Circulating DNA from plasma

User manual
NucleoSpin® Plasma XS

June 2017 / Rev. 05
## Circulating DNA from plasma

### Protocol-at-a-glance (Rev. 05)

<table>
<thead>
<tr>
<th>NucleoSpin® Plasma XS</th>
<th>High Sensitivity protocol</th>
<th>Rapid protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> Prepare sample</td>
<td>Use up to 240 μL plasma</td>
<td>Use up to 200 μL plasma</td>
</tr>
<tr>
<td><strong>1a</strong> Optional: Proteinase K treatment</td>
<td>Add 20 μL Proteinase K Mix Incubate at 37 °C for 10 min</td>
<td>/</td>
</tr>
<tr>
<td><strong>2</strong> Adjust binding conditions</td>
<td>Add 360 μL BB</td>
<td>Add 300 μL BB</td>
</tr>
<tr>
<td><strong>3</strong> Mix sample</td>
<td>Invert tube 3 x Vortex 3 s Spin down briefly</td>
<td>Invert tube 3 x Vortex 3 s Spin down briefly</td>
</tr>
<tr>
<td><strong>4</strong> Bind DNA</td>
<td>Load lysate 2,000 x g, 30 s 11,000 x g, 5 s</td>
<td>Load lysate 11,000 x g, 30 s</td>
</tr>
<tr>
<td><strong>5</strong> Wash and dry silica membrane</td>
<td>1st wash 500 μL WB 11,000 x g, 30 s 2nd wash 250 μL WB 11,000 x g, 3 min</td>
<td>1st wash 500 μL WB 11,000 x g, 30 s 2nd wash 250 μL WB 11,000 x g, 3 min</td>
</tr>
<tr>
<td><strong>6</strong> Elute DNA</td>
<td>20 μL Elution Buffer 11,000 x g, 30 s</td>
<td>20 μL Elution Buffer 11,000 x g, 30 s</td>
</tr>
<tr>
<td><strong>7</strong> Removal of residual ethanol</td>
<td>90 °C, 8 min</td>
<td>/</td>
</tr>
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# Components

## 1.1 Kit contents

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<tr>
<th>NucleoSpin® Plasma XS</th>
<th>10 preps</th>
<th>50 preps</th>
<th>250 preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding Buffer BB</td>
<td>5 mL</td>
<td>22 mL</td>
<td>110 mL</td>
</tr>
<tr>
<td>Wash Buffer WB</td>
<td>10 mL</td>
<td>50 mL</td>
<td>250 mL</td>
</tr>
<tr>
<td>Elution Buffer*</td>
<td>13 mL</td>
<td>13 mL</td>
<td>13 mL</td>
</tr>
<tr>
<td>Proteinase K (lyophilized)**</td>
<td>6 mg</td>
<td>30 mg</td>
<td>2 x 75 mg</td>
</tr>
<tr>
<td>Proteinase Buffer PB</td>
<td>1.8 mL</td>
<td>1.8 mL</td>
<td>8 mL</td>
</tr>
<tr>
<td>NucleoSpin® Plasma XS Columns</td>
<td>10</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>(red rings – plus Collection</td>
<td>Collection Tubes</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Tubes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>User manual</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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

** For preparation of working solutions and storage conditions see section 3.
1.2 Consumables and equipment to be supplied by user

Consumables
• 1.5 mL microcentrifuge tubes
• Disposable pipette tips

Equipment
• Manual pipettors
• Centrifuge for microcentrifuge tubes
• Vortex mixer
• Heating-block for incubation at 90 °C
• Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

The manual provides two procedures differing in the number of handling steps, speed and performance. The **high sensitivity procedure** is recommended if highest DNA yield and concentration is required. The **rapid procedure** is recommended if shortest preparation time is required.

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Plasma XS** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at [www.mn-net.com](http://www.mn-net.com).

