Bioanalysis

MACHEREY-NAGEL

User manual

Genomic DNA from forensic samples

■ NucleoSpin® 96 Trace

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www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Trace	
REF	**2 × 96 preps 740726.2**	**4 × 96 preps 740726.4**
Lysis Buffer FLB	200 mL	2 × 200 mL
Wash Buffer B5 (Concentrate) [1]	100 mL	2 × 100 mL
Elution Buffer BE [2]	125 mL	2 × 125 mL
Proteinase K (lyophilized) [1]	2 × 33 mg	4 × 33 mg
Proteinase Buffer PB	8 mL	15 mL
NucleoSpin® Trace Binding Plates (gray rings)	2	4
MN Wash Plates (including six paper sheets)	2	4
MN Square-well Bocks	2	4
Rack of Tube Strips [3]	2	4
User manual	1	1

1.2 Reagent to be supplied by user

- 96 – 100 % ethanol

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

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.

[1] For preparation of working solutions and storage conditions, see section 3.

[2] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

[3] Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

2 Product description

2.1 The basic principle

With the **NucleoSpin® 96 Trace** method, genomic DNA is prepared from forensic samples. Lysis is achieved by incubation of samples in a solution containing chaotropic ions in the presence of Proteinase K at room temperature. Appropriate conditions for binding of DNA to the silica membrane in the NucleoSpin® Trace Binding Plate are created by addition of isopropanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by two washing steps with ethanolic buffer. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® 96 Trace** is designed for the rapid, small-scale preparation of highly pure genomic DNA from forensic samples. The obtained DNA can be used directly as template for PCR.
- Typically yields of 1 – 2 µg genomic DNA can be purified from buccal swabs.
- The final concentration of eluted DNA is 10 – 20 ng/µL (depending on elution buffer volume). Typically, the A_{260}/A_{280} ratio is 1.8 – 1.9.
- **NucleoSpin® 96 Trace** can be processed under vacuum or in a centrifuge (see section 2.3).

Table 1: Kit specifications at a glance

Parameters	NucleoSpin® 96 Trace
Technology	Silica-membrane technology
Format	96-well plate
Processing	Manual or automated, vacuum or centrifugation
Sample material	Forensic samples, buccal swabs, blood spots
Fragment size	200 bp–approx. 50 kbp
Typical yield	Depending on sample amount
A_{260}/A_{280}	1.8 – 1.9
Elution volume	50 – 100 µL
Preparation time	70 min/plate (excl. lysis)
Binding capacity	20 µg
Use	For research use only

Forensic quality product:

NucleoSpin® 96 Trace is certified as forensic quality product. Consumables used in forensics need to be treated carefully to prevent DNA contamination. MACHEREY-NAGEL therefore has a stringently controlled production process to avoid DNA contamination of consumables. Further, MACHEREY-NAGEL uses ethylene oxide (EO) treatment to remove amplifiable DNA, which might still be introduced during the manufacturing process. MACHEREY-NAGEL products carrying the forensic quality seal, contain plastic materials that are EO treated. This means, DNA of any kind, which might still be introduced into plastic consumables during the production process, is inactivated by means of the treatment with ethylene oxide, in order to prevent the generation of accidental human profile by PCR amplification. Ethylene oxide treatment has been shown to be the method of choice to prevent DNA profiles due to DNA contamination. (Shaw *et al.*, 2008).

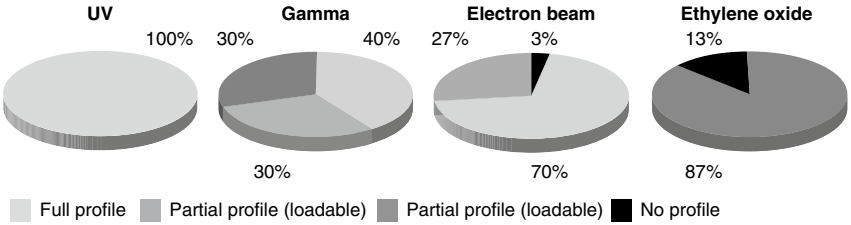


Figure 1 According to Shaw *et al.*, 2008, Comparison of the effects of sterilization techniques on subsequent DNA profiling. Int J Legal Med 122: 29 – 33.

