



# **Genomic DNA from forensic samples**

## **User manual** NucleoSpin® 8 Trace

April 2014 / Rev. 04

**MACHEREY-NAGEL**

***www.mn-net.com***



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# 1 Components

## 1.1 Kit contents

| NucleoSpin® 8 Trace                           |                          |                          |
|-----------------------------------------------|--------------------------|--------------------------|
| REF                                           | 12 x 8 preps<br>740722.1 | 60 x 8 preps<br>740722.5 |
| Lysis Buffer FLB                              | 125 mL                   | 500 mL                   |
| Wash Buffer B5 (Concentrate) <sup>1</sup>     | 50 mL                    | 2 x 100 mL               |
| Elution Buffer BE <sup>2</sup>                | 60 mL                    | 250 mL                   |
| Proteinase K (lyophilized) <sup>1</sup>       | 33 mg                    | 5 x 33 mg                |
| Proteinase Buffer PB                          | 2 x 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 <sup>3</sup>              | 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.

<sup>1</sup> For preparation of working solutions and storage conditions, see section 3.

<sup>2</sup> Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

<sup>3</sup> 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.

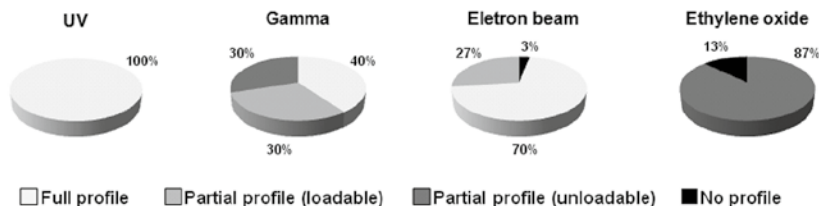
### 2.2 Kit specifications

- **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                                         |

- **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 x 8 preps                           | 60 x 8 preps                                            |
|                                 | 740722.1                               | 740722.5                                                |
| Wash Buffer B5<br>(Concentrate) | 50 mL<br>Add 200 mL ethanol            | 2 x 100 mL<br>Add 400 mL ethanol<br>to each bottle      |
| Proteinase K                    | 33 mg<br>Add 3 mL<br>Proteinase Buffer | 5 x 33 mg<br>Add 3 mL<br>Proteinase Buffer to each vial |

## 4 Safety instructions

The following components of the **NucleoSpin® 8 Trace** kits contain hazardous contents.

*Wear gloves and goggles and follow the safety instructions given in this section.*

### GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

| Component    | Hazard contents                                                      | GHS symbol                                                                                                                                                             | Hazard phrases          | Precaution phrases                                                               |
|--------------|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|----------------------------------------------------------------------------------|
| Inhalt       | Gefahrstoff                                                          | GHS Symbol                                                                                                                                                             | H-Sätze                 | P-Sätze                                                                          |
| FLB          | Guanidine hydrochloride 1–10 %<br><i>Guanidinhydrochlorid 1–10 %</i> | Substance does not have to be specially labeled as hazardous<br><i>Die Substanz muss nicht als gefährlich gekennzeichnet werden.</i>                                   |                         |                                                                                  |
| Proteinase K | Proteinase K, lyophilized<br><i>Proteinase K, lyophilisiert</i>      | <br> | Danger<br><i>Gefahr</i> | 315, 319, 334, 335                                                               |
|              |                                                                      |                                                                                                                                                                        |                         | 261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 403+233 |

### Hazard phrases

|       |                                                                                                                                                                          |
|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| H 315 | Causes skin irritation.<br><i>Verursacht Hautreizungen.</i>                                                                                                              |
| H 319 | Causes serious eye irritation.<br><i>Verursacht schwere Augenreizung.</i>                                                                                                |
| H 334 | May cause allergy or asthma symptoms or breathing difficulties if inhaled.<br><i>Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.</i> |
| H 335 | May cause respiratory irritation.<br><i>Kann die Atemwege reizen.</i>                                                                                                    |

### Precaution phrases

|           |                                                                                                            |
|-----------|------------------------------------------------------------------------------------------------------------|
| P 261     | Avoid breathing dust.<br><i>Einatmen von Staub vermeiden.</i>                                              |
| P 280     | Wear protective gloves / eye protection.<br><i>Schutzhandschuhe / Augenschutz tragen.</i>                  |
| P 302+352 | IF ON SKIN: Wash with plenty of water/...<br><i>Bei Kontakt mit der Haut: Mit viel Wasser/... waschen.</i> |

### Precaution phrases

- P 304+340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.  
*BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
- P 305+351+338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.  
*BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.*
- P 312 Call a POISON CENTER/ doctor/.../if you feel unwell.  
*Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /... anrufen.*
- P 332+313 If skin irritation occurs: Get medical advice / attention.  
*Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 337+313 Get medical advice / attention.  
*Bei anhaltender Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/...  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM / Arzt/... anrufen.*
- P 403+233 Store in a well ventilated place. Keep container tightly closed.  
*Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ([www.mn-net.com](http://www.mn-net.com)).  
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ([www.mn-net.com](http://www.mn-net.com)).

