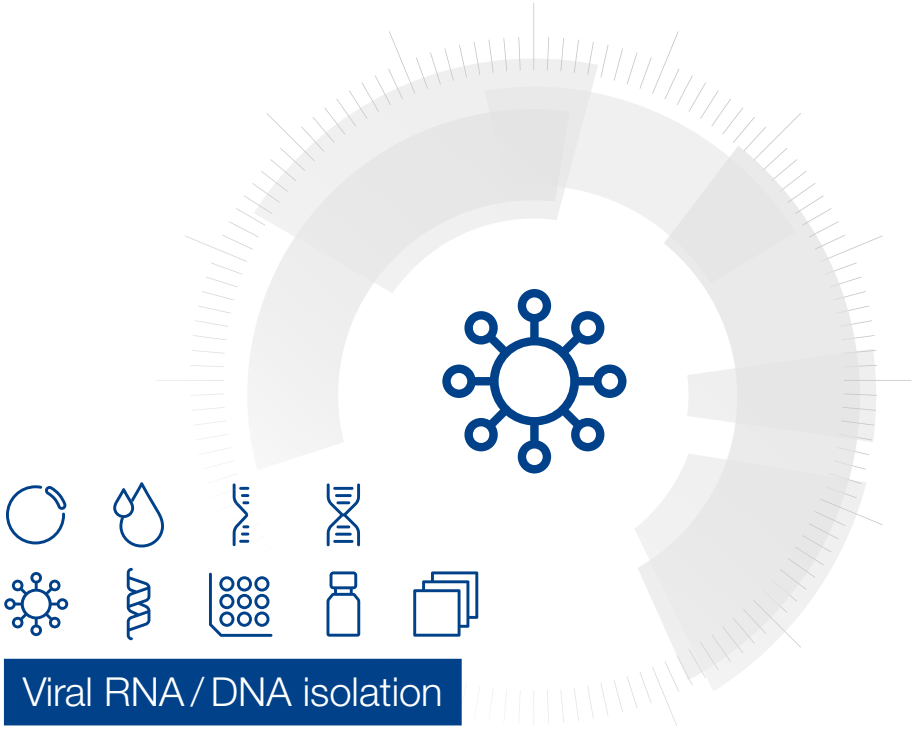
Bioanalysis

MACHEREY-NAGEL

User manual



Viral RNA / DNA isolation

■ NucleoMag® Virus

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1 Components

1.1 Kit contents

	NucleoMag® Virus	
REF	**1 × 96 preps 744800.1**	**4 × 96 preps 744800.4**
NucleoMag® V-Beads	2 × 1.5 mL	12 mL
Lysis Buffer MVL	2 × 13 mL	125 mL
Binding Buffer MV2	75 mL	300 mL
Wash Buffer MV3	75 mL	300 mL
Wash Buffer MV4	75 mL	300 mL
Wash Buffer MV5	60 mL	250 mL
Elution Buffer MV6	13 mL	60 mL
Carrier RNA*	400 µg	4 × 400 µg
Carrier RNA Buffer	500 µL	4 × 500 µL
Proteinase K (lyophilized)*	20 mg	4 × 20 mg
Proteinase Buffer PB	1.8 mL	8 mL
User manual	1	1

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

1.2 Material to be supplied by user

Product	REF	Pack of
Separation plate for magnetic beads separation, e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
Lysis tubes for incubation of samples and lysis, e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Elution plate for collecting purified nucleic acids, e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24
e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells)	740673	20
For use of kit on KingFisher® Flex instrument: 96-well Accessory Kit A for KingFisher® (Square-well Blocks, Deep-well Tip Combs, Plates for 4 × 96 NucleoMag® Virus preps using KingFisher® 96/Flex platform)	744950	1 set

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also 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 or updated revisions

2 Product description

2.1 The basic principle

The **NucleoMag® Virus** kit is designed for the isolation of viral DNA or RNA from cell-free body fluids such as serum, plasma, saliva and swab washes. This kit provides reagents and magnetic beads for isolation of 96 samples from 200 µL cell-free body fluid. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a new and improved Lysis Buffer MVL containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads, Binding Buffer MV2 and the NucleoMag® V-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers MV3 and MV4. Residual ethanol from previous wash steps is removed by a short incubation of the beads in Wash Buffer MV5. Finally, highly pure viral RNA / DNA is eluted with low salt Elution Buffer MV6 or water. Purified viral RNA / DNA can directly be used for downstream applications. It is recommended to use suitable controls for downstream applications (e.g., internal controls, extraction controls, positive / negative controls).The **NucleoMag® Virus** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag® Virus is designed for rapid manual and automated small-scale preparation of viral RNA / DNA from cell-free body fluids such as serum, plasma, saliva and swab washes.

