier RNA stock solution (1 µg/µL) to the tube.

Mix

Mix the tube content by vortexing or pipetting up and down.

RT, 3 min

Incubate for **3 min** at **room temperature**.

~ 1 s, ~ 2,000 x *g*

If neccesary, briefly centrifuge the Collection Tube **(~ 1 s at ~ 2,000 x *g*)** to remove drops from the lid (short spin only).

2 Adjust binding conditions

Add **200 µL ethanol** (96 – 100 %) to the tube and mix by vortexing (10 – 15 s).

+200 µL EtOH

RT, 5 min

Incubate for **5 min** at **room temperature.**

~ 1 s, ~ 2,000 x *g*

Briefly centrifuge the Collection Tube (**~ 1 s** at **~ 2,000 x *g***) to remove drops from the lid (short spin only).

Do not centrifuge at a higher **g**-force in this step!

3 Bind viral RNA / DNA

Load sample

Load the lysate (610 µL) onto a NucleoSpin® Virus Column and centrifuge **3 min** at **4,000 x *g***.

3 min, 4,000 x *g*

If the lysate is not completely drawn through the membrane, repeat the centrifugation at higher g-forces (15,000 – 20,800 x g for 1 min). In case the lysate still does not pass the membrane completely, discard the sample and repeat the isolation with new sample material.

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

4 Wash and dry silica membrane

1 st wash

+400 µL VW1

Add **400 µL Wash Buffer VW1** to the NucleoSpin® Virus Column.

30 s, 11,000 x *g*

Centrifuge **30 s** at **11,000 x *g***.

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

2nd wash

+400 µL VW2

Add **400 µL Wash Buffer VW2** to the NucleoSpin® Virus Column.

30 s, 11,000 x *g*

Centrifuge **30 s** at **11,000 x *g***.

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

Note: Make sure that residual buffer from the previous step is washed away with Buffer VW2, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim, flush it with Buffer VW2.

+200 µL VW2

3rd wash

5 min, 20,000 x *g*

Add **200 µL Wash Buffer VW2** to the NucleoSpin® Virus Column.

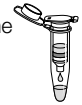
Centrifuge **for 5 min** at **20,000 x *g*** (or full speed).

Place the NucleoSpin® Virus Column in a clean Elution Tube (1.5 mL, provided) and discard the Collection Tube with flow-through from the previous step.

Incubate the assembly for **5 min** at **56 °C** with open column lid.

56 °C, 5 min

5 Elute RNA / DNA



+30 µL RNase-free H_2O (70 °C)

Add **30 µL RNase-free H_2O** (pre-heated to 70 °C) onto the column.

Incubate for **3 min** at **room temperature**.

RT, 3 min

Centrifuge **3 min** at **20,000 x *g*** to elute nucleic acid from the column.

3 min, 20,000 x *g*

Keep eluted RNA / DNA on ice or freeze for storage.

5.2 Viral RNA and DNA purification from 400 µL serum, plasma, or cell-free biological fluids

Before starting the preparation:

- Check if Wash Buffer VW2 was prepared according to section 3.
- Check if Carrier RNA is dissolved in water (stock solution).
- The complete procedure should be performed at room temperature.
- Preheat RNase-free H_2O (for elution) to 70 °C.

1 Lyse viruses

Provide **10 µL Liquid Proteinase K** in a Collection Tube (1.5 mL, provided).

10 µL Proteinase K

Proteinase K may be pipetted into the inside of the lid or onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube or lid!

Add **400 µL sample** to the tube and mix moderately.

+400 µL sample

Add **400 µL Lysis Buffer VL**.

+400 µL VL

Mix the tube content moderately by vortexing (10 – 15 s).

Mix

If necessary, briefly centrifuge the Collection Tube (**~ 1 s** at **~ 2,000 x *g***) to remove drops from the lid (short spin only).

~ 1 s, ~ 2,000 x *g*

Add **11.2 µL Carrier RNA stock solution** (1 µg/µL) to the tube.

+11.2 µL Carrier RNA

<u>Note:</u> Alternatively, 5.6 µg Carrier RNA can be used. Influence of Carrier RNA amount on downstream applications is typically low, but might vary between different downstream applications.

Mix the tube content by vortexing or pipetting up and down.

Mix

Incubate for **3 min** at r**oom temperature**.

RT, 3 min

If necessary, briefly centrifuge the Collection Tube (**~ 1 s** at **~ 2,000 x *g***) to remove drops from the lid (short spin only).

~ 1 s, ~ 2,000 x *g*

2 Adjust binding conditions

Add **400 µL ethanol** (96 – 100 %) to the tube and mix by vortexing (10 – 15 s).

+400 µL EtOH

Incubate for **5 min** at **room temperature.**

RT, 5 min

Briefly centrifuge the Collection Tube (**~ 1 s** at **~ 2,000 x *g***) to remove drops from the lid (short spin only).

~ 1 s, ~ 2,000 x *g*

3 Bind viral RNA / DNA

Load sample stepwise

Load 600 µL lysate onto a NucleoSpin® Virus Column and centrifuge **3 min** at **4,000 x *g***.