2.3 Required hardware

Vacuum processing

The **NucleoSpin® 96 Trace** kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information, section 6.2). When using **NucleoSpin® 96 Trace** with less than 96 samples, Self-adhering PE Foil (see ordering information) should be used in order to close and protect non-used wells of the NucleoSpin® Trace Binding Plate and thus guarantee proper vacuum.

Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information, section 6.2) is recommended. Alternatively, adjust the vacuum so that during the purification the sample flows through the column with a rate of 1 – 2 drops per second. Depending on the amount of sample being used, the vacuum times may need to be increased for complete filtration.

Centrifugation

For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accomodate the NucleoSpin® Trace Binding Plate stacked on a Round- or Square-well Block and reach accelerations of 5,600 – 6,000 x *g* is required (bucket height: 85 mm).

3 Storage conditions and preparation of working solutions

Attention: *Buffer FLB contains chaotropic salts! Wear gloves and goggles!*

CAUTION: Buffer FLB contains guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components can be stored at room temperature (18 – 25 °C) and are stable for at least one year.
- Upon storage, especially at low temperatures, a white precipitate may form in Lysis Buffer FLB. Such precipitates have to be dissolved by incubating at 45 – 50 °C for 10 min before use.

Before starting any **NucleoSpin® 96 Trace** protocol, prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96 – 100 %) to **Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer B5 at room temperature (18 – 25 °C) for at least one year.
- Before first use of the kit, add the indicated volume (see table below or on the bottle) of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for at least 6 months.

	NucleoSpin® 96 Trace	
REF	**2 × 96 preps 740726.2**	**4 × 96 preps 740726.4**
Wash Buffer B5 (Concentrate)	100 mL Add 400 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle
Proteinase K	2 × 33 mg Add 3 mL Proteinase Buffer to each vial	4 × 33 mg Add 3 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoSpin® 96 Trace** 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***).

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

4.1 Disposal

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

5 Protocols

5.1 NucleoSpin® 96 Trace – vacuum processing

Protocol-at-a-glance

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 10.
- For detailed information on each step, see page 11.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.

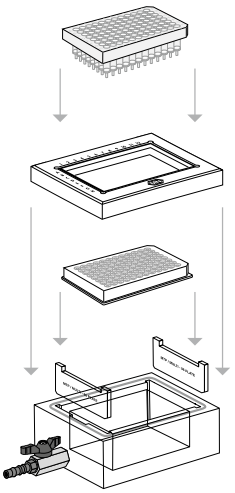
1	**Lyse** samples	**125 – 600 µL FLB** **25 µL Proteinase K** **Mix** **RT, several hours or overnight**
2	**Adjust** DNA binding conditions	**1 vol. isopropanol (per 2 vol. lysate)** **Mix** **Prepare the NucleoVac 96 Vacuum Manifold**
3	**Transfer** lysates to NucleoSpin® Trace Binding Plate	
4	**Bind** DNA to silica membrane of the NucleoSpin® Trace Binding plate	**-0.2 bar*, 2 min**
5	**Wash** silica membrane	**900 µL B5 – 0.2 bar*, 1 min** **900 µL B5 – 0.2 bar*, 1 min** **Remove MN Wash Plate**
6	**Dry** silica membrane	**-0.6 bar*, 10 min**

* Reduction of atmospheric pressure

7	**Elute** DNA	**50 – 200 µL BE**	**-0.4 bar*, 2 min**

Setup of vacuum manifold:

Binding / Washing steps

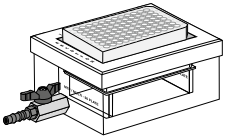


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

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

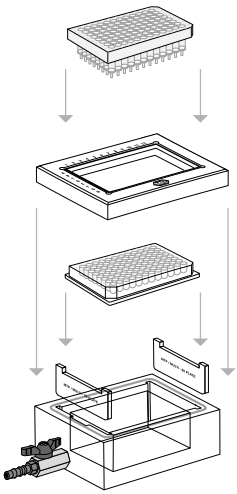
Step 2:
Place the MN Wash Plate in the manifold.

