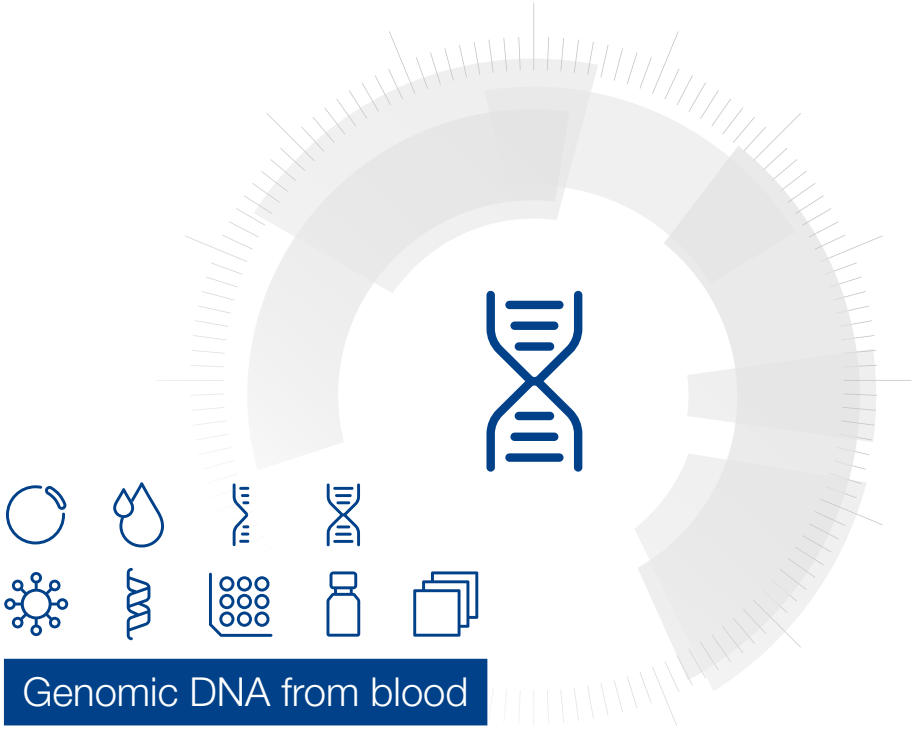
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from blood

■ NucleoSpin® Blood L Vacuum

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1 Components

1.1 Kit contents

	NucleoSpin® Blood L Vacuum
REF	**24 preps 740954.24**
Lysis Buffer BLV1	25 mL
Binding Buffer BLV2	125 mL
Wash Buffer BLV3	125 mL
Wash Buffer BLV4 (Concentrate)*	25 mL
Elution Buffer BLV5**	30 mL
Liquid Proteinase K	1.4 mL
NucleoSpin® Blood L Columns	24
Collection Tubes (1.5 mL)	24
Lysis Block	1
Sample Waste Block	1
User manual	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer BLV5: 5 mM Tris/HCl, pH 8.5

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

Reagents

- 96–100 % ethanol
- Phosphate-buffered saline (PBS) may be required for some samples

Consumables

- Disposable pipette tips

Equipment

- NucleoVac 96 Vacuum Manifold
- Starter Set Midi (see ordering information)
- Thermal heating shaker
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Blood L Vacuum** kit is used for the first time. All technical literature is available on the internet at www.mn-net.com.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Blood L Vacuum** method, genomic DNA is prepared from whole blood. Lysis is achieved by incubation of whole blood in a solution containing large amounts of chaotropic ions in the presence of Proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding **NucleoSpin® Blood L Columns** are achieved by addition of Binding Buffer BLV2 to the lysate. The binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® Blood L Vacuum** kits are designed for the rapid isolation of highly pure genomic DNA from up to 2 mL whole blood.
- DNA can be purified successfully from blood samples treated with EDTA, citrate, or heparin. If leukocyte rich materials like buffy coat are used, apply smaller volumes and dilute the samples with sterile PBS (dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 in 800 mL H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 liter).
- The kits allow purification of highly pure genomic DNA with an A_{260}/A_{280} ratio between 1.60 and 1.90 and a typical yield of 40–80 µg dependent on sample material.
- The obtained DNA is ready to use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reactions.
- For research use only.

