# Circulating DNA from plasma

## User manual

NucleoSpin® Plasma XS

November 2012 / Rev. 03

MACHEREY-NAGEL

# Circulating DNA from plasma

## Protocol-at-a-glance (Rev. 03)

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

**MACHEREY-NAGEL GmbH & Co. KG** · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-270 · Fax: +49 24 21 969-199 · tech-bio@mn-net.com · ***www.mn-net.com***

# Table of contents

# 1 Components

## 1.1 Kit contents

| | NucleoSpin® Plasma XS | | |
|---|---|---|---|
| | **10 preps** | **50 preps** | **250 preps** |
| **REF** | **740900.10** | **740900.50** | **740900.250** |
| Binding Buffer BB | 4.5 mL | 22 mL | 110 mL |
| Wash Buffer WB | 10 mL | 2 x 25 mL | 250 mL |
| Elution Buffer* | 15 mL | 15 mL | 15 mL |
| Proteinase K (lyophilized)** | 6 mg | 30 mg | 2 x 75 mg |
| Proteinase Buffer PB | 0.8 mL | 1.8 mL | 8 mL |
| NucleoSpin® Plasma XS Columns (red rings – plus Collection Tubes) | 10 | 50 | 250 |
| Collection Tubes (2 mL) | 20 | 100 | 500 |
| User manual | 1 | 1 | 1 |

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

** For preparation of working solutions and storage condistions 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***.

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.
- 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.

| Table 1: Kit specifications at a glance | |
|---|---|
| **Parameter** | **NucleoSpin® Plasma XS** |
| Sample material | Up to 240 µL EDTA plasma (single column loading) |
| Average yield | Typically in a range of 0.1–100 ng per mL plasma, depending on sample<br>(depending on kind of patient samples, yield can be much higher). |
| Elution volume | 5–30 µL |
| Preparation time | High sensitivity procedure: 22–27 min/6 preps<br>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 per 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.

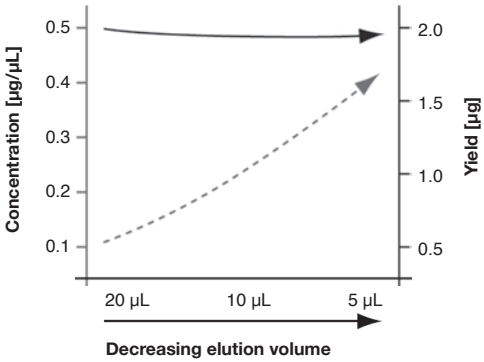



**Figure 1: Correlation between elution volume and DNA concentration (NucleoSpin® Plasma XS Columns)**

## 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 LightCycler™ PCR (Roche) with DyNAmo™ Capillary SYBR® Green qPCR Kit (Finnzymes).

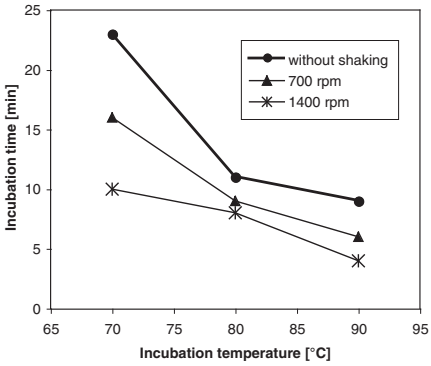



**Figure 2: Removal of residual ethanol from the elution fraction by heat treatment.**

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.

| | **NucleoSpin® Plasma XS** | | |
|---|---|---|---|
| | **10 preps** | **50 preps** | **250 preps** |
| **REF** | **740900.10** | **740900.50** | **740900.250** |
| Proteinase K (lyophilized) | 6 mg<br>Add 260 µL Proteinase Buffer | 30 mg<br>Add 1.35 mL Proteinase Buffer | 2 x 75 mg<br>Add 3.35 mL Proteinase Buffer to each vial |