## 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 13.
- 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 | <b>Lyse</b> samples                                                           | 125–600 µL FLB                              |
|   |                                                                               | 25 µL Proteinase K                          |
|   |                                                                               | Mix                                         |
|   |                                                                               | RT, several hours or overnight              |
| 2 | <b>Adjust</b> DNA binding conditions                                          | 1 vol. isopropanol<br>(per 2 vol. lysate)   |
|   |                                                                               | Mix                                         |
|   |                                                                               | Prepare the NucleoVac 96<br>Vacuum Manifold |
| 3 | <b>Transfer</b> lysates to NucleoSpin® Trace Binding Strips                   |                                             |
| 4 | <b>Bind</b> DNA to silica membrane of the<br>NucleoSpin® Trace Binding Strips | -0.2 bar*,<br>2 min                         |
| 5 | <b>Wash</b> silica membrane                                                   | 900 µL B5                                   |
|   |                                                                               | -0.2 bar*,<br>1 min                         |
|   |                                                                               | 900 µL B5                                   |
|   |                                                                               | -0.2 bar*,<br>1 min                         |

\* Reduction of atmospheric pressure

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**Remove MN Wash Plate**

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**6    Dry silica membrane**

**-0.6 bar\*,  
10 min**

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**7    Elute DNA**

**50–200 µL BE**

**-0.4 bar\*,  
2 min**

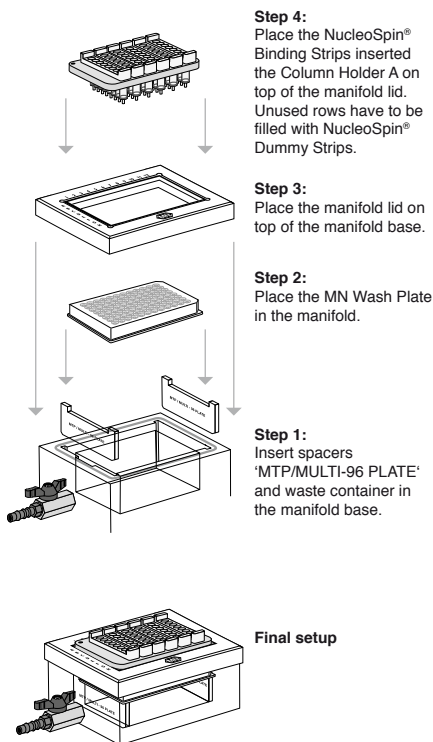
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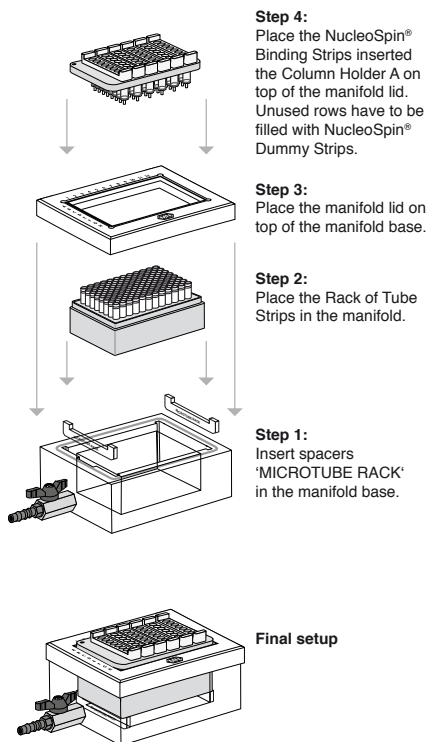
\* Reduction of atmospheric pressure

## 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  $n \times 8$  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 13.