2.3 Required hardware

Vacuum processing

The NucleoSpin® Blood L Vacuum kit is used with the NucleoVac 96 Vacuum Manifold (see ordering information). Additional to the vacuum manifold, special adapter frames (included in the Starter Set Midi) are needed for processing up to 24 NucleoSpin® Blood L Columns on the NucleoVac 96 Vacuum Manifold. The Starter Set Midi (see ordering information) contains a Column Holder Midi for holding up to 24 NucleoSpin® Blood L Columns, a Wash Plate Midi, for preventing cross-contamination, and the Elution Tube Holder Midi for holding the Elution Tubes inside the vacuum manifold. For the use of less than 24 columns, Dummy Columns are included.

The manifold may be used with a vacuum pump, house vacuum, or water respirator. We recommend a vacuum of -0.2 to -0.6 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended.

2.4 Storage of blood samples

For the isolation of genomic DNA from blood treated with anticoagulants (heparin, citrate, or EDTA) using a **NucleoSpin® Blood L Vacuum** kit the blood samples can be stored at room temperature, +4 °C, or frozen.

Blood samples stored at room temperature or +4 °C for up to several days or weeks, respectively, will still allow DNA isolation. However, DNA yield and quality will slowly decrease due to prolonged storage of blood samples under these conditions.

Blood stored frozen for years is well suited for DNA isolation.

Highest yields and quality of DNA are obtained from fresh blood.

2.5 Elution procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 70–90 %) there are several modifications possible.

- **High yield:** Use elution buffer preheated to 70 °C to obtain higher yields.
- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution (around 100 %).

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and / or multi use storage at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in subsequent downstream applications we recommend elution with the supplied elution buffer and storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g., > 10 kbp) or detection sensitivity of trace amount of DNA species might be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at +4 °C or room temperature due to DNA shearing or adsorption to surfaces.

3 Storage conditions and preparation of working solutions

Attention: *Buffers BLV1, BLV2, and BLV3 contain chaotropic salt. Wear gloves and goggles!*

CAUTION: Buffer BLV1, BLV2, and BLV3 contain 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.

- Buffers and consumables can be stored at 15–25 °C and are stable until: see package label.
- NucleoSpin® Blood L Columns have to be stored at 4 °C and are stable up to one year.
- During storage, especially at low temperatures, a white precipitate may form in Buffer BLV1. Such precipitates can be easily dissolved by incubating the bottle at 70 °C before use.

Before starting any **NucleoSpin® Blood L Vacuum** protocol prepare the following:

- **Wash Buffer BLV4:** Add the indicated volume of ethanol (96–100 %) to **Wash Buffer BLV4 (Concentrate)**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer BLV4 at 15–25 °C for up to one year.
- **Liquid Proteinase K:** Liquid Proteinase K is ready to use. After first time use, store Liquid Proteinase K at 4 °C or -20 °C.

	NucleoSpin® Blood L Vacuum
REF	**24 preps** **740954.24**
Wash Buffer BLV4 (Concentrate)	25 mL Add 100 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® Blood L Vacuum** 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 BLV1, BLV2 and buffer BLV3 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® Blood L Vacuum** 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 treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

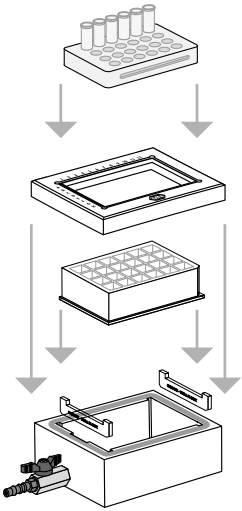
4.1 Disposal

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

5 Protocol – Genomic DNA purification with NucleoSpin® Blood L Vacuum

Setup of vacuum manifold:

Binding step

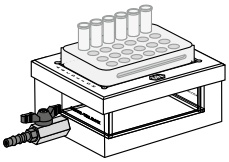


Step 4:
Place the NucleoSpin® L Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.

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

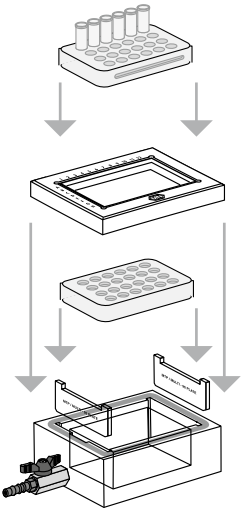
Step 2:
Place the Sample Waste Block in the manifold.