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

2.1 The basic principle

The NucleoSpin® Plasma XS kit is designed for the efficient isolation of circulating DNA from human blood plasma. Fragmented DNA as small as 50–1000 bp can be purified with high efficiency. Due to a special funnel design the NucleoSpin® Plasma XS Columns allow very small elution volumes (5–30 μL) which results in highly concentrated DNA.

The protocol follows state-of-the-art bind-wash-elute procedures: After mixing of a plasma sample with the binding buffer, the mixture is applied to the NucleoSpin® Plasma XS Column. Upon loading of the mixture DNA binds to a silica membrane. Two subsequent washing steps efficiently remove contaminations and highly pure DNA is finally eluted with 5–30 μL of a slightly alkaline elution buffer of low ionic strength (5 mM Tris-HCl, pH 8.5).

2.2 Kit specifications

- The NucleoSpin® Plasma XS kit is recommended for the isolation of fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage.

- The NucleoSpin® Plasma XS kit is designed for high recovery, especially of fragmented DNA in a range of 50–1000 bp.

- Up to 240 μL plasma can be used as sample material with a single column loading step. DNA yield strongly depends on the individual sample, but is typically in the range of 0.1 to 100 ng DNA per mL plasma. Up to 720 μL plasma can be used with three column loadings. If more than 240 μL plasma is processed, additional Lysis Buffer BB is required (see ordering information).

- Elution can be performed with as little as 5–30 μL elution buffer. DNA is ready to use for downstream applications like real-time PCR or others.

- The preparation time is approximately 15–30 min for 6–12 plasma samples.
Circulating DNA from plasma

Table 1: Kit specifications at a glance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NucleoSpin® Plasma XS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample material</td>
<td>Up to 240 μL EDTA plasma (single column loading)</td>
</tr>
<tr>
<td>Average yield</td>
<td>Typically in a range of 0.1–100 ng per mL plasma, depending on sample (depending on kind of patient samples, yield can be much higher).</td>
</tr>
<tr>
<td>Elution volume</td>
<td>5–30 μL</td>
</tr>
</tbody>
</table>
| Preparation time   | High sensitivity procedure: 22–27 min/6 preps  
|                    | Rapid procedure: 15–20 min/6 preps |
| Format             | XS spin column |

DNA yield from human plasma

DNA amounts from less than 0.1 ng DNA per mL of plasma up to several 100 ng DNA/mL of plasma have been reported (Chiu et al. 2006; Chun et al. 2006; Fatouros et al. 2006; Lazar et al. 2006; Rainer et al. 2006; Rhodes et al. 2006; Schmidt et al. 2005).

The content of DNA in plasma depends on: condition of the donor, sampling and handling of the blood, plasma preparation and DNA isolation method, DNA quantification method, and others.

Size of circulating DNA

A good portion of the cell-free DNA in plasma results from apoptotic cells. Therefore, a considerable percentage of this circulating nucleosomal DNA is known to be highly fragmented. However, the degree of fragmentation and the ratio of fragmented DNA to high molecular weight DNA depends on several parameters like origin of the DNA (e.g., fetal, tumor, microbial DNA), health of the blood donor, blood sampling procedure, and handling of the sample.

The performance of many downstream applications depends on the efficient isolation even of smallest DNA fragments (Chan et al. 2006, 2005, 2004, 2003; Deligezer et al. 2006; Giacona et al. 1998; Hanley et al. 2006; Hromadnikova et al. 2006; Jiang et al. 2006; Koide et al. 2005; Li et al. 2006, 2005, 2004; Wang et al. 2004). According to this the NucleoSpin® Plasma XS purification system is designed for the efficient isolation of highly fragmented DNA in a range of 50–1000 bp. Within this range fragments are recovered with similar high efficiency.
2.3 Handling of sample material

Several publications indicate strong influence of blood sampling, handling, storage, and plasma preparation on DNA yield and DNA quality (Page et al. 2006; Sozzi et al. 2005; Chan et al. 2005; Lam et al. 2004; Jung et al. 2003). Therefore it is highly recommended keeping blood sampling procedure, handling, storage, and plasma preparation method constant in order to achieve highest reproducibility.

