Genomic DNA from blood

User manual

NucleoSpin® Dx Blood

CE

IVD *In Vitro* Diagnostic Medical Device

REF 740899.50, 740899.250

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1 Components

1.1 Kit contents

		NucleoSpin® Dx Blood	
		50 preps	250 preps
REF		740899.50	740899.250
Buffer B3	BUF B3	13 mL	65 mL
Wash Buffer BW	BUF BW	30 mL	2 x 75 mL
Wash Buffer B5 (Concentrate)*	BUF B5 conc.	7 mL	2 x 20 mL
Elution Buffer BE**	BUF BE	13 mL	60 mL
Proteinase Buffer PB	BUF PB	1.8 mL	8 mL
Proteinase K (lyophilized)*	Proteinase K	30 mg	2 x 75 mg
NucleoSpin® Dx Blood Columns (red rings - plus Collection Tubes)	Dx Blood Columns	50	250
Collection Tubes (2 mL)	Collection Tubes	3 x 50	3 x 250
Lysis Tubes (1.5 mL)	Lysis Tubes	50	5 x 50
Elution Tubes (1.5 mL)	Elution Tubes	50	5 x 50
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* For preparation of working solutions and storage conditions see section 3.

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

			LOT	REF			IVD			
EN	Do not reuse	Use by	Batch code	Catalogue number	Contains sufficient for <n> tests	Manufacturer	In Vitro Diagnostic Medical Device	Consult Instructions for use	Temperature limitation	Irritant
DE	Nicht zur Wiederverwendung	Verwendbar bis	Chargenbezeichnung	Bestellnummer	Inhalt ausreichend für <n> Tests	Hersteller	In vitro-Diagnostikum	Gebrauchsanweisung beachten	Temperaturbegrenzung	Reizend
ES	No reutilizar	Fecha de caducidad	Código de lote	Número de catálogo	Contenido suficiente para <n> ensayos	Fabricante	Producto sanitario para diagnóstico in vitro	Consulte las instrucciones de uso	Límite de temperatura	Irritante
IT	Non riutilizzare	Utilizzare entro	Codice del lotto	Numero di catalogo	Contenuto sufficiente per “n” saggi	Fabbricante	Dispositivo medico-diagnostico in vitro	Consultare le istruzioni per l’uso	Limiti di temperatura	Irritant
FR	Ne pas réutiliser	Utiliser jusque	Code du lot	Référence du catalogue	Contenu suffisant pour “n” tests	Fabricant	Dispositif médical de diagnostic in vitro	Consulter les instructions d’utilisation	Limites de température	Irritante
NL	Niet opnieuw gebruiken	Houdbaar tot	Lot nummer	Catalogus nummer	Inhoud voldoende voor “n” testen	Fabrikant	Medisch hulpmiddel voor in vitro diagnostiek	Raadpleeg de gebruiksaanwijzing	Temperatuurlimiet	Irritierend
DA	Må ikke genbruges	Holdbar til	Lotnummer	Katalognummer	Indeholder tilstrækkeligt til „n“ test	Producent	Medicinsk udstyr til in vitro-diagnostik	Se brugsanvisning	Temperaturbegrænsning	Lokalirriterende
EL	Μην κάνετε επα-να-ληπτική χρήση	Ημερομηνία λήξης	Αριθμός Παρτίδας	Αριθμός καταλόγου	Περιεχόμενο επαρκές για «ν» εξετάσεις	Κατασκευαστής	In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν	Συμβουλευτείτε τις οδηγίες χρήσης	Περιορισμοί θερμοκρασίας	Διαβρωτικό
PT	Não reutilizar	Prazo de validade	Código do lote	Referência de catálogo	Conteúdo suficiente para “n” ensaios	Fabricante	Dispositivo médico para diagnóstico in vitro	Consulte as instruções de utilização	Limites de temperatura	Irritante

										
SV	Återan-vänd ej	Använd för	Lot num-mer	Katalog-nummer	Räcker till „n“ antal tester	Tillver-kare	Medicin-tekniska produk-ter för in vitro diagnos-tik	Se handha-vande-beskriv-ningen	Tempe-raturbe-gräns-ning	Irritan-derende

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to adjust DNA binding conditions and to prepare Wash Buffer B5)

Consumables

- Disposable pipet tips (aerosol barrier pipet tips are recommended to avoid cross-contamination)

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Thermal heating block or water bath (for samples lysis at 70 °C)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® Dx Blood** kit read the detailed protocol sections of this user manual. 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 Intended use

The **NucleoSpin® Dx Blood** kit is a generic system for the isolation and purification of genomic DNA from human whole blood samples for subsequent *in vitro* diagnostic purposes. The kit can be used with fresh and frozen human whole blood treated with EDTA, citrate, or heparin, from common blood collection systems.