Step 1:
Insert spacers 'SQUARE-WELL BLOCK' in the manifold.



Final setup

Washing step



Step 4:
Place the NucleoSpin® L Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.

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

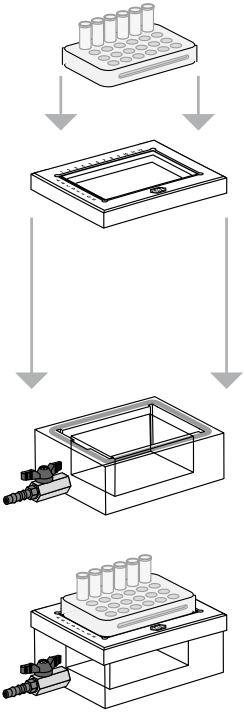
Step 2:
Place the Wash Plate Midi in the manifold.

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

Final setup

Setup of vacuum manifold:

Drying step



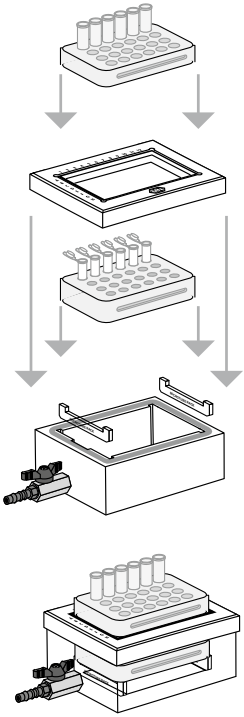
Step 3:
Place the NucleoSpin® L Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.

Step 2:
Place the manifold lid on top of the manifold base.

Step 1:
Insert the equiped waste container in the manifold base.

Final setup

Elution step



Step 4:
Place the NucleoSpin® L Columns inserted in the Column Holder Midi on top of the manifold lid. Unused positions have to be filled with Dummy Columns.

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

Step 2:
Place the 1.5 mL Collection Tubes inserted the Elution Tube Holder Midi in the manifold.

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

Final setup

Before starting the preparation:

- For hardware requirements, refer to section 2.3.
- For detailed information regarding vacuum manifold, see page 10 and 11.
- Check if Buffer BLV4 was prepared according to section 3.
- Set a thermal heating shaker to 56 °C.

1	**Lyse blood sample** Pipette up to **2 mL blood** sample (equilibrated to room temperature) and **50 µL Liquid Proteinase K** (if processing less than 2 mL blood, add the adapted volume of liquid Proteinase K) into the Lysis Block. Add **750 µL Lysis Buffer BLV1** (if processing less than 2 mL blood, add the adapted volume of Buffer BLV1). Incubate samples at **56 °C** and **1200 rpm** for **30 min**. Let the samples cool down to room temperature before proceeding with addition of Binding Buffer BLV2. *The lysate should become brownish during incubation with Buffer BLV1. Increase incubation time with Proteinase K (up to 60 min) and mix once or twice during incubation if older or clotted blood samples are processed.*	**2 mL blood** **+ 50 µL Liquid Proteinase K** **+ 750 µL BLV1** **Shaking 30 min** **56 °C, 1200 rpm**
	Place spacers 'SQUARE-WELL BLOCK' and Sample Waste Block, into vacuum manifold base. Place the manifold lid and the NucleoSpin® L Columns inserted in the Column Holder Midi on the top of the manifold lid.	
2	**Adjust DNA binding conditions** Add **4 mL BLV2** (if processing less than 2 mL blood, add the adapted volume of BLV2) to each sample and mix by pipetting up and down 5 times. *Be sure that the lysate has cooled down to room temperature before loading it onto the column. Loading of hot lysate may lead to diminished yields.*	**+ 4 mL BLV2** **Mix 5 times**

3 Bind DNA

For preparation, take the required number of **NucleoSpin® Blood L Columns** and place them into Column Holder Midi. Place Dummy Columns in unused positions of the adapter.

3.5 mL lysate + 300 µL BLV4 overlay

Transfer **3.5 mL of lysate** carefully to **NucleoSpin® Blood L Columns**. Overlay lysate slowly with **300 µL Buffer BLV4**.