Plasma can be isolated according to protocols described in literature (e.g., Chiu and Lo 2006; Birch et al. 2005) or according to the following recommendation:

**Preparation of plasma from human EDTA blood**

1. Centrifuge fresh blood sample for 10 min at 2,000 x g.
2. Remove the plasma without disturbing sedimented cells.
3. Freeze plasma at -20 °C for storage upon DNA isolation.
4. Thaw frozen plasma samples prior to DNA isolation and centrifuge for 3 min at ≥ 11,000 x g in order to remove residual cells, cell debris, and particulate matter. Use the supernatant for DNA isolation.

2.4 Elution procedures

The recommended standard elution volume is 20 μL. A reduction of the elution volume to 5–15 μL will increase DNA concentration, the total DNA yield is decreased by this reduction however. An increase of the elution volume to 30 μL or more will only slightly increase total DNA yield, but reduce DNA concentration. Figure 1 gives a graphic description of the correlation between elution volume and DNA concentration to help finding the optimized elution volume for your individual application.

![Figure 1 Correlation between elution volume and DNA concentration](NucleoSpin® Plasma XS Columns)
2.5 Removal of residual traces of ethanol for highest PCR sensitivity

A reduction of the 20 μL standard elution volume will increase the concentration of residual ethanol in the eluate. For 20 μL elution volume a heat incubation of the elution fraction (incubate eluate with open lid for 8 min at 90 °C) is recommended if the eluate comprises more than 20 % of the final PCR volume, in order to avoid an inhibition of sensitive downstream reactions. In this context, please mind the remarks below:

- An incubation of the elution fraction at higher temperatures will increase signal output in PCR. This is of importance especially if the template represents more than 20 % of the total PCR reaction volume (e.g., more than 4 μL eluate used as template in a PCR reaction with a total volume of 20 μL).

  The template may represent up to 40 %* of the total PCR reaction volume, if the eluate is incubated at increased temperature as described.

- A volume of 20 μL used for elution will evaporate to 12–14 μL during a heat incubation for 8 min at 90 °C. If a higher final volume is required, please increase the initial volume of elution buffer, for example from 20 μL to 30 μL.

- An incubation of the elution fraction for 8 min at 90 °C will denature DNA. If non denatured DNA is required (e.g., for downstream applications other than PCR; like ligation or cloning), we recommend an incubation for longer time at a temperature below 80 °C as most of the DNA has a melting point above 80 °C. Suggestion: Incubate for 17 min at 75 °C.

- The incubation of the eluate at higher temperatures may be adjusted according to Figure 2. The incubation times and conditions shown will reduce an initial elution volume of 20 μL to about 12–14 μL and will effectively remove traces of ethanol as described above.

- If the initial volume of elution buffer applied to the column is less than 20 μL, time of heat incubation should be reduced to avoid complete dryness.

* The maximum percentage of template volume in a PCR reaction may vary depending on the robustness of the PCR system; 40 % template volume were tested using LightCyclerTM PCR (Roche) with DyNAmoTM Capillary SYBR® Green qPCR Kit (Finnzymes).
Figure 2 Removal of residual ethanol from the elution fraction by heat treatment. 
In order to obtain highest PCR sensitivity, heat incubation of the eluate is recommended. 
Heat incubation may be performed at temperatures of 70–90 °C in a heat block with or 
without shaking. Effective conditions (temperature, time, and shaking rate) for ethanol 
removal can be read from the diagram; an initial volume of 20 μL will evaporate to 
12–14 μL during the described incubation.

2.6 Stability of isolated DNA

Due to the typically low DNA content in plasma and the resulting low total amount of isolated 
DNA, its fragmentation, and the absence of DNase inhibitors (the elution buffer does NOT 
contain EDTA) the eluates should be placed on ice for short term and frozen at -20 °C for 
long term storage.
3 Storage conditions and preparation of working solutions

Attention: The Buffer BB contains chaotropic salt and ethanol! Wear gloves and goggles!

CAUTION: Buffer BB contains guanidinium thiocyanate 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.