# 4 Safety instructions – risk and safety phrases

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.*

## 4.1 Risk and safety phrases

| Component<br>*Inhalt* | Hazard contents<br>*Gefahrstoff* | Hazard symbol<br>*Gefahrstoff-symbol* | Risk phrases<br>*R-Sätze* | Safety phrases<br>*S-Sätze* |
|---|---|---|---|---|
| BB | Guanidinium thiocyanate 30–60 % + ethanol 35–55 %<br>*Guanidiniumthiocyanat 30–60 % + Ethanol 35–55 %* | Xn* | R 10-20/21/22-32-52/53 | S 13-16-61 |
| WB | Ethanol 55–75 %<br>*Ethanol 55–75 %* | F** | R 11 | S 7-16 |
| Proteinase K | Proteinase K, lyophilized<br>*Proteinase K, lyophilisiert* | Xn | R 36/37/38-42 | S 22-24-26-36/37 |

**Risk phrases**

| | |
|---|---|
| R 10 | Flammable.<br>*Entzündlich* |
| R 11 | Highly flammable.<br>*Leichtentzündlich.* |
| R 20/21/22 | Harmful if swallowed.<br>*Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.* |
| R 32 | Contact with acids liberates very toxic gas.<br>*Entwickelt bei Berührung mit Säure sehr giftige Gase.* |
| R 36/37/38 | Irritating to eyes, respiratory system, and skin.<br>*Reizt die Augen, Atmungsorgane und die Haut.* |
| R 42 | May cause sensitization by inhalation<br>*Sensibilisierung durch Einatmen möglich.* |
| R 52/53 | Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.<br>*Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.* |

* Hazard labeling not neccessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not neccessary if quantity per bottle below 25 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

**Safety phrases**

| | |
|---|---|
| S 7 | Keep container tightly closed.<br>*Behälter dicht geschlossen halten.* |
| S 13 | Keep away from food, drink and animal feedstuffs.<br>*Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.* |
| S 16 | Keep away from sources of ignition – No smoking!<br>*Keep away from sources of ignition – No smoking.* |
| S 22 | Do not breathe dust.<br>*Staub nicht einatmen.* |
| S 24 | Avoid contact with the skin.<br>*Berührung mit der Haut vermeiden.* |
| S 26 | In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.<br>*Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren.* |
| S 36/37 | Wear suitable protective clothing and gloves.<br>*Bei der Arbeit geeignete Schutzhandschuhe und Schutzkleidung tragen.* |
| S 61 | Avoid release to the environment. Refer to special instructions safety data sheets.<br>*Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen/ Sicherheitsdatenblatt zu Rate ziehen.* |

## 4.2 GHS classification

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

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

| **Component**<br>***Inhalt*** | **Hazard contents**<br>***Gefahrstoff*** | **GHS symbol**<br>***GHS Symbol*** | **Hazard phrases**<br>***H-Sätze*** | **Precaution phrases**<br>***P-Sätze*** |
|---|---|---|---|---|
| BB | Guanidinium thiocyanate 30–60 % + ethanol 35–55 %<br>*Guanidiniumthiocyanat 30–60 % + Ethanol 35–55 %* |  Warning<br>*Achtung* | 226, 302, 412, EUH031 | 210, 233, 260D, 273, 301+312, 330, 403+235 |
| WB | Ethanol 55–75 %<br>*Ethanol 55–75 %* |  Warning<br>*Achtung* | 225 | 210, 233, 403+235 |
| Proteinase K | Proteinase K, lyophilized<br>*Proteinase K, lyophilisiert* |  Danger<br>*Gefahr* | 315, 317, 319, 334, 335 | 261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 363, 403+233 |

### Hazard phrases

| | |
|---|---|
| H 225 | Highly flammable liquid and vapour.<br>*Flüssigkeit und Dampf leicht entzündbar.* |
| H 226 | Flammable liquid and vapour.<br>*Flüssigkeit und Dampf entzündbar.* |
| H 302 | Harmful if swallowed.<br>*Gesundheitsschädlich bei Verschlucken.* |
| H 315 | Causes skin irritation.<br>*Verursacht Hautreizungen.* |
| H 317 | May cause an allergic skin reaction.<br>*Kann allergische Hautreaktionen verursachen.* |
| H 319 | Causes serious eye irritation.<br>*Verursacht schwere Augenreizung.* |
| H 334 | May cause allergy or asthma symptoms or breathing difficulties if inhaled.<br>*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.* |
| H 335 | May cause respiratory irritation.<br>*Kann die Atemwege reizen.* |
| H 412 | Harmful to aquatic life with long lasting effects.<br>*Schädlich für Wasserorganismen, mit langfristiger Wirkung.* |
| EUH031 | Contact with acids liberates toxic gas.<br>*Entwickelt bei Berührung mit Säure giftige Gase.* |