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



Final setup

Elution step

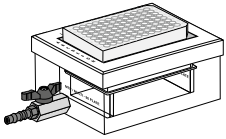


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

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

Step 2:
Place the Elution Plate in the manifold.

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



Final setup

Detailed protocol

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 11.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.

1 Lyse samples

Premix **25 µL Proteinase K** and at least **125 – 600 µL Buffer FLB** and pipette it to the sample.

Incubate several hours or overnight at room temperature.

Optional: Separate lysate from sample material. See section 5.3 for use of the ***NucleoSpin® Trace Filter Plate*** *(see ordering information).*

2 Adjust DNA binding conditions

Add **1 vol.** (e.g., 300 µL) isopropanol to **2 vol.** (e.g., 600 µL) lysate, mix 3 times, and transfer to NucleoSpin® Trace Binding Plate.

Prepare the NucleoVac 96 Vacuum Manifold:

Place waste tray into vacuum manifold base. Insert spacers labeled 'MTP/MULTI-96 PLATE' notched side up and place the MN Wash Plate on them. Close the manifold with the manifold lid.

Place the NucleoSpin® Trace Binding Plate on top of the manifold.

3 Transfer lysates

Transfer the lysates resulting from step 2 carefully into the wells of the NucleoSpin® Trace Binding Plate. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination.

4 Bind DNA to silica membrane

Apply vacuum until all lysates have passed through the wells of the NucleoSpin® Trace Binding Plate **(-0.2 bar*; 2 min)**. Release the vacuum.

* Reduction of atmospheric pressure

5 Wash silica membrane*

1st wash

Add **900 µL Buffer B5** to each well of the NucleoSpin® Trace Binding Plate. Apply vacuum **(-0.2 bar*; 1 min)** until all buffer has passed through the wells of the NucleoSpin® Trace Binding Plate. Release the vacuum.

2nd wash

Add **900 µL Buffer B5** to each well of the NucleoSpin® Trace Binding Plate. Apply vacuum **(-0.2 bar*; 1 min)** until all buffer has passed through the wells of the NucleoSpin® Trace Binding Plate. Release the vacuum.

Remove MN Wash Plate

After the final washing step, close the valve, release the vacuum, and remove the NucleoSpin® Trace Binding Plate from the vacuum manifold. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

6 Dry silica membrane

Remove any residual washing buffer from the outlets of the NucleoSpin® Trace Binding Plate. If necessary, tap the outlets onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the Column Holder A with the NucleoSpin® Trace Binding Plate again into the lid and close the manifold. 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.

Note: The ethanol in Buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

7 Elute DNA

Insert spacers 'MICROTUBE RACK' into the NucleoVac 96 Vacuum Manifold's short sides. Place a Rack of Tube Strips onto the spacer. Close the vacuum manifold and place the NucleoSpin® Trace Binding Plate on top. Dispense 50 – 200 µL Buffer BE directly to the bottom of each well. Incubate for 5 min at room temperature. Apply vacuum for elution (-0.6 bar*; 1 min). Release vacuum.

Finally, close Tube Strips with Cap Strips for storage.

Centrifuge the Rack of Tube Strips shortly to collect all sample at the bottom of the Tube Strips.

Note: Elution with a centrifuge is recommended (see section 5.2).

* Reduction of atmospheric pressure

5.2 NucleoSpin® 96 Trace – centrifuge processing

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 Buffer B5 and Proteinase K were prepared according to section 3.