Storage conditions:

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.
- If there is any precipitate present in the buffers, warm the buffer up to 25–37 °C to dissolve the precipitate before use.

Before starting any NucleoSpin® Plasma XS protocol prepare the following:

- Before first use of the kit, add the indicated volume of Proteinase Buffer PB to dissolve lyophilized Proteinase K (see bottle or table below). Proteinase K solution is stable at -20 °C for at least 6 months.

<table>
<thead>
<tr>
<th>NucleoSpin® Plasma XS</th>
<th>10 preps</th>
<th>50 preps</th>
<th>250 preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>740900.10</td>
<td>740900.50</td>
<td>740900.250</td>
</tr>
<tr>
<td>Proteinase K (lyophilized)</td>
<td>6 mg</td>
<td>30 mg</td>
<td>2 x 75 mg</td>
</tr>
<tr>
<td>Add 260 µL Proteinase Buffer</td>
<td></td>
<td>Add 1.35 mL Proteinase Buffer</td>
<td>Add 3.35 mL Proteinase Buffer to each vial</td>
</tr>
</tbody>
</table>
4 Safety instructions

The following components of the NucleoSpin® Plasma XS 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Hazard contents</th>
<th>GHS symbol</th>
<th>Hazard phrases</th>
<th>Precaution phrases</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>Guanidinium thiocyanate 30–45 % + ethanol 35–55 %</td>
<td><img src="image" alt="WARNING ACHTUNG" /></td>
<td>226, 302, 412, EUH031</td>
<td>210, 260, 264, 273, 301+312, 330</td>
</tr>
<tr>
<td></td>
<td>CAS 593-84-0, 64-17-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td>Ethanol 55–75 %</td>
<td><img src="image" alt="DANGER GEFahr" /></td>
<td>225</td>
<td>210, 233</td>
</tr>
<tr>
<td></td>
<td>CAS 64-17-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinase K</td>
<td>Proteinase K, lyophilized 90–100 %</td>
<td><img src="image" alt="DANGER GEFahr" /></td>
<td>317, 334</td>
<td>261, 280, 342+311</td>
</tr>
<tr>
<td></td>
<td>Proteinase K, lyophilisiert 90–100 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAS 39450-01-6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The symbol shown on labels refers to further safety information in this section. Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

**Hazard phrases**

H 225 Highly flammable liquid and vapour.

Flüssigkeit und Dampf leicht entzündbar.

H 226 Flammable liquid and vapour.

Flüssigkeit und Dampf entzündbar.

H 302 Harmful if swallowed.

Gesundheitsschädlich bei Verschlucken.

H 317 May cause an allergic skin reaction.

Kann allergische Hautreaktionen verursachen.

H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.

H 412 Harmful to aquatic life with long lasting effects.

Schädlich für Wasserorganismen, mit langfristiger Wirkung.
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EUH031 Contact with acids liberates toxic gas.
Entwickelt bei Berührung mit Säure giftige Gase.

Precaution phrases

P 210 Keep away from heat/sparks/open flames/hot surfaces — No smoking.

P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.

P 260D Do not breathe vapours.
Dampf nicht einatmen.

P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.

P 264 Wash … thoroughly after handling.
Nach Handhabung … gründlich waschen.

P 273 Avoid release to the environment.
Freisetzung in die Umwelt vermeiden.

P 280 Wear protective gloves/eye protection.
Schutzhandschuhe/Augenschutz tragen.

P 301+312 IF SWALLOWED: Call a POISON CENTER/doctor/…/if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM /Arzt/… anrufen.

P 330 Rinse mouth.
Mund ausspülen.

P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor/…
Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/… anrufen.
## 5 Protocols

### Before starting the preparation:

Equilibrate sample to room temperature (18–25 °C) and make sure that the sample is cleared from residual cells, cell debris, and particular matter (e.g., by centrifugation of the plasma sample for 3 min at ≥ 11,000 x g).

For the high sensitivity procedure: Set the thermal heating block to 75–90 °C for final ethanol removal (see section 2.6 for details).

