



Circulating DNA from plasma

User manual

NucleoSpin[®] cfDNA XS

October 2019 / Rev. 07

Circulating DNA from plasma

Protocol at a glance (Rev.07)

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

Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Consumables and equipment to be supplied by user	5
1.3	About this user manual	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Handling of sample material	8
2.4	Elution procedures	8
2.5	Removal of residual traces of ethanol for highest PCR sensitivity	9
2.6	Stability of isolated DNA	10
3	Storage conditions and preparation of working solutions	11
4	Safety instructions	12
5	Protocols	14
5.1	High sensitivity protocol for the isolation of cfDNA	14
5.2	Rapid protocol for the isolation of cfDNA	16
6	Appendix	18
6.1	Troubleshooting	18
6.2	Ordering information	19
6.3	References	19
6.4	Product use restriction / warranty	21

1 Components

1.1 Kit contents

NucleoSpin® cfDNA XS			
REF	10 preps 740900.10	50 preps 740900.50	250 preps 740900.250
Binding Buffer BB	5 mL	22 mL	110 mL
Wash Buffer WB	10 mL	50 mL	250 mL
Elution Buffer*	13 mL	13 mL	13 mL
Proteinase K (lyophilized)**	6 mg	30 mg	2 x 75 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
NucleoSpin® cfDNA XS Columns (red rings – incl. 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 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® cfDNA 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® cfDNA XS** kit is designed for the efficient isolation of circulating DNA from human blood plasma or other types of cell-free fluids. Fragmented DNA as small as 50–1000 bp can be purified with high efficiency. The special funnel design of the **NucleoSpin® cfDNA XS Columns** allows very small elution volumes (5–30 µL) resulting in highly concentrated DNA.

The protocol follows state-of-the-art bind-wash-elute procedures: After mixing of the sample with the binding buffer, the mixture is applied to the **NucleoSpin® cfDNA 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 elution buffer (5 mM Tris-HCl, pH 8.5).

2.2 Kit specifications

- The **NucleoSpin® cfDNA XS** kit is recommended for the isolation of fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage. It has been successfully used for other cell-free fluids including urine (Outinen *et al.* 2012) and follicular fluid (Kansaku *et al.* 2018).
- The **NucleoSpin® cfDNA XS** kit is designed for high recovery, especially of fragmented DNA in a range of 50–1000 bp.
- Up to 240 µL sample can be used with a single column loading step. DNA yield strongly depends on the individual sample. For plasma it is typically in the range of 0.1 ng up to several 100 ng DNA per mL sample. Up to 720 µL sample can be used with three column loadings. If more than 240 µL sample 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 samples.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® cfDNA XS
Sample material	EDTA plasma, serum, bronchial lavage, urine, other cell-free fluids
Sample volume	standard volume: 240 µL (one column loading step) max. volume: 720 µL (three column loading steps)
Average yield	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).
Elution volume	5–30 µL
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® cfDNA 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.

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

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 $\geq 11,000 \times g$ in order to remove residual cells, cell debris, and particulate matter. Use the supernatant for DNA isolation.

Preparation of other cell-free liquid samples (e.g., urine)

Clarify the sample by centrifugation (e.g., 5 min at 4,500 x *g*) in order to pellet cells or other solid particles suspended in the sample. Use only the supernatant for cfDNA 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.

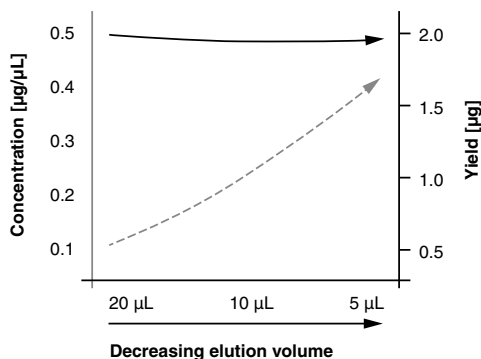


Figure 1 Correlation between elution volume and DNA concentration (NucleoSpin® cfDNA XS Columns)

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).