The kit is designed to be used with any downstream application employing enzymatic amplification and detection of DNA (e.g., PCR). Any diagnostic results generated using the DNA isolated with the **NucleoSpin® Dx Blood** kit in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to additional clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications (e.g., extraction controls, positive / negative controls) should be used.

The **NucleoSpin® Dx Blood** kit is intended for use by professional users such as technicians and physicians experienced and trained in molecular biological techniques including experience with whole blood samples and DNA isolation.

The **NucleoSpin® Dx Blood** kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

2.2 Product use limitations

The **NucleoSpin® Dx Blood** kit is not for use with tissue or stool samples, cell-free body fluids such as plasma, serum, urine, or cerebrospinal fluid. The kit performance has not been evaluated with buffy coat, cultured or isolated cells, swabs, dried blood spots, and viral DNA. The kit is also not specified for the isolation and purification of bacterial, fungal, or parasite nucleic acids.

2.3 Quality control

In accordance with MACHEREY-NAGEL´s Quality Management System, each lot of **NucleoSpin® Dx Blood** kit is tested against predetermined specifications to ensure consistent product quality.

2.4 Introduction and kit specifications

NucleoSpin® Dx Blood is based on well-established NucleoSpin® silica-membrane technology and provides an easy way to isolate genomic DNA from 200 µL of whole blood samples. Purified DNA is ready-to-use for downstream PCR amplification.

The **NucleoSpin® Dx Blood** procedure is based on a series of simple steps:

First, the blood samples are lysed in the presence of chaotropic salts and Proteinase K. The genomic DNA in the lysate is then bound to a **NucleoSpin® Dx Blood Column**. Subsequently, the membrane with bound nucleic acids is washed and finally highly pure genomic DNA is eluted.

Samples

The kit can be used with 200 µL fresh or frozen human whole blood treated with EDTA, citrate, or heparin, from common blood collection systems. Cryoprecipitates formed during thawing of frozen samples may clog the **NucleoSpin® Dx Blood Column**. If such precipitates are visible avoid aspirating them when loading the lysate to the binding column.

Typically, 200 µL human whole blood will yield 3–5 µg genomic DNA, depending on the white blood cell count of the sample.

A selection of suitable blood collection devices is shown below:

Table 1: Selection of suitable blood collection systems

Blood collecting system	Manufacturer
S-Monovette® Li-Heparin	Sarstedt
S-Monovette® EDTA	Sarstedt
S-Monovette® Citrat	Sarstedt
VACUETTE® EDTA	GREINER BIO-ONE
BD VACUTAINER® K2E	BD
K3 EDTA	DELTA LAB
K2 EDTA	APTACA

Table 2: Kit specifications at a glance	
Parameter	**NucleoSpin® Dx Blood**
Sample material	Fresh and frozen human whole blood treated with EDTA, citrate, or heparin, from common blood collection systems
Sample volume	200 µL
Typical DNA yield	3–5 µg depending on white blood cell count
Typical DNA quality	Ratio A_{260}/A_{280} 1.7–1.9 Ratio A_{260}/A_{230} 1.8–2.3
Elution volume	50–200 µL
Typical DNA concentration	40–60 ng/µL
Processing	Centrifugation

2.5 Elution procedures

DNA is eluted from the **NucleoSpin® Dx Blood Columns** with 50 to 200 µL Elution Buffer BE. Overall DNA yield increases with increasing elution volume, whereas the DNA concentration decreases (see Figure 1).

Typically, up to 10 µL of the eluate can be used as template in a 50 µL PCR mix without affecting PCR performance. It is recommended storing eluted DNA at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

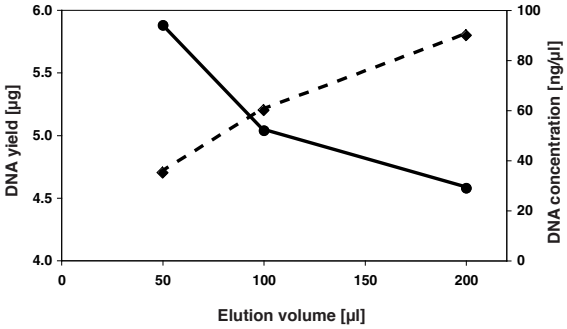

Figure 1: Impact of elution volume on overall DNA yield (dashed line) and concentration (solid line). Elution was performed with 50, 100, and 200 µL.