### 5.1 High sensitivity protocol for the isolation of DNA from plasma

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
</table>
| **1** | **Prepare sample**  
Add 240 μL plasma to a microcentrifuge tube (not provided).  
*Less than 240 μL may be used. Adopt the binding buffer volume accordingly (see below).* |
| **1a** | **Optional: Proteinase K treatment**  
Add 20 μL Proteinase K to the plasma sample, mix, and incubate at 37 °C for 10 min.  
*Depending on the plasma sample and the PCR conditions, the proteinase treatment of the plasma sample provokes a increase of the PCR signal of 0.5–1.5 cycles, i.e. the cycle threshold (Ct-value)/crossing point (Cp-value) is reached 0.5–1.5 cycles earlier. The proteinase treatment may however alter the ratio of high to low molecular weight DNA.* |
| **2** | **Adjust DNA binding conditions**  
Add 360 μL Buffer BB.  
*If less than 240 μL plasma is used, adjust the binding buffer volume accordingly. A ratio of 1:1.5 (v/v) for plasma and binding buffer has to be ensured.* |
| **3** | **Mix sample**  
Invert the tube 3 x and vortex for 3 s. Centrifuge the tube briefly to clean the lid.  
**Optional:**  
+ 20 μL Proteinase K  
+ 360 μL BB  
Mix sample |
### 4 Bind DNA

For each sample, load the mixture (620 μL) to a **NucleoSpin® Plasma XS Column** placed in a Collection Tube (2 mL).

**Centrifuge at 2,000 x g for 30 s**, increase centrifuge force to **11,000 x g** for further 5 s. Discard Collection Tube with flow-through and place column into new Collection Tube (provided).

*The maximal column volume is approximately 600 μL. Do not apply a higher volume in order to avoid spillage. If larger plasma sample volumes have to be processed, the loading step may be repeated. Be aware of an increased risk of membrane clogging in case of multiple column loading steps. If the solution has not completely passed the column, centrifuge for an additional 60 s at 11,000 x g.*

### 5 Wash and dry silica membrane

**1st wash**

Pipette 500 μL **Buffer WB** onto the NucleoSpin® Plasma XS Column. **Centrifuge for 30 s at 11,000 x g.** Discard Collection Tube with flow-through and place the column into new Collection Tube (provided).

**2nd wash**

Add 250 μL **Buffer WB** to the NucleoSpin® Plasma XS Column. **Centrifuge for 3 min at 11,000 x g.** Discard Collection Tube with flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not provided).

### 6 Elute DNA

Add 20 μL **Elution Buffer** to the NucleoSpin® Plasma XS Column. **Centrifuge for 30 s at 11,000 x g.**

*Elution volume may be varied in range of 5–30 μL. For a correlation of elution volume, DNA concentration, and DNA amount eluted from the column see section 2.4.*

### 7 Removal of residual ethanol

Incubate elution fraction with open lid for 8 min at 90 °C.

*See section 2.5 for further comments and alternative incubation times and temperatures for a removal of residual ethanol.*
5.2 **Rapid protocol for the isolation of DNA from plasma**

The rapid procedure represents a good compromise between DNA yield and concentration as well as ease and speed of nucleic acid extraction.

1 **Prepare sample**

Add 200 μL plasma to a microcentrifuge tube (not provided).

Less than 240 μL may be used. Adopt the binding buffer volume accordingly (see below).

2 **Adjust DNA binding conditions**

Add 300 μL Buffer BB.

If less than 200 μL plasma is used, adjust the binding buffer volume accordingly. A ratio of 1:1.5 (v/v) for plasma and binding buffer has to be ensured.

3 **Mix sample**

Invert the tube 3 x and vortex for 3 s. Centrifuge the tube briefly to clean the lid.

4 **Bind DNA**

For each sample, load the mixture (500 μL) to a NucleoSpin® Plasma XS Column placed in a Collection Tube (2 mL).

Centrifuge at 11,000 x g for 30 s. Discard Collection Tube with flow-through and place column into new Collection Tube (provided).