### 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**.

*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 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\*

#### 1<sup>st</sup> 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.

#### 2<sup>nd</sup> 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.

---

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\* Reduction of atmospheric pressure

## 6 Dry silica membrane

Remove any residual washing buffer from the NucleoSpin® Trace Binding Strips. If necessary, tap the outlets of the NucleoSpin® Trace Binding Strips 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.

---

## 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 Column Holder A with 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).*

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\* Reduction of atmospheric pressure

## 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 18.

#### Before starting the preparation:

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

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

## 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  $n \times 8$  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).*

---

#### 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

##### **1<sup>st</sup> 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**.

##### **2<sup>nd</sup> 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**).

---

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                               | <p><i>Reagents not applied or prepared properly</i></p> <ul style="list-style-type: none"> <li>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.</li> </ul>                                                                                                            |
|                                                         | <p><i>Kit storage</i></p> <ul style="list-style-type: none"> <li>Store aliquots of the reconstituted Proteinase K at -20 °C.</li> <li>Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.</li> <li>Keep bottles tightly closed in order to prevent evaporation or contamination.</li> </ul>                                          |
|                                                         | <p><i>Suboptimal elution</i></p> <ul style="list-style-type: none"> <li>Elution efficiencies decrease dramatically if elution is performed with buffers with pH &lt; 7.0. Use slightly alkaline elution buffer like BE (pH 8.5).</li> <li>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.</li> </ul> |
| Suboptimal performance of DNA in downstream experiments | <p><i>Carry-over of ethanol</i></p> <ul style="list-style-type: none"> <li>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.</li> </ul>                                                                                                                                     |
| Insufficient vacuum pressure                            | <p><i>Vacuum pressure is not sufficient</i></p> <ul style="list-style-type: none"> <li>Check if the vacuum manifold lid fits tightly to the manifold base if vacuum is turned on.</li> </ul>                                                                                                                                                                                               |
| Insufficient buffer volumes                             | <p><i>Buffer volumes are not enough</i></p> <ul style="list-style-type: none"> <li>Buffers are delivered in sufficient, but limited amounts. Calculate the needed buffer volumes and pour an additional amount of 10 % into the reservoirs.</li> </ul>                                                                                                                                     |

| <b>Problem</b>                             | <b>Possible cause and suggestions</b>                                                                                                                                                                                                      |
|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Insufficient buffer volumes<br>(continued) | <ul style="list-style-type: none"> <li>Do not fill back unused buffer from reservoir to the flask to avoid contaminations. Ask technical service for extended buffer volumes.</li> </ul>                                                   |
| Cross-contamination                        | <p><i>Cross-contamination during transfer of lysate.</i></p> <ul style="list-style-type: none"> <li>Be sure that no liquid drops out of the tips while moving the tips with samples above the NucleoSpin® Trace Binding Strips.</li> </ul> |

## 6.2 Ordering information

| <b>Product</b>                                    | <b>REF</b>            | <b>Pack of</b>               |
|---------------------------------------------------|-----------------------|------------------------------|
| NucleoSpin® 8 Trace                               | 740722.1<br>740722.5  | 12 x 8 preps<br>60 x 8 preps |
| NucleoSpin® 96 Trace                              | 740726.2<br>740726.4  | 2 x 96 preps<br>4 x 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)<br>(for 100 mL Buffer B5) | 740322.500            | 500 mL                       |
| Proteinase K                                      | 740506                | 100 mg                       |
| MN Wash Plate                                     | 740675                | 1                            |

| Product                                                                                                    | REF                 | Pack of           |
|------------------------------------------------------------------------------------------------------------|---------------------|-------------------|
| Rack of Tube Strips<br>(set consists of 1 Rack,<br>12 Tube Strips with 8 tubes each,<br>and 12 Cap Strips) | 740477<br>740477.24 | 4 sets<br>24 sets |
| MN Square-well Block                                                                                       | 740476<br>740476.24 | 4 sets<br>24 sets |
| Self-adhering PE Foil                                                                                      | 740676              | 50                |
| Starter Set A<br>(for processing NucleoSpin® 8-well<br>strips on NucleoVac 96 Vacuum<br>Manifold)          | 740682              | 1                 |
| Starter Set C<br>(for processing NucleoSpin® 8-well<br>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](http://www.mn-net.com) for more detailed product information.

### 6.3 Product use restriction/warranty

**NucleoSpin® 8 Trace** components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

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Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

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Please contact:

MACHEREY-NAGEL GmbH & Co. KG

Tel.: +49 (0) 24 21 969 270

e-mail: tech-bio@mn-net.com