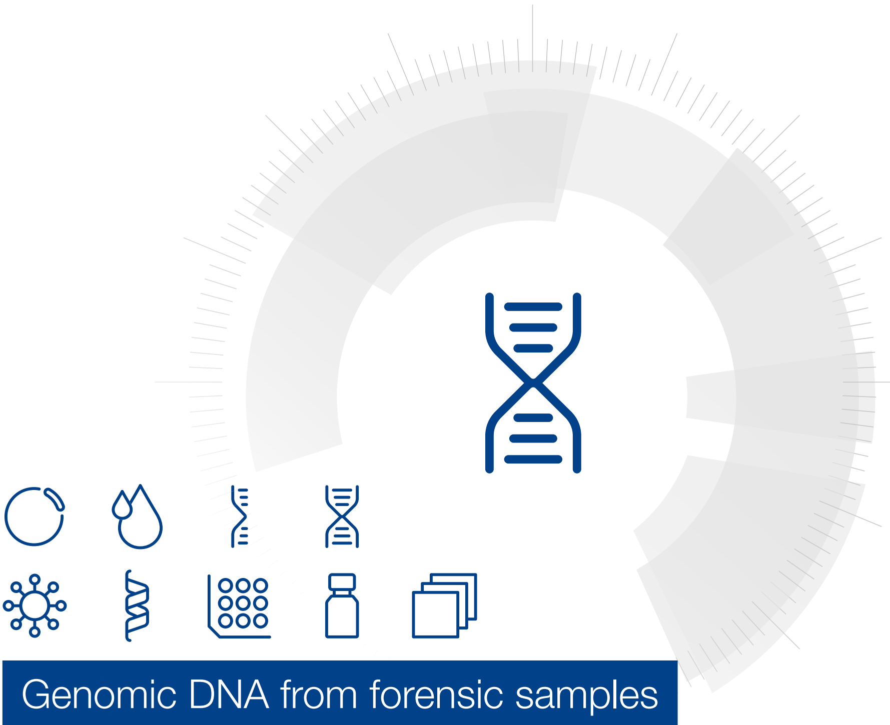
MACHEREY-NAGEL

User manual

Bioanalysis



Genomic DNA from forensic samples

- NucleoSpin® 8 Trace

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MN

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Table of contents

1 Components 4
1.1 Kit contents 4
1.2 Reagent to be supplied by user 4
1.3 About this user Manual 4
2 Product description 5
2.1 The basic principle 5
2.2 Kit specifications 5
2.3 Required hardware 6
3 Storage conditions and preparation of working solutions 7
4 Safety instructions 8
4.1 Disposal 8
5 Protocols 9
5.1 NucleoSpin® 8 Trace – vacuum processing 9
5.2 NucleoSpin® 8 Trace – centrifuge processing 14
5.3 NucleoSpin® 8 Trace – use of the NucleoSpin® Trace Filter Plate 17
6 Appendix 18
6.1 Troubleshooting 18
6.2 Ordering information 19
6.3 Product use restriction / warranty 20

1 Components

1.1 Kit contents

	NucleoSpin® 8 Trace	
REF	12 × 96 preps 740722.1	60 × 8 preps 740722.5
Lysis Buffer FLB	125 mL	500 mL
Wash Buffer B5 (Concentrate) [1]	50 mL	2 × 100 mL
Elution Buffer BE [2]	60 mL	250 mL
Proteinase K (lyophilized) [1]	33 mg	5 × 33 mg
Proteinase Buffer PB	2 × 1.8 mL	35 mL
NucleoSpin® Trace Binding Strips (gray rings)	12	60
MN Wash Plates (including six paper sheets)	2	10
MN Square-well Bocks	2	10
Rack of Tube Strips [3]	1	5
Self-adhering PE Foils	2	10
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® 8 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 Strips 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® 8 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® 8 Trace** can be processed under vacuum or in a centrifuge (see section 2.3).

Table 1: Kit specifications at a glance

Parameters	NucleoSpin® 8 Trace
Technology	Silica-membrane technology
Format	8-well strips
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	30 min/6 strips (excl. lysis)
Binding capacity	20 μg
Use	For research use only

Forensic quality product:

NucleoSpin® 8 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).

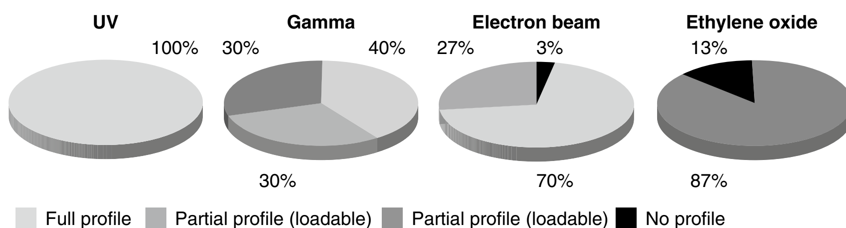


Figure 1 According to Shaw *et al.*, 2008, Comparison of the effects of sterilization techniques on subsequent DNA profiling. Int J Legal Med 122: 29 – 33.