3 Storage conditions and preparation of working solutions

Attention:

- Check all components for damages after receiving the kit. If kit contents, like buffer bottles or blister packages are damaged, contact MACHEREY-NAGEL technical support and customer service, or your local distributor.
- Do not use damaged kit components.
- Upon arrival the **NucleoSpin® Dx Blood** kit should be stored at room temperature (18–25 °C). It is NOT required to open the kit on delivery and remove individual components for separate storage.
- **NucleoSpin® Dx Blood Columns** can be used until the expiration date specified on the kit box.

Before starting the **NucleoSpin® Dx Blood** protocol prepare the following:

- **Wash Buffer B5**: Add the indicated volume of ethanol (96–100 %, see table below or on the bottle) to **Wash Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer B5 at room temperature (18–25 °C) until the expiration date.
- Lyophilized **Proteinase K** can be stored at room temperature (18–25 °C) until the expiration date without decrease in performance. Before first use of the kit, add the indicated volume of **Proteinase Buffer PB** to dissolve lyophilized Proteinase K. Reconstituted Proteinase K should be stored at -20 °C for up to 6 months, but only until the expiration date.
- During storage, especially at low temperatures, a white precipitate may form in Buffer B3 and Buffer BW. Such precipitates can be easily dissolved by incubating the bottle at 70 °C for 5 min before use.

	NucleoSpin® Dx Blood	
	50 preps	**250 preps**
REF	**740899.50**	**740899.250**
Wash Buffer B5 (Concentrate)	7 mL Add 28 mL ethanol	2 x 20 mL Add 80 mL ethanol to each bottle
Proteinase K	30 mg Add 1.35 mL Proteinase Buffer	2 x 75 mg Add 3.35 mL Proteinase Buffer to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoBond®** kits contain hazardous contents.
Wear gloves and goggles and follow the safety instructions given in this section.

4.1 Risk and safety phrases

Component *Inhalt*	Hazard contents *Gefahrstoff*	Hazard symbol *Gefahrstoff-symbol*	Risk phrases *R-Sätze*	Safety phrases *S-Sätze*
B3	Guanidine hydrochloride *Guanidinhydrochlorid*	Xn*	22-36	26-39
BW	Guanidine hydrochloride *Guanidinhydrochlorid*	 Xn*	10-22-36	16-26-39
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert*	 Xn	36/37/38-42	22-26-36/37

Risk phrases

R 10 Flammable.
Entzündlich.

R 22 Harmful if swallowed.
Gesundheitsschädlich beim Verschlucken.

R 36 Irritating to eyes.
Reizt die Augen.

R 36/37/38 Irritating to eyes, respiratory system, and skin.
Reizt die Augen, Atmungsorgane und die Haut.

R 42 May cause sensitization by inhalation
Sensibilisierung durch Einatmen möglich.

Safety phrases

S 16 Keep, away from source of ignition – No smoking!
Von Zündquellen fernhalten.

S 22 Do not breathe dust.
Staub nicht einatmen.

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren.

* Hazard labeling not necessary 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.

S 36/37	Wear suitable protective clothing and gloves. *Bei der Arbeit geeignete Schutzhandschuhe und Schutzkleidung tragen.*
S 39	Wear eye / face protection. *Schutzbrille / Gesichtsschutz tragen.*

4.2 GHS classification

Only harmful features need not 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 *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
B3	Guanidine hydrochloride 36–50 % *Guanidinhydrochlorid 36–50 %*	 Warning *Achtung*	302-319	280-301+312-305+351+338-330-337+313
BW	Guanidine hydrochloride 36–50 % + isopropanol 20–50 % *Guanidinhydrochlorid 36–50 % + Isopropanol 20–50 %*	 Warning *Achtung*	226-302-319	210-233-280-301+312-305+351+338-330-337+313-403+235
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert*	 Danger *Gefahr*	334	261-304+341-342+311

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 319	Causes serious eye irritations. *Verursacht schwere Augenreizung.*
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. *Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*

Precaution phrases

P 210	Keep away from heat / sparks / open flames / hot surfaces. – No smoking. *Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten. – Nicht rauchen.*
P 233	Keep container tightly closed. *Behälter dicht verschlossen halten.*
P 261	Avoid breathing dust. *Einatmen von Staub vermeiden.*
P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*

P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell. *Bei Verschlucken: Bei Unwohlsein Giftinformationszentrum oder Arzt anrufen.*
P 304+341	IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing. *Bei Einatmen: Bei Atembeschwerden an die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
P 305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. *Bei Kontakt mit den Augen: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 337+313	If eye irritation persists: Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician. *Bei Symptomen der Atemwege: Giftinformationszentrum oder Arzt anrufen.*
P 403+235	Store in a well ventilated place. Keep cool. *An einem gut belüfteten Ort lagern. Kühl halten.*

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

When working with the **NucleoSpin® Dx Blood** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***http://www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in Lysis Buffer B3 and Wash Buffer BW can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® Dx Blood** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according to local safety regulations.