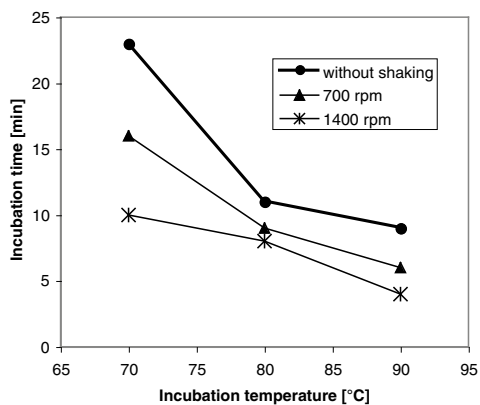


Figure 2 Removal of residual ethanol from the elution fraction by heat treatment.

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 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 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® cfDNA 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® cfDNA XS			
	10 preps	50 preps	250 preps
REF	740900.10	740900.50	740900.250
Proteinase K (lyophilized)	6 mg Add 260 µL Proteinase Buffer	30 mg Add 1.35 mL Proteinase Buffer	2 x 75 mg Add 3.35 mL Proteinase Buffer to each vial

4 Safety instructions

The following components of the **NucleoSpin® cfDNA XS** kits contain hazardous contents.

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

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
BB	Guanidinium thiocyanate 30–45 % + ethanol 35–55 % <i>Guanidiniumthiocyanat 30–45 % + Ethanol 35–55 %</i> CAS 593-84-0, 64-17-5	 WARNING ACHTUNG	226, 302, 412	210, 264W, 273, 301+312, 330
WB	Ethanol 55–75 % <i>Ethanol 55–75 %</i> CAS 64-17-5	 DANGER GEFAHR	225	210, 233
Proteinase K	Proteinase K, lyophilized 90–100 % <i>Proteinase K, lyophilisiert 90–100 %</i> CAS 39450-01-6	 DANGER GEFAHR	315, 319, 334	261sh, 280sh, 342+311



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 vapor. <i>Flüssigkeit und Dampf leicht entzündbar.</i>
H 226	Flammable liquid and vapor. <i>Flüssigkeit und Dampf entzündbar.</i>
H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 315	Causes skin irritation. <i>Verursacht Hautreizungen.</i>
H 319	Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i>
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. <i>Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.</i>
H 412	Harmful to aquatic life with long lasting effects. <i>Schädlich für Wasserorganismen, mit langfristiger Wirkung.</i>

Precaution phrases

P 210	Keep away from heat/sparks/open flames/hot surfaces. No smoking. <i>Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.</i>
P 233	Keep container tightly closed. <i>Behälter dicht verschlossen halten.</i>
P 264W	Wash with water thoroughly after handling <i>Nach Gebrauch mit Wasser gründlich waschen.</i>
P 261sh	Avoid breathing dust / vapors. <i>Einatmen von Staub / Dampf vermeiden.</i>
P 273	Avoid release to the environment. <i>Freisetzung in die Umwelt vermeiden.</i>
P 280sh	Wear protective gloves / eye protection. <i>Schutzhandschuhe / Augenschutz tragen.</i>
P 301+312	IF SWALLOWED: Call a POISON CENTER / doctor / ... / if you feel unwell. <i>BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.</i>
P 330	Rinse mouth. <i>Mund ausspülen.</i>
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / ... <i>Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.</i>

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 particulate matter (e.g., by centrifugation of the plasma sample for 3 min at $\geq 11,000 \times g$).

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 cfDNA

1 Prepare sample

Add **240 µL plasma or alternative cell-free fluid** to a microcentrifuge tube (not provided).

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



**240 µL
sample**

1a *Optional: Proteinase K treatment*

Add **20 µL Proteinase K** to the sample, mix, and incubate at 37 °C for 10 min.