### Precaution phrases

| | |
|---|---|
| P 210 | Keep away from heat / sparks /open flames / hot surfaces – No smoking.<br>*Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten. Nicht rauchen.* |
| P 233 | Keep container tightly closed.<br>*Behälter dicht verschlossen halten.* |
| P 260D | Do not breathe vapours.<br>*Dampf nichht einatmen.* |
| P 261 | Avoid breathing dust.<br>*Einatmen von Staub vermeiden.* |
| P 273 | Avoid release to the environment.<br>*Freisetzung in die Umwelt vermeiden.* |
| P 280 | Wear protective gloves / eye protection.<br>*Schutzhandschuhe / Augenschutz tragen.* |
| P 301+312 | IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell.<br>*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.* |
| P 302+352 | IF ON SKIN: Wash with plenty of soap and water.<br>*BEI KONTAKT MIT DER HAUT: Mit viel Wasser und Seife waschen.* |
| P 304+340 | IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing.<br>*BEI EIANTMEN: Bei Atembeschwerden 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.<br>*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 or doctor / physician if you feel unwell.<br>*Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.* |

**Precaution phrases**

| | |
|---|---|
| P 330 | Rinse mouth.<br>*Mund ausspülen.* |
| P 332+313 | If skin irritation occurs: Get medical advice / attention.<br>*Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.* |
| P 337+313 | Get medical advice / attention.<br>*Bei anhaltende Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.* |
| P 342+311 | If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.<br>*Bei Symptomen der Atemwege: Giftinformationszentrum oder Arzt anrufen.* |
| P 363 | Wash contaminated clothing before reuse.<br>*Kontaminierte Kleidung vor erneutem Tragen waschen.* |
| P 403+233 | Store in a well ventilated place. Keep container tightly closed.<br>*Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.* |
| P 403+235 | Store in a well ventilated place. Keep cool.<br>*Kühl an einem gut belüfteten Ort aufbewahren.* |

# 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

**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).*

**240 µL plasma**

***1 a Optional: Proteinase K treatment***

*Add **20 µL Proteinase K t**o 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.*

***Optional: + 20 µL Proteinase K***

**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.*

**+ 360 µL BB**

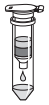
**3 Mix sample**

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

**Mix sample**

**4 Bind DNA**

For each sample, load the mixture **(600 µL)** to a **NucleoSpin® Plasma XS Column** placed in a Collection Tube (2 mL).



**Load lysate**

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).

**2,000 x *g*, 30 s**

**11,000 x *g*, 5 s**

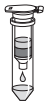
*The maximal column volume is approximately 600 µL. Do not apply a higher volume in order to avoid spillage. If larger plasma sample volumes have to be processed, the loading step may be repeated. Be aware of an increased risk of membrane clogging in case of multiple column loading steps. If the solution 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**

**+ 500 µL WB**

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).



**11,000 x *g*, 30 s**

**2nd wash**

**+ 250 µL WB**

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).

**11,000 x *g*, 3 min**

**6 Elute DNA**

**+ 20 µL Elution Buffer**

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

**11,000 x *g*, 30 s**

*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**

**90 °C, 8 min**

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).*

**200 µL plasma**

**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.*

**+ 300 µL BB**

**3 Mix sample**

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

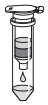
**Mix sample**

**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.



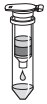
**Load lysate**

**11,000 x *g*, 30 s**

## 5 Wash and dry silica membrane

**1st wash** — **+ 500 µL WB**

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).



**11,000 x *g*, 30 s**

**2nd wash** — **+ 250 µL WB**

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).