The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). NucleoMag® V-Beads are highly reactive superparamagnetic beads. The theoretical binding capacity is approx. 0.2 µg of nucleic acid per 1 µL of NucleoMag® V-Bead Suspension. Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA / DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

NucleoMag® Virus allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on the automation platform.

For research use only

2.3 Magnetic separation systems

For use of **NucleoMag® Virus**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins / rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

- Load 1000 µL (for checking the settings for the binding step) or 600 µL (for checking the settings for the washing steps) dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100 µL dyed water to the wells of the collection plate and proceed as described above.

2.5 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads / binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

2.6 Elution procedures

Purified viral RNA / DNA can be eluted directly with the supplied Elution Buffer MV6. Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, high elution volumes might be necessary to cover the whole pellet.

* 8-channel pipetting device

3 Storage conditions and preparation of working solutions

Attention: Buffers MVL, MV2, and MV3 contain chaotropic salt! Wear gloves and goggles!

- All components of the **NucleoMag® Virus** kit should be stored at 15 – 25 °C and are stable for up to one year.
- All buffers are delivered ready to use.
- **Lysis Buffer MVL:**
  Lysis Buffer MVL may form salt precipitates upon storage. To re-dissolve the salt precipitate incubate the buffer bottle at 40 °C until all of the precipitate is re-dissolved.
  Lysis Buffer MVL with Carrier RNA: Lysis Buffer MVL with added Carrier RNA can be stored at room temperature for 1 – 2 weeks.

Frequent warming, temperatures > 80 °C, and extended heat incubation will cause degradation of the Carrier RNA. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular, if low-titer samples are used. Do not warm Buffer MVL containing Carrier RNA more than 6 times!

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

- **Proteinase K**: Before first use of the kit, add 1.1 mL Proteinase Buffer PB to each vial of the **lyophilized Proteinase K**. Dissolved Proteinase K solution should be stored in aliquots at - 20 °C for up to 6 months.
- **Carrier RNA**: Before first use of the kit, add 440 µL Carrier RNA Buffer to each vial **lyophilized Carrier RNA**. Store dissolved Carrier RNA solution in aliquots at - 20 °C for up to 6 months.

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

	NucleoMag® Virus	
REF	**1 × 96 preps 744800.1**	**4 × 96 preps 744800.4**
Proteinase K (lyophilized)	1 vial (20 mg) Add 1.1 mL Proteinase Buffer	4 vials (20 mg/vial) Add 1.1 mL Proteinase Buffer to each vial
Carrier RNA (lyophilized)	1 vial (400 µg) Add 440 µL Carrier RNA Buffer	4 vials (400 µg/vial) Add 440 µL Carrier RNA Buffer to each vial

4 Safety instructions

When working with the **NucleoMag® 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 MVL, sodium perchlorate in buffer MV2 and MV3, and guanidinium thiocyanate in buffer Carrier RNA Buffer 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 **NucleoMag® Virus** 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.

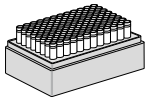
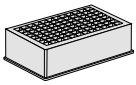
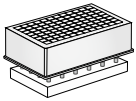
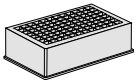
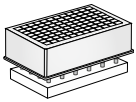
5 Protocol for the isolation of viral RNA / DNA from cell-free body fluids

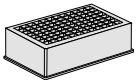
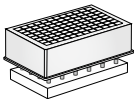
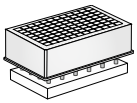
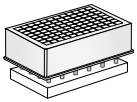
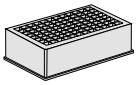
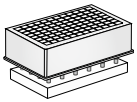
Protocol at a glance

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 14.

Before starting the preparation:

- Check if Proteinase K and Carrier RNA were prepared according to section 3.