5 Genomic DNA purification with NucleoSpin® Dx Blood

The procedure below provides instructions for processing a single blood sample. However, several samples can be processed at the same time; the number depends on the capacity of the microcentrifuge used.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check that 96–100 % ethanol (denatured or non-denatured) is available to adjust DNA binding conditions.
- Set an incubator (e.g., heating block) or water bath to 70 °C.
- Equilibrate the blood samples to room temperature (18–25 °C). Make sure that the samples are mixed well.
- If a precipitate has formed in Lysis Buffer B3 or Buffer BW, incubate the buffer at 70 °C until the precipitate is dissolved.
- Generally, do not mix reagents and columns from different kits and lots.
- Equilibrate Elution Buffer BE to room temperature (18–25 °C).
- Do not add Proteinase K solution directly to Lysis Buffer B3. Proteinase K has to be mixed with the blood sample before addition of Buffer B3.
- All centrifugation steps should be carried out at room temperature (18–25 °C).

5.1 Protocol-at-a-glance

Supplemental protocol-overview:
Carefully read the detailed protocol (section 5.2) before starting the procedure.

Lyse blood samples	**1**	25 µL Proteinase K	
	2	200 µL blood	
	3	200 µL B3, mix	
	4	RT, 5 min	
	5	7 °C, 10 min, mix	
	6	2,000 x *g*, 1 s	
Adjust DNA binding conditions	**7**	210 µL ethanol, mix	
	8	2,000 x *g*, 1 s	
Bind DNA	**9**	Load lysate	
	10	11,000 x *g*, 1 min	
	11	Transfer the NucleoSpin® Dx Blood Column to a new Collection Tube	
Wash silica membrane	**12**	500 µL BW	
	13	11,000 x *g*, 1 min	
	14	Transfer the NucleoSpin® Dx Blood Column to a new Collection Tube	
	15	600 µL B5	
	16	11,000 x *g*, 1 min	
Dry silica membrane	**17**	Transfer the NucleoSpin® Dx Blood Column to a new Collection Tube	
	18	11,000 x *g*, 1 min	
Elute DNA	**19**	Transfer the NucleoSpin® Dx Blood Column to an Elution Tube	
	20	50 – 200 µL BE	
	21	11,000 x *g*, 1 min	

5.2 Procedure

1. Pipette **25 µL Proteinase K** solution into a Lysis Tube (1.5 mL, provided).
2. Add **200 µL blood** sample into the Lysis Tube. Mix.
3. Add **200 µL Buffer B3** to the Lysis Tube, close the lid, and mix by pulse-**vortexing** vigorously for 10 s.
   Do not premix Buffer B3 and Proteinase K!
4. Incubate at **room temperature** (18–25 °C) for **5 min** (±1 min).
5. Incubate the Lysis Tube at **70 °C** (±2 °C) for **10 min** (±1 min). After incubation mix by pulse-**vortexing** vigorously for 5 s.
6. **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).
7. Add **210 µL ethanol** (96–100 %) to the sample. Close the lid and mix by pulse-vortexing for 5 s.
   Make sure that the ethanol and the lysate is mixed well.
8. **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).
9. Carefully **load the entire lysate** to the **NucleoSpin® Dx Blood Column** placed in a Collection Tube and close the lid.
10. **Centrifuge 1 min** at **11,000 x *g***.
    If the lysate is not completely drawn through the membrane, repeat the centrifugation at higher g-force (15,000–20,800 x g *for 1 min). If the lysate still does not pass the membrane completely, discard the sample and repeat the isolation with new sample material.*
11. Place the **NucleoSpin® Dx Blood Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
12. Open the **NucleoSpin® Dx Blood Column** and add **500 µL Buffer BW** to the column. Close the lid.
    Note: Make sure that residual lysate is washed away with Buffer BW.
13. **Centrifuge 1 min** at **11,000 x *g***.
14. Place the **NucleoSpin® Dx Blood Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

15. Open the **NucleoSpin® Dx Blood Column** and add **600 µL Buffer B5** to the column. Close the lid.

    Note: Make sure that residual wash buffer from the previous step is washed away with Buffer B5.

16. **Centrifuge 1 min** at **11,000 x *g***.

17. Place the **NucleoSpin® Dx Blood Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

18. **Centrifuge 1 min** at **11,000 x *g***.

    Residual ethanol is removed during this step.