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

3 min, 4,000 x *g*

Load the remaining lysate onto the NucleoSpin® Virus Column and centrifuge **3 min** at **4,000 x *g***.

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, not provided) and discard the Collection Tube with flow-through from the previous step.

If the lysate is not completely drawn through the membrane, repeat the centrifugation at higher g-forces (15,000 – 20,800 x g for 1 min). In case the lysate still does not pass the membrane completely, discard the sample and repeat the isolation with new sample material.

4 Wash and dry silica membrane

+400 µL VW1

30 s, 11,000 x *g*

1 st wash

Add **400 µL Wash Buffer VW1** to the NucleoSpin® Virus Column.

Centrifuge **30 s** at **11,000 x *g***.

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

2nd wash

Add **400 µL Wash Buffer VW2** to the NucleoSpin® Virus Column.

+400 µL VW2

Centrifuge **30 s** at **11,000 x *g***.

30 s, 11,000 x *g*

Place the NucleoSpin® Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

Note: Make sure that residual buffer from the previous step is washed away with Buffer VW2, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim, flush it with Buffer VW2.

3rd wash

Add **200 µL Wash Buffer VW2** to the NucleoSpin® Virus Column.

+200 µL VW2

Centrifuge for **5 min** at **20,000 x *g***.

5 min, 20,000 x *g*

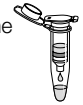
Place the NucleoSpin® Virus Column in a clean Elution Tube (1.5 mL, provided) and discard the Collection Tube with flow-through from the previous step.

Incubate the assembly for **5 min** at **56 °C** with open column lid.

56 °C, 5 min

5 Elute RNA / DNA

Add **30 µL RNase-free H_2O** (pre-heated to 70 °C) onto the column.



+30 µL RNase-free H_2O (70 °C)

Incubate for **3 min** at **room temperature**.

RT, 3 min

Centrifuge **3 min** at **20,000 x *g*** to elute nucleic acid from the column.

3 min, 20,000 x *g*

Keep eluted RNA / DNA on ice or freeze for storage.

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 was not added. **Viral nucleic acids degraded** • Samples should be processed immediately. Ensure appropriate storage conditions up to the processing. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer VL, Carrier RNA, and RNase-free water.
Problems with subsequent detection	**Reduced sensitivity** • Change the volume of eluate added to the PCR / RT-PCR. **Ethanol carry-over** • Prolong centrifugation steps in order to remove Buffer VW2 completely. **Carrier RNA interference with detection method** • Check if Carrier RNA interferes the detection method. Some detection methods tolerate only limited amounts of carrier RNA.
General problems	**Clogged membrane** • Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding NucleoSpin® Virus Columns.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Virus	740983.10 / .50 / .250	10 / 50 / 250
NucleoSpin® Dx Virus	740895.50	50
NucleoSpin® RNA Virus F	740958	25
NucleoSpin® Funnel Columns	740959	30 sets
NucleoSpin® 8 Virus	740643 / .5	12 × 8 / 60 × 8
NucleoSpin® 96 Virus	740691.2 / .4	2 × 96 / 4 × 96
NucleoMag® Virus	744800.1 / .4	1 × 96 / 4 × 96
NucleoSpin® Blood	740951.10 / .50 / .250	10 / 50 / 250
NucleoSpin® Tissue	740952.10 / .50 / .250	10 / 50 / 250
NucleoSpin® RNA	740955.10 / .50 / .250	10 / 50 / 250
NucleoSpin® RNA Blood	740200.10 / .50	10 / 50
NucleoSpin® RNA Blood Midi	740210.20	20
Collection Tubes (2 mL)	740600	1000

Visit ***www.mn-net.com*** for more detailed product information.

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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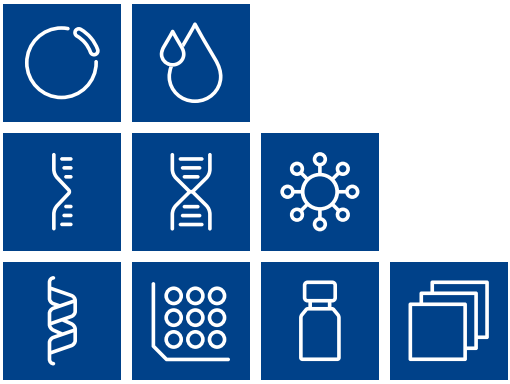
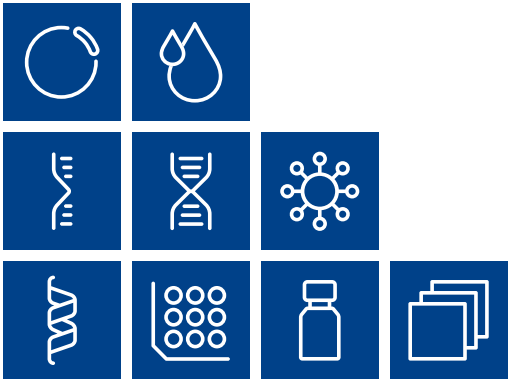


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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