1	**Lyse** samples	**125 – 600 µL FLB** **25 µL Proteinase K** **Mix** **RT, several hours or overnight**
2	**Adjust** DNA binding conditions	**1 vol. isopropanol (per 2 vol. lysate)** **Mix**
3	**Transfer** lysates to NucleoSpin® Trace Binding Plate	
4	**Bind** DNA to silica membrane of the NucleoSpin® Trace Binding Plate	**5,600 – 6,000 x *g*, 3 min**
5	**Wash** silica membrane	**900 µL B5** **5,600 – 6,000 x *g*, 2 min** **900 µL B5** **5,600 – 6,000 x *g*, 10 min**
6	**Dry** silica membrane	**Not necessary – see prolonged centrifugation at step 5 (2nd wash step)**
7	**Elute** DNA	**50 – 200 µL BE** **5,600 – 6,000 x *g*, 3 min**

Detailed protocol

- For hardware requirements, refer to section 2.3.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.

1 Lyse samples

Premix **25 µL Proteinase K** and at least **125 µL Buffer FLB** and pipette it to the sample.

Incubate several hours or overnight at room temperature.

Optional: Separate lysate from sample material. See section 5.3 for use of the ***NucleoSpin® Trace Filter Plate*** *(see ordering information, section 6.2).*

2 Adjust DNA binding conditions

Add 1 vol. (e.g., 300 µL) isopropanol to 2 vol. (e.g., 600 µL) lysate, mix 3 times and transfer to NucleoSpin® Trace Binding Plate.

3 Transfer lysates

Remove the first Cap Strip and transfer the lysates resulting from step 2 into the wells of the NucleoSpin® Trace Binding Plate. Continue with the next eight samples. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross-contamination during centrifugation. After transfer, seal the openings of the plate with Self-adhering PE Foil.

4 Bind DNA to silica membrane

Place the MN Square-well Blocks with NucleoSpin® Trace Binding Plates onto the centrifuge carriers and insert them into the rotor buckets. Centrifuge at **5,600 – 6,000 x g** for **3 min**.

5 Wash silica membrane

1st wash

Remove the Self-adhering PE Foil and add **900 µL Buffer B5** to each well of the NucleoSpin® Trace Binding Plate. Seal plate with a new Self-adhering PE Foil and centrifuge again at **5,600 – 6,000 x g** for **2 min**.

2nd wash

Remove the Self-adhering PE Foil and add **900 µL Buffer B5** to each well of the NucleoSpin® Trace Binding Plate. Seal plate with a new Self-adhering PE Foil and centrifuge again at **5,600 – 6,000 x *g*** for 10 min.

During this step, as much ethanolic Buffer B5 as possible is removed by centrifugation.

6 **Dry silica membrane**

Residual washing buffer from the NucleoSpin® Trace Binding Plate is removed by the prolonged centrifugation time of 10 min after adding the Buffer B5 as described in step 5. This prolonged time is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

7 **Elute DNA**

For elution, place the NucleoSpin® Trace Binding Plate on the Rack of Tube Strips and pipette **50 – 200 µL Buffer BE** directly to the bottom of each well. Incubate **5 min** at **room temperature** and centrifuge at **5,600 – 6,000 x *g*** for **3 min**. Close the Tube Strips with Cap Strips for storage.

Be sure that all of the water gets into contact with the silica membrane: no water drops should stick to the walls of the columns.

5.3 NucleoSpin® 96 Trace – use of the NucleoSpin® Trace Filter Plate

- For hardware requirements, refer to section 2.3.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.

1 Place the **NucleoSpin® Trace Filter Plate** onto a square-well block. Add forensic material (e.g., buccal swabs) to the wells of the NucleoSpin® Trace Filter Plate. Premix **25 µL Proteinase K** and the **minimum volume of Buffer FLB** necessary to soak the material completely to the sample. Incubate several hours or overnight at room temperature.

2 After incubation, separate the lysate containing DNA from the forensic material by centrifugation (**5 min, 5,600 – 6,000 x *g***).