Depending on the sample and the PCR conditions, the proteinase treatment provokes an 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 sample is used, adjust the binding buffer volume accordingly. A ratio of 1:1.5 (v/v) for sample 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 (**620 µL**) to a **NucleoSpin® cfDNA 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 flowthrough and place column into new Collection Tube (provided).



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

The maximal column volume is approximately 600 µL. Do not apply a higher volume in order to avoid spillage. If larger 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.



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

5 Wash and dry silica membrane

1st wash

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



**+ 500 µL
WB**

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

2nd wash

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



**+ 250 µL
WB**

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

6 Elute DNA

Add **20 µL Elution Buffer** to the NucleoSpin® cfDNA 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**

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.



**90 °C,
8 min**

5.2 Rapid protocol for the isolation of cfDNA

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 or alternative cell-free fluid** to a microcentrifuge tube (not provided).

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



**200 µL
sample**

2 Adjust DNA binding conditions

Add **300 µL Buffer BB**.

If less than 200 µL sample is used, adjust the binding buffer volume accordingly. A ratio of 1:1.5 (v/v) for sample 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® cfDNA XS Column** placed in a Collection Tube (2 mL).

Centrifuge at **11,000 x g** for **30 s**. Discard Collection Tube with flowthrough 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 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

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



**+ 500 µL
WB**
**11,000 x g,
30 s**

2nd wash

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



**+ 250 µL
WB**
**11,000 x g,
3 min**

6 Elute DNA

Add **20 µL Elution Buffer** to the NucleoSpin® cfDNA 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	<i>Low DNA content of the sample</i>
	- The content of cell-free DNA in human plasma and other cell-free bodily fluids 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	<i>Sample contains residual cell debris or cells</i>
	The sample may have contained residual cells or cell debris. Make sure to use only cleared samples (see remarks in section 2.3).
No increase of PCR signal despite of an increased volume of eluate used as template in PCR	<i>Residual ethanol in eluate</i>
	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	<i>Silica abrasion from the membrane</i>
	Due to the typically low DNA content in plasma (and other cell free fluids) 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 $> 11.000 \times g$ and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen® fluorescent dye).

*Measurement not in the range of photometer detection limit*Unexpected
 A_{260}/A_{280} ratio

- 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

Product	REF	Pack of
NucleoSpin® cfDNA XS	740900.10 / .50 / .250	10 / 50 / 250
NucleoSpin® DNA cfDNA Midi	740303.48	48
NucleoSpin® DNA cfDNA Midi Core Kit	740302.48	48
NucleoSnap® cfDNA	740300.10 / .50	10 / 50
NucleoSpin® 96 cfDNA	740873.1 / .4	1 x 96 / 4 x 96
NucleoSpin® 96 cfDNA Core Kit	740874.1 / .4	1 x 96 / 4 x 96
Buffer BB	740394.22	22 mL
Buffer WB	740331	250 mL
Collection Tubes (2 mL)	740600	1000

Note: This product has been formerly distributed under the name NucleoSpin® Plasma XS. The product code (REF) and kit content have not been changed.

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, Codel 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.
- Kansaku K, Munakata Y, Itami N, Shirasuna K, Kuwayama T, Iwata H:** Mitochondrial dysfunction in cumulus-oocyte complexes increases cell-free mitochondrial DNA. *J Reprod Dev.* 2018; 64: 261–266.
- 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.

Outinen TK, Kuparinen T, Jylhava J, Leppanen S, Mustonen J, Makela S, *et al.* . Plasma cell-free DNA levels are elevated in acute Puumala Hantavirus infection. *PLoS ONE*, 2012, 7:e31455.

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® cfDNA 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

Trademarks/Disclaimer:

DyNAmo is a trademark of Finnzymes Oy

LightCycler is a trademark of a member of the Roche Group

NucleoSpin is a trademark of MACHEREY-NAGEL GmbH & Co KG

PicoGreen is a registered trademark of Molecular Probes, Inc.

SYBR is a registered trademark of Molecular Probes, Inc.

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.



www.mn-net.com

MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany

DE/International:

Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:

Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:

Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:

Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com



A034262/1190.5