19. Place the **NucleoSpin® Dx Blood Column** in a clean Elution Tube (1.5 mL, provided) and discard the Collection Tube from the previous step.

20. Open the **NucleoSpin® Dx Blood Column** and **add 50–200 µL Buffer BE** directly onto the center of the membrane.

21. **Centrifuge 1 min** at **11,000 x *g*** to elute the DNA from the column.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Low concentration of white blood cells in sample* • The DNA yield depends on the number of white blood cells per sample. Blood samples with low white blood cell count yield low DNA amounts. *Incomplete sample lysis* • Inhomogeneous blood sample or blood clots within the sample: Make sure that blood samples are collected following the instructions of the manufacturer of the blood collection tube. Make sure that only blood which can be easily transferred by pipetting is used as sample material. If necessary, homogenize the blood sample before use. • Sample not thoroughly mixed with Proteinase K and lysis buffer. The mixture has to be vortexed vigorously immediately after addition of Lysis Buffer B3. • Proteinase K digestion not optimal. Never add Proteinase K directly to Lysis Buffer B3. *Reagents not applied properly* • Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysate before loading lysate on the column. *Unappropriate centrifugation* • Do not extend centrifugation time and speed in step 6 and 8. Only use a short spin to remove droplets from the lid. *Suboptimal elution of DNA from the column* • Elution efficiency depends on elution buffer volume. For highest elution efficiency use 200 µL elution buffer; for highest DNA concentration use 50 µL elution buffer.

Problem	Possible cause and suggestions
Clogged DNA binding column	*Inhomogeneous blood sample* • Cryoprecipitate formed during thawing of frozen samples may clog the NucleoSpin® Dx Blood Column. If such precipitates are visible avoid aspirating them when loading the lysate to the binding column. Precipitates may also form in fresh blood samples. Make sure that the samples are mixed well. If the column clogs during the DNA binding step repeat the centrifugation at higher *g*-force (15,000–20,800 x *g* for 1 min).
Poor DNA quality	*Reagents not applied properly* • Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysate and mix before loading them on columns. *Incomplete sample lysis* • Sample not thoroughly mixed with Proteinase K solution and lysis buffer. The mixture has to be vortexed vigorously immediately after addition of lysis buffer. • Proteinase K digestion not optimal. Do not add Proteinase K directly to Lysis Buffer B3. *Old or clotted blood samples processed* • Make sure that only blood is used as sample material which can be easily transferred by pipetting. If necessary homogenize the blood sample before use.
Suboptimal performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol* • Be sure to remove all of ethanolic Buffer B5 before eluting the DNA. If the filling level of Wash Buffer B5 flow-through after the second wash reaches the column outlet for any reason, discard flow-through, place the column back into the Collection Tube, and centrifuge again. • DNA eluates may contain traces of ethanol. However, no decrease in PCR performance was observed using DNA eluate of up to 20 % of the PCR final volume as template (e.g., using 4 µL from 100 µL eluate as template in a 20 µL PCR). The maximum percentage of template volume in a PCR may vary depending on the robustness of the PCR system and has to be determined by the user.

Problem	Possible cause and suggestions
Suboptimal performance of genomic DNA in enzymatic reactions *(continued)*	*Contamination of DNA with inhibitory substances* • If preparing DNA from old or clotted blood samples, make sure that only blood is used as sample material which can be easily transferred by pipetting. If necessary, homogenize the blood sample before use.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Dx Blood	740899.50/.250	50/250

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction/warranty

The **NucleoSpin® Dx Blood** Kit is a generic system for the isolation and purification of genomic DNA from human whole blood samples for subsequent *in vitro* diagnostic purposes.

The kit is designed to be used with downstream applications employing enzymatic amplification and detection of DNA (e.g., PCR). Any and all diagnostic results generated using the DNA isolated with the NucleoSpin® Dx Blood kit in conjunction with a diagnostic assay should be interpreted with regard to additional clinical or laboratory findings. The NucleoSpin® Dx Blood Kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream in vitro diagnostic assay. ONLY MACHEREY-NAGEL products specially labeled as IVD are suitable for *In vitro*-diagnostic use.

NucleoSpin® Dx Blood kit is intended for use by professional users such as technicians and physicians experienced in and trained in molecular biological techniques including experience with whole blood samples and DNA isolation. For safety instructions please refer to the respective chapter in the user manual. NucleoSpin® Dx Blood kit shall exclusively be used in an adequate test environment, i.e. a suitable laboratory setting.

The respective user is liable for any and all damages resulting from application of the NucleoSpin® Dx Blood kit for use deviating from the intended use as specified in the user manual.

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.

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