Apply vacuum **(-0.2 to -0.4 bar*; 5 min)** until all lysates have passed through the **NucleoSpin® Blood L Columns**. Release vacuum.

Vacuum -0.2 to -0.4 bar*, 5 min

Note: Do not moisten the rims of the individual columns while dispensing the lysate. Moisten rims may cause cross-contamination.

Remaining lysate

Load **all of the remaining lysate** in a second step to the respective **NucleoSpin® Blood L Column**, avoiding moistening the rim.

+ 300 µL BLV4 overlay

Overlay lysate slowly with **300 µL Buffer BLV4**.

Vacuum -0.2 to -0.4 bar*, 5 min

Apply vacuum (-0.2 to -0.4 bar*; 5 min) until all lysates have passed through the **NucleoSpin® Blood L Columns**.

Close the valve, release the vacuum, and remove **Column Holder Midi** including **NucleoSpin® Blood L Columns** from the vacuum manifold. Put the Column Holder Midi on a clean paper towel to remove residual lysate.

Remove manifold lid and Sample Waste Block. Place spacers 'MTP/MULTI-96 PLATE', waste container, and Wash Plate Midi into the vacuum manifold base.

* Reduction of atmospheric pressure

4 Wash silica membrane

1st wash

Add **4 mL Buffer BLV3** to each **NucleoSpin® Blood L** Column. I**ncubate** for **5 min** at room temperature to wash away lysate residuals from the columns sides.

+ 4 mL BLV3

Incubate at RT, 5 min

Apply vacuum **(-0.4 to -0.6 bar*; 2 min)** until all buffer has passed through **NucleoSpin® Blood L Columns**. Release the vacuum.

Vacuum -0.4 to -0.6 bar*, 2 min

2nd wash

Add **2 mL Buffer BLV4** to each **NucleoSpin® Blood L** Column.

+ 2 mL BLV4

Vacuum -0.4 to -0.6 bar*, 2 min

Apply vacuum **(-0.4 to -0.6 bar*; 2 min)** until all buffer has passed through **NucleoSpin® Blood L Columns**. Release the vacuum.

3rd wash

Add **2 mL Buffer BLV4** to each **NucleoSpin® Blood L Column**.

+ 2 mL BLV4

Vacuum -0.4 to -0.6 bar*, 2 min

Apply vacuum **(-0.4 to -0.6 bar*; 2 min)** until all buffer has passed through **NucleoSpin® Blood L Columns**. Release the vacuum.

After the final washing step, close the valve, release the vacuum, and remove **Column Holder Midi** including **NucleoSpin® Blood L Columns** from the vacuum manifold. Put it on a clean paper towel to remove residual ethanol containing wash buffer.

Remove manifold lid, Wash Plate Midi, and waste container from the vacuum manifold.

Remove any residual washing buffer from the outlets of **NucleoSpin® Blood L Columns**. If necessary, tap the outlets onto a clean paper sheet or soft tissue until no drops come out. Insert emptied and cleaned wash container into the manifold base. Put **Column Holder Midi** including the **NucleoSpin® Blood L Columns** onto the manifold lid and place afterwards the lid onto the manifold base.

* Reduction of atmospheric pressure

5	**Dry silica membrane** Apply maximum vacuum **(at least - 0.6 bar*)** for 10 min to dry the membrane completely. This step is necessary to eliminate traces of ethanol. Finally, release the vacuum. *Note: The ethanol in Buffer BLV4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.*	**Vacuum at least - 0.6 bar*, 10 min**
	After drying step close the valve, release the vacuum, and remove the **Column Holder Midi** including **NucleoSpin® Blood L Columns** from the vacuum manifold. Put it on a clean paper towel. Insert spacers 'MICROTUBE RACK' and **Elution Tube Holder Midi** including 1.5 mL Collection Tubes into vacuum manifold base. Put **Column Holder Midi** including the **NucleoSpin® Blood L Columns** onto the manifold lid and place afterwards the lid onto the manifold base.	
6	**Elute highly pure DNA** Add **300 µL Buffer BLV5** directly to the center of the silica membrane. Incubate at **room temperature** for **2 min**. Apply vacuum **(- 0.4 bar*; 30 s and - 0.6 bar*; 40 s)** until all buffer has passed through **NucleoSpin® Blood L Columns**. Release the vacuum. Add **300 µL Buffer BLV5** directly to the center of the silica membrane. Incubate at **room temperature** for **2 min**. Apply vacuum **(- 0.4 bar*; 30 s and - 0.6 bar*; 40 s)** until all buffer has passed through **NucleoSpin® Blood L Columns.** Release the vacuum. *For alternative elution procedures see section 2.4.*	**+ 300 µL BLV5 Incubate at RT, 2 min Vacuum - 0.4 bar*, 30 s, + - 0.6 bar*, 40 s,** **+ 300 µL BLV5 Incubate at RT, 2 min Vacuum - 0.4 bar*, 30 s, + - 0.6 bar*, 40 s,**