2.3 Required hardware

The NucleoSpin® 8 Trace kit can be used manually with the NucleoVac 96 Vacuum Manifold (REF 740681) by using the Starter Set A containing Column Holders A and NucleoSpin® Dummy Strips (see ordering information).

For automation on laboratory platforms with standard 96-well plate vacuum chambers, the use of the Starter Set A is also required.

Processing of the NucleoSpin® 8 Trace kit under centrifugation is possible by using the Starter Set C (see ordering information), containing Column Holders C, MN Square-well Blocks, and Racks of Tube Strips. For detailed information, refer to the Starter Set C manual.

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® 8 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® 8 Trace	
REF	**12 × 8 preps 740726.2**	**60 × 8 preps 740722.5**
Wash Buffer B5 (Concentrate)	50 mL Add 200 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle
Proteinase K	33 mg Add 3 mL Proteinase Buffer	5 × 33 mg Add 3 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoSpin® 8 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***).

Caution: Guanidine hydrochloride in buffer FLB 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® 8 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® 8 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.

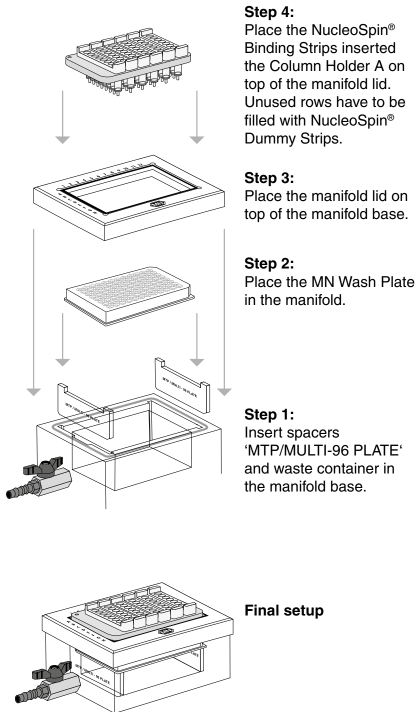
1	**Lyse** samples	**125 – 600 µL FLB** **25 µL Proteinase K** **Mix** **RT, several hours or overnight**
2	**Adjust** DNA binding conditions	**1 vol. isopropanol (per 2 vol. lysate)** **Mix** **Prepare the NucleoVac 96 Vacuum Manifold**
3	**Transfer** lysates to NucleoSpin® Trace Binding Strips	
4	**Bind** DNA to silica membrane of the NucleoSpin® Trace Binding Strips	**-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

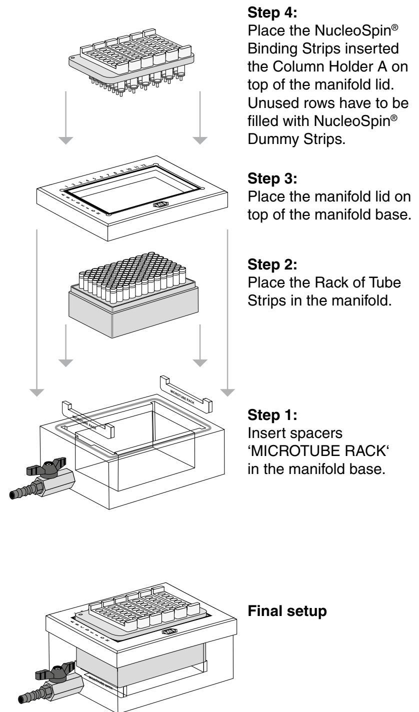
7	**Elute** DNA	**50 – 200 µL BE** **-0.4 bar*, 2 min**

Setup of vacuum manifold:

Binding / Washing steps



Elution step



Detailed protocol

- For hardware requirements, refer to section 2.3.
  For processing of NucleoSpin® 8 Trace under vacuum, the NucleoVac 96 Vacuum Manifold and the Starter Kit A are required (see ordering information). Starter Kit A contains the Column Holders A and NucleoSpin® Dummy Strips to seal unused rows.
  The use of NucleoSpin® Trace Binding Strips in a Column Holder A allows the isolation of up to n x 8 samples (n = 1 to 6). Insert as many NucleoSpin® Trace Binding Strips as required into the reusable column holder. Seal unused wells of NucleoSpin® Trace Binding Strips with Self-adhering PE-Foil and close unused wells with NucleoSpin® Dummy Strips. Place the Column Holder on the NucleoVac 96 manifold.
- 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 **125 – 600 µL Buffer FLB** and pipette it to the sample.

Incubate several hours or overnight at room temperature.

*<u>Optional:</u> 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 Strips.

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.

Insert desired number of NucleoSpin® Trace Binding Strips in the Column Holder A. Use NucleoSpin® Dummy Strips to seal unused positions in the column holder.