3 Proceed with step 2 of the general procedure (section 5.1 or 5.2, addition of isopropanol).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA quality or yield	*Reagents not applied or prepared properly* • Reagents were not properly prepared. Add the indicated volume of Proteinase Buffer PB to the Proteinase K vial and 96 – 100 % ethanol to Buffer Concentrate B5 and mix. *Kit storage* • Store aliquots of the reconstituted Proteinase K at -20 °C. • Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. *Suboptimal elution* • Elution efficiencies decrease dramatically if elution is performed with buffers with pH < 7.0. Use slightly alkaline elution buffer like BE (pH 8.5). • Be sure that all of the elution buffer gets into contact with the silica membrane. No drops should stick to the walls of the columns.
Suboptimal performance of DNA in downstream experiments	*Carry-over of ethanol* • Be sure to remove all of the ethanolic Buffer B5 after the final washing step. Dry the NucleoSpin® Trace Binding Plate for at least 10 min with maximum vacuum.
Insufficient vacuum pressure	*Vacuum pressure is not sufficient* • Check if the vacuum manifold lid fits tightly to the manifold base if vacuum is turned on.
Insufficient buffer volumes	*Buffer volumes are not enough* • Buffers are delivered in sufficient, but limited amounts. Calculate the needed buffer volumes and pour an additional amount of 10 % into the reservoirs. • Do not fill back unused buffer from reservoir to the flask to avoid contaminations. Ask technical service for extended buffer volumes.

Problem	Possible cause and suggestions
Cross-contamination	*Cross-contamination during transfer of lysate.* • Be sure that no liquid drops out of the tips while moving the tips with samples above the NucleoSpin® Trace Binding Plate.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 Trace	740726.2 740726.4	2 × 96 preps 4 × 96 preps
NucleoSpin® 8 Trace	740722.1 740722.5	12 x preps 60 x preps
NucleoSpin® Trace Filter Plate	740677	20
NucleoSpin® Forensic Filters	740988.10 / .50 / .250	10 / 50 / 250 pieces
NucleoSpin® Forensic Filters (Bulk)	740988.50B / .250B / .1000B	50 / 250 / 1000 pieces
Buffer FLB	740322.500	500 mL
Buffer BW	740922	100 mL
Buffer B5 (Concentrate) (for 100 mL Buffer B5)	740322.500	500 mL
Proteinase K	740506	100 mg
MN Wash Plate	740675	1
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
MN Square-well Block	740476 740476.24	4 sets 24 sets
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Self-adhering PE Foil	740676	50

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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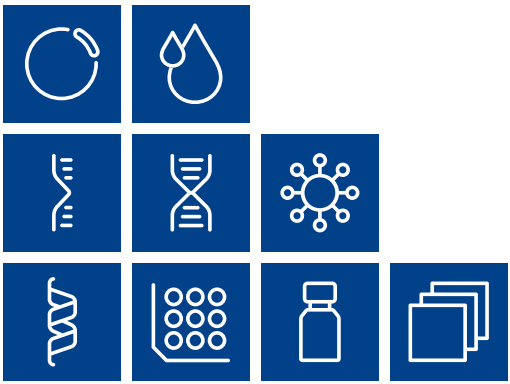
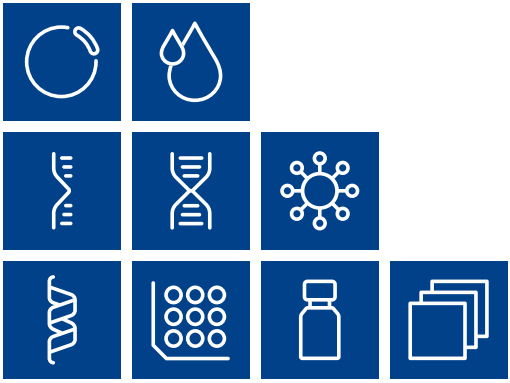


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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