The maximal column volume is approximately 600 μL. Do not apply a higher volume in order to avoid spillage. If larger plasma sample volumes have to be processed, the loading step may be repeated. Be aware of an increased risk of membrane clogging in case of multiple column loading steps. If the solution has not completely passed the column, centrifuge for an additional 60 s at 11,000 x g.
5 Wash and dry silica membrane

**1st wash**

Pipette **500 μL Buffer WB** onto the NucleoSpin® Plasma XS Column. Centrifuge for **30 s** at **11,000 x g**. Discard Collection Tube with flow-through and place the column into new Collection Tube (provided).

**2nd wash**

Add **250 μL Buffer WB** to the NucleoSpin® Plasma XS Column. Centrifuge for **3 min** at **11,000 x g**. Discard Collection Tube with flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not provided).

6 Elute DNA

Add **20 μL Elution Buffer** to the NucleoSpin® Plasma XS Column. Centrifuge for **30 s** at **11,000 x g**. Elution volume may be varied in range of 5–30 μL. For a correlation of elution volume, DNA concentration, and DNA amount eluted from the column see section 2.4.
## 6 Appendix

### 6.1 Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause and suggestions</th>
</tr>
</thead>
</table>
| Low DNA yield | **Low DNA content of the sample**  
  - The content of cell-free DNA in human plasma may vary over several orders of magnitude. DNA contents from approximately 0.1–100 ng DNA per mL of plasma have been reported (see remarks in section 2.2).  
  - If the DNA concentration is measured with double strand specific dyes, e.g., PicoGreen, and step 7 “Removal of residual ethanol” with incubation at 90 °C is performed, the measured yield is below the actual value. This is due to the denaturation of DNA during the heat incubation step and the double strand specificity of certain DNA dyes, e.g., PicoGreen. |
| Column clogging | **Sample contains residual cell debris or cells**  
  - The plasma sample may have contained residual cells or cell debris. Make sure to use only clear plasma samples (see remarks in section 2.3). |
| No increase of PCR signal despite of an increased volume of eluate used as template in PCR | **Residual ethanol in eluate**  
  - Please see the detailed description of removal of residual traces of ethanol in section 2.5. |
| Discrepancy between A\textsubscript{260} quantification values and PCR quantification values | **Silica abrasion from the membrane**  
  - Due to the typically low DNA content in plasma and the resulting low total amount of isolated DNA, a DNA quantification via A\textsubscript{260} absorption measurement is often hampered due to the low sensitivity of the absorption measurement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect A\textsubscript{260} quantification of small DNA amounts, centrifuge the eluate for 30 s at > 11,000 x g and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen® fluorescent dye). |
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**Measurement not in the range of photometer detection limit**

- In order to obtain a significant $A_{260}/A_{280}$ ratio, it is necessary that the initially measured $A_{260}$ and $A_{280}$ values are significantly above the detection limit of the photometer used. An $A_{280}$ value close to the background noise of the photometer will cause unexpected $A_{260}/A_{280}$ ratios.

### 6.2 Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>REF</th>
<th>Pack of</th>
</tr>
</thead>
<tbody>
<tr>
<td>NucleoSpin® Plasma XS</td>
<td>740900.10/.50/.250</td>
<td>10/50/250</td>
</tr>
<tr>
<td>Buffer BB</td>
<td>740394.22</td>
<td>22 mL</td>
</tr>
<tr>
<td>Buffer WB</td>
<td>740331</td>
<td>250 mL</td>
</tr>
<tr>
<td>Collection Tubes (2 mL)</td>
<td>740600</td>
<td>1000</td>
</tr>
</tbody>
</table>

### 6.3 References


**Chan** KC, Lo YM: Clinical applications of plasma Epstein-Barr virus DNA analysis and protocols for the quantitative analysis of the size of circulating Epstein-Barr virus DNA. Methods Mol Biol. 2006;336:111-21.


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6.4 Product use restriction / warranty

NucleoSpin® Plasma XS kit 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 SUITABLE 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).
Circulating DNA from plasma

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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