1	Lyse sample	**10 µL Proteinase K** **200 µL sample** **4 µL Carrier RNA** **200 µL MVL** **Mix** **56 °C, 10 min**	
2	Bind viral RNA / DNA to NucleoMag® V-Beads	**30 µL V-Beads** **600 µL MV2**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Remove supernatant after 2 min separation	
3	Wash with MV3	**Remove Square-well Block from NucleoMag® SEP** **500 µL MV3**	
		Resuspend: Shake 1 min at RT	⟷
		Remove supernatant after 2 min separation	

4	Wash with MV4	**Remove Square-well Block from NucleoMag® SEP** **500 µL MV4**	
		Resuspend: Shake 1 min at RT	
		Remove supernatant after 2 min separation	
5	Wash with MV5	**550 µL MV5** **Incubate for 45 – 60 s** *Note: Do not resuspend the beads in Buffer MV5!*	
		Remove supernatant	
6	Elute RNA / DNA	**Remove Square-well Block from NucleoMag® SEP** **50-100 µL MV6**	
		Shake 5 min at 56 °C *(Optional: Mix by pipetting up and down)*	
		Separate 2 min and transfer viral RNA / DNA into elution plate / tubes	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers. It is recommended using a Square-well Block for separation (see section 6.2 ordering information). Alternatively, isolation of viral RNA / DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

1 Lyse sample

Predispense **10 µL Proteinase K** and **200 µL of sample** to a suitable reaction tube. Add **200 µL Buffer MVL** (with added Carrier RNA) to the reaction tube (If Carrier RNA is not premixed with the Buffer MVL, add 4 µL of the stock solution to the reaction tube). Mix well by repeated pipetting up and down and incubate at **56 °C** for **10 min.** Alternatively, lysis step can be performed in Tube Strips (see section 6.2 ordering information).

For higher convenience a premix of Proteinase K, Buffer MVL, and Carrier RNA can be prepared. This premix should be added to the sample immediately (within 15 min after preparation).

Following the lysis incubation, spin down to collect any sample from the lysis tube lids and transfer each lysate to the wells of a Square-well Block.

2 Bind viral RNA / DNA to magnetic beads

Add **30 µL resuspended V-Beads** and **600 µL Buffer MV2** to the lysed sample.

Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

NucleoMag® V-Beads and Buffer MV2 can be premixed.

Be sure to resuspend the NucleoMag® V-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP a magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Do not disturb the attracted beads while aspirating the supernatant.

3 Wash with MV3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **500 µL Buffer MV3** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

4 Wash with MV4

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **500 µL Buffer MV4** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

5 Wash with MV5

Leave the Square-well Block on the NucleoMag® SEP magnetic separator. Gently add **550 µL Buffer MV5** to each well and incubate for **45 – 60 s** while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

Do not resuspend the beads in Buffer MV5. This step is to remove traces of ethanol and eliminates a drying step. Do not exceed incubation time of max. 1 min.

6 Elution

Add desired volume of **Buffer MV6 (50 – 100 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at **56 °C**. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 min** at **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified viral RNA / DNA to either microtubes or Tube Strips (see section 6.2 ordering information).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor yield/ low sensitivity	*Insufficient elution buffer volume* • Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation too short or aspiration speed too high.
Low purity / low sensitivity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
Poor performance of RNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution Buffer MV4, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* • Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.

Problem	Possible cause and suggestions
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® Virus	744800.1 744800.4	1 × 96 preps 4 × 96 preps
NucleoMag® SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Self adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
96-well Accessory Kit A for KingFisher (set consists of Square-well Blocks, Deep-well Tip Combs, Elution Plates for 4 × 96 NucleoMag® Virus preps using King Fisher® Flex platform)	744950	1 set

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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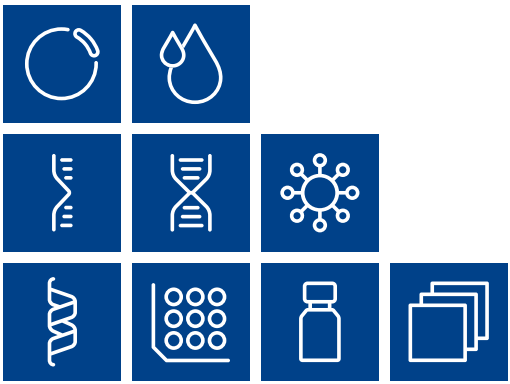
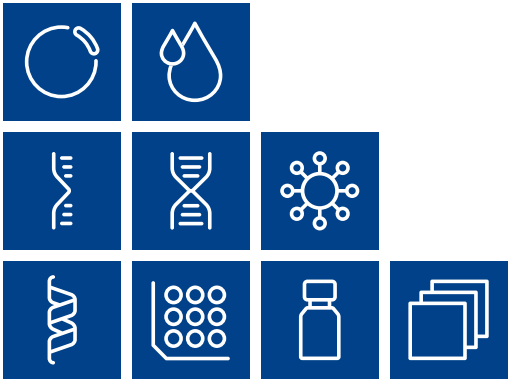


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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