Place Column Holder A with inserted NucleoSpin® Trace Binding Strips on top of the manifold.

3 Transfer lysates

Transfer the lysates resulting from step 2 carefully into the wells of the NucleoSpin® Trace Binding Strips. When using the Rack of Tube Strips, remove the first Cap Strip and transfer lysates before removing the next Cap Strip. 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 Strips **(-0.2 bar*; 2 min)**. Release the vacuum.

5 Wash silica membrane*

1st wash

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

2nd wash

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

Remove MN Wash Plate

After the final washing step, close the valve, release the vacuum, and remove the Column Holder A with inserted NucleoSpin® Trace Binding Strips 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 NucleoSpin® Trace Binding Strips. 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 with NucleoSpin® Trace Binding Strips into the lid and close the manifold. Apply maximum vacuum ca. -**0.6 bar*** for at least **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.

* Reduction of atmospheric pressure

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 Strips 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 **(-0.4 bar*; 2 min)**. Release vacuum and repeat elution step once.

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).

5.2 NucleoSpin® 8 Trace – centrifuge processing

Protocol-at-a-glance

For hardware requirements, refer to section 2.3

For detailed information on each step, see page 15.

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 Strips	
4	**Bind** DNA to silica membrane of the NucleoSpin® Trace Binding Strips	**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.
  For processing of NucleoSpin® 8 Trace with a centrifuge, the NucleoVac 96 Vacuum Manifold and the Starter Kit C are required (see ordering information).
  The use of NucleoSpin® Trace Binding Strips in a Column Holder C allows the isolation of up to n x 8 samples (n = 1 to 6). Insert as many of the NucleoSpin® Trace Binding Strips as required into the same positions of each one of the two reusable column holders and place column holders onto the MN Square-well Blocks. Label the column holders or 8-well strips for later identification. Always use 2 Column Holders C containing identical numbers of NucleoSpin® Trace Binding Strips for centrifugation. This avoids the need to balance the centrifuge, and allows multiples of 16 samples to be processed in parallel. We recommend inserting the NucleoSpin® Trace Binding Strips around the center of the column holder

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, 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 Strips.

3 Transfer lysates

Transfer the lysates resulting from step 2 carefully into the wells of the NucleoSpin® Trace Binding Strips. When using the Rack of Tube Strips, remove the first Cap Strip and transfer lysates before removing the next Cap Strip.

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

Place the MN Square-well Block with Column Holder C 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 Strips. Seal strips 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 Strips. Seal strips 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 NucleoSpin® Trace Binding Strips is removed by the prolonged centrifugation time of 10 min after adding 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

Dispense **50–200 µL Buffer BE** to each well of the NucleoSpin® Trace Binding Strips. Dispense the buffer directly onto the membrane. Incubate at room temperature for 5 min. Centrifuge at **5,600–6,000 x g** for **3 min**. Remove Column Holder C with inserted NucleoSpin® Trace Binding Strips from the Rack

of Tube Strips. Close the Tube Strips with Cap Strips for storage.

If elution in small volume tubes is desired, place a 96 PCR plate (not supplied) on top of a Round-well Block or a Rack of Tube Strips and elute into the PCR plate.

5.3 NucleoSpin® 8 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 Strips. 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 Strips 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 Strips.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 8 Trace	740722.1 740722.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 96 Trace	740726.2 740726.4	2 × 96 preps 4 × 96 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
Self-adhering PE Foil	740676	50
Starter Set A (for processing NucleoSpin® 8-well strips on NucleoVac 96 Vacuum Manifold)	740682	1

Product	REF	Pack of
Starter Set C (for processing NucleoSpin® 8-well strips under centrifugation)	740684	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
MN Frame	740680	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-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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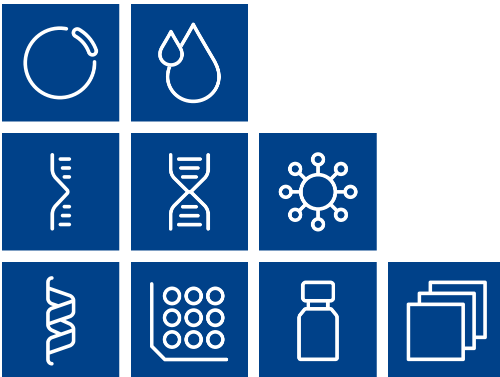
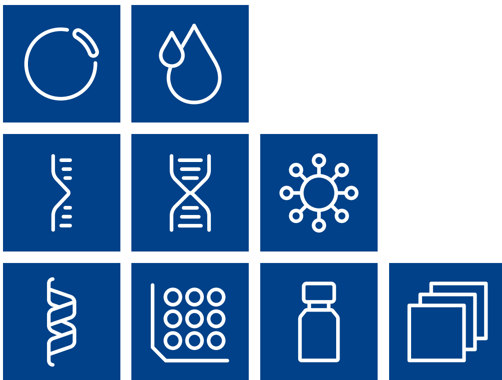


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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