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Low concentration of leukocytes in sample* Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature (3,300 x *g*; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (= buffy coat). *Reagents not applied properly* Prepare buffers according to instructions (section 3). Add Binding Buffer BLV2 to lysates before loading them on columns. *Suboptimal elution of DNA from the column* Preheat Buffer BLV5 to 70 °C before elution. Apply Buffer BLV5 directly onto the center of the silica membrane. Elution efficiencies decrease dramatically if elution is performed with buffers of pH < 7.0. Use slightly alkaline elution buffer like Buffer BLV5 (pH 8.5). Mix by pipetting up and down during the 56 °C incubation step especially when working with older or clotted blood samples.
Poor DNA quality	*Reagents not applied properly* Prepare buffers according to instructions (section 3). Add Binding Buffer BLV2 to lysates and mix before loading them on columns. *Incomplete cell lysis* Sample not thoroughly mixed with lysis buffer / Proteinase K. The sample has to be mixed vigorously immediately after addition of lysis buffer.

Problem	Possible cause and suggestions
Poor DNA quality (continued)	*RNA in sample* If RNA-free DNA is desired, add 20 µL RNase A solution (20 mg/mL; not supplied with the kit, see ordering information) before addition of lysis buffer. *Old or clotted blood samples processed* For isolation of DNA from older or clotted blood samples, we recommend prolonging Proteinase K incubation to 40–60 min and mix by pipetting up and down several times during this step. Clear lysate before addition of Binding Buffer BLV2. It is recommended performing a short centrifugation step of about 30–60 s after the lysis of the sample material (before addition of Binding Buffer BLV2) in order to pellet non-lysed clumps.
Suboptimal performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol* Be sure to remove all of ethanolic Buffer BLV4 before eluting the DNA. *Contamination of DNA with inhibitory substances* If DNA has been eluted with Tris/EDTA-buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in Buffer BLV5. If the A_{260}/A_{280} ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of Buffer BLV1 plus 1 volume of Buffer BLV2 to the eluate, load on NucleoSpin® Blood L Column, and proceed with step 3 of the corresponding protocol.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Blood L Vacuum	740954.24	24
Liquid Proteinase K	740396	5 mL
RNase A	740505.50 740505	50 mg 100 mg
Starter Set Midi	740744	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1

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-NAGELS 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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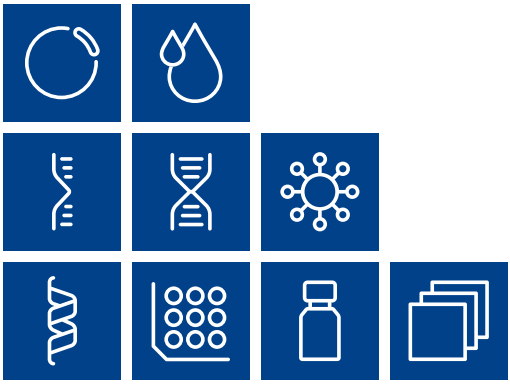
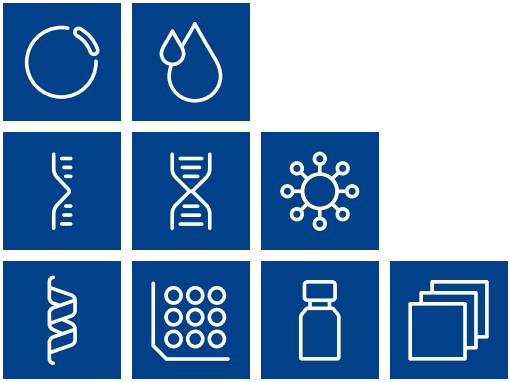


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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