**11,000 x *g*, 3 min**

## 6 Elute DNA

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

**+ 20 µL Elution Buffer**

*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.*

**11,000 x *g*, 30 s**

# 6 Appendix

## 6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Low DNA yield | *Low DNA content of the sample*<br>• The content of cell-free DNA in human plasma may vary over several orders of magnitude. DNA contents from approximately 0.1–1000 ng DNA per mL of plasma have been reported (see remarks in section 2.2). |
| Column clogging | *Sample contains residual cell debris or cells*<br>• 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*<br>• Please see the detailed description of removal of residual traces of ethanol in section 2.5. |
| Discrepancy between $A_{260}$ quantification values and PCR quantification values | *Silica abrasion from the membrane*<br>• Due to the typically low DNA content in plasma and the resulting low total amount of isolated DNA, a DNA quantification via $A_{260}$ 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 $A_{260}$-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® fluorecent dye). |

| | |
|---|---|
| Unexpected $A_{260}/A_{280}$ ratio | *Measurement not in the range of photometer detection limit*<br>• 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 unexpeced $A_{260}/A_{280}$ ratios. |

## 6.2 Ordering information

| Product | REF | Pack of |
|---|---|---|
| NucleoSpin® Plasma XS | 740900.10/.50/.250 | 10/50/250 |
| Buffer BB | 740394.22 | 22 mL |
| Buffer BW | 740922 / .500 | 100 / 500 mL |
| Collection Tubes (2 mL) | 740600 | 1000 |

## 6.3 References

**Birch** L, English CA, O'Donoghue K, Barigye O, Fisk NM, Keer JT: Accurate and robust quantification of circulating fetal and total DNA in maternal plasma from 5 to 41 weeks of gestation. Clin Chem. 2005 Feb;51(2):312-20. Epub 2004 Dec 17.

**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.

**Chan** KC, Yeung SW, Lui WB, Rainer TH, Lo YM: Effects of preanalytical factors on the molecular size of cell-free DNA in blood. Clin Chem. 2005 Apr;51(4):781-4. Epub 2005 Feb 11.

**Chan** KC, Zhang J, Chan AT, Lei KI, Leung SF, Chan LY, Chow KC, Lo YM: Molecular characterization of circulating EBV DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients. Cancer Res. 2003 May 1;63(9):2028-32.

**Chan** KC, Zhang J, Hui AB, Wong N, Lau TK, Leung TN, Lo KW, Huang DW, Lo YM: Size distributions of maternal and fetal DNA in maternal plasma. Clin Chem. 2004 Jan;50(1):88-92.

**Chiu** RW, Lo YM: Noninvasive prenatal diagnosis by analysis of fetal DNA in maternal plasma. Methods Mol Biol. 2006;336:101-9.

**Chiu** TW, Young R, Chan LY, Burd A, Lo DY: Plasma cell-free DNA as an indicator of severity of injury in burn patients. Clin Chem Lab Med. 2006;44(1):13-7.

**Chun** FK, Muller I, Lange I, Friedrich MG, Erbersdobler A, Karakiewicz PI, Graefen M, Pantel K, Huland H, Schwarzenbach H: Circulating tumour-associated plasma DNA represents an independent and informative predictor of prostate cancer. BJU Int. 2006 Sep;98(3):544-8.

**Deligezer** U, Erten N, Akisik EE, Dalay N: Circulating fragmented nucleosomal DNA and caspase-3 mRNA in patients with lymphoma and myeloma. Exp Mol Pathol. 2006 Feb;80(1):72-6. Epub 2005 Jun 15.

**Fatouros** IG, Destouni A, Margonis K, Jamurtas AZ, Vrettou C, Kouretas D, Mastorakos G, Mitrakou A, Taxildaris K, Kanavakis E, Papassotiriou I: Cell-free plasma DNA as a novel marker of aseptic inflammation severity related to exercise overtraining. Clin Chem. 2006 Sep;52(9):1820-4. Epub 2006 Jul 13.

**Giacona** MB, Ruben GC, Iczkowski KA, Roos TB, Porter DM, Sorenson GD: Cell-free DNA in human blood plasma: length measurements in patients with pancreatic cancer and healthy controls. Pancreas. 1998 Jul;17(1):89-97.

**Hanley** R, Rieger-Christ KM, Canes D, Emara NR, Shuber AP, Boynton KA, Libertino JA, Summerhayes IC: DNA integrity assay: a plasma-based screening tool for the detection of prostate cancer. Clin Cancer Res. 2006 Aug 1;12(15):4569-74.

**Hromadnikova** I, Zejskova L, Doucha J, Codl D: Quantification of fetal and total circulatory DNA in maternal plasma samples before and after size fractionation by agarose gel electrophoresis. DNA Cell Biol. 2006 Nov;25(11):635-40.

**Jiang** WW, Zahurak M, Goldenberg D, Milman Y, Park HL, Westra WH, Koch W, Sidransky D, Califano J: Increased plasma DNA integrity index in head and neck cancer patients. Int J Cancer. 2006 Dec 1;119(11):2673-6.

**Jung** M, Klotzek S, Lewandowski M, Fleischhacker M, Jung K: Changes in concentration of DNA in serum and plasma during storage of blood samples. Clin Chem. 2003 Jun;49(6 Pt 1):1028-9.

**Koide** K, Sekizawa A, Iwasaki M, Matsuoka R, Honma S, Farina A, Saito H, Okai T: Fragmentation of cell-free fetal DNA in plasma and urine of pregnant women. Prenat Diagn. 2005 Jul;25(7):604-7.

**Lam** NY, Rainer TH, Chiu RW, Lo YM: EDTA is a better anticoagulant than heparin or citrate for delayed blood processing for plasma DNA analysis. Clin Chem. 2004 Jan;50(1):256-7.

**Lazar** L, Nagy B, Ban Z, Nagy GR, Papp Z: Presence of cell-free fetal DNA in plasma of women with ectopic pregnancies. Clin Chem. 2006 Aug;52(8):1599-601. Epub 2006 Jun 1.

**Li** Y, Di Naro E, Vitucci A, Zimmermann B, Holzgreve W, Hahn S: Detection of paternally inherited fetal point mutations for beta-thalassemia using size-fractionated cell-free

DNA in maternal plasma. JAMA. 2005 Feb 16;293(7):843-9. Erratum in: JAMA. 2005 Apr 13;293(14):1728.

**Li** Y, Holzgreve W, DI Naro E, Vitucci A, Hahn S: Cell-free DNA in maternal plasma: is it all a question of size? Ann N Y Acad Sci. 2006 Sep;1075:81-7.

**Li** Y, Holzgreve W, Page-Christiaens GC, Gille JJ, Hahn S: Improved prenatal detection of a fetal point mutation for achondroplasia by the use of size-fractionated circulatory DNA in maternal plasma--case report. Prenat Diagn. 2004 Nov;24(11):896-8.

**Li** Y, Wenzel F, Holzgreve W, Hahn S: Genotyping fetal paternally inherited SNPs by MALDI-TOF MS using cell-free fetal DNA in maternal plasma: influence of size fractionation. Electrophoresis. 2006 Oct;27(19):3889-96.

**Li** Y, Zimmermann B, Rusterholz C, Kang A, Holzgreve W, Hahn S: Size separation of circulatory DNA in maternal plasma permits ready detection of fetal DNA polymorphisms. Clin Chem. 2004 Jun;50(6):1002-11. Epub 2004 Apr 8.

**Page** K, Powles T, Slade MJ, DE Bella MT, Walker RA, Coombes RC, Shaw JA: The Importance of Careful Blood Processing in Isolation of Cell-Free DNA. Ann N Y Acad Sci. 2006 Sep; 1075:313-317.

**Rainer** TH, Lam NY, Man CY, Chiu RW, Woo KS, Lo YM: Plasma beta-globin DNA as a prognostic marker in chest pain patients. Clin Chim Acta. 2006 Jun;368(1-2):110-3. Epub 2006 Feb 14.

**Rhodes** A, Wort SJ, Thomas H, Collinson P, Bennett ED: Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. Crit Care. 2006;10(2): R60.

**Schmidt** B, Weickmann S, Witt C, Fleischhacker M: Improved method for isolating cell-free DNA. Clin Chem. 2005 Aug;51(8):1561-3.

**Sozzi** G, Roz L, Conte D, Mariani L, Andriani F, Verderio P, Pastorino U: Effects of prolonged storage of whole plasma or isolated plasma DNA on the results of circulating DNA quantification assays. J Natl Cancer Inst. 2005 Dec 21;97(24):1848-50.

**Wang** M, Block TM, Steel L, Brenner DE, Su YH: Preferential isolation of fragmented DNA enhances the detection of circulating mutated k-ras DNA. Clin Chem. 2004 Jan;50(1):211-3.

## 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 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.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR

USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

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.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
e-mail: tech-bio@mn-net.com