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1 Components

1.1 Kit contents

		NucleoSpir	n [®] Dx Blood
REF		50 preps 740899.50	250 preps 740899.250
Buffer B3	BUF B3	15 mL	60 mL
Wash Buffer BW	BUF BW	30 mL	150 mL
Wash Buffer B5 (Concentrate)*	BUF B5 conc.	12 mL	50 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
User manual	ī	1	1

^{*} For preparation of working solutions and storage conditions see section 3.

^{**} Composition of Elution Buffer BE: 5 mM Tris/HCI, pH 8.5

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 crosscontamination)

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 to read the detailed protocol section of this user manual. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

MACHEREY-NAGEL user manuals are 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.

Contact information

MACHEREY-NAGEL GmbH & Co. KG Valencienner Str. 11 52355 Duren, Germany Tel.: +49 24 21 969-0 Toll-free: 0800 26 16 000 (Germany only) E-mail: info@mn-net.com

Technical Support Bioanalysis

Tel.: +49 24 21 969-270 E-mail: tech-bio@mn-net.com

Benutzerhandbücher in weiteren Sprachen sind im Download-Bereich auf der Produktseite verfügbar.

Les manuels d'utilisation dans d'autres langues sont disponibles dans la section Telechargements de la page du produit.

Los manuales de usuario en otros idiomas estan disponibles en la seccion de descargas de la pagina del producto.



2 Product description

2.1 Intended use

NucleoSpin® Dx Blood is a kit for the isolation of genomic DNA from fresh and frozen human whole blood, stabilized with either EDTA, citrate or heparin from common blood collection systems for the subsequent *in-vitro* analysis. The product provides purified genomic DNA to be used for subsequent down-stream analysis such as PCR, qPCR or sequencing to obtain information about the genomic DNA in the sample. The product is used by professional users in diagnostic laboratories.

The **NucleoSpin® Dx Blood** kit is not suitable for self-testing or near-patient testing. The user should have experience with molecular biological techniques including experience with whole blood and other potentially infectious human sample materials.

The use of suitable controls such as internal controls, extraction controls, positive/negative controls is recommended.

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 μ L fresh or frozen human whole blood treated with EDTA, citrate, or heparin, from common blood collection systems.

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		

Sample limitations

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.

Obviously haemolytic and hyperlipidemic samples may impact DNA yield and purity.

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	$3-5\mu g$ 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 Analytical performance

Within run repeatability was calculated from parallel isolation of 12 identical blood sample aliquots. The average DNA yield was 5.2 μ g ± 0.3, corresponding to a CV of 6%. Between run repeatability was tested in two independent runs. In each run, DNA was isolated from one blood sample with 6 replicates each. The difference between the average yields of the two runs was 0.2 μ g DNA corresponding to 6%. For batch-to-batch repeatability, three batches of NucleoSpin Dx Blood were compared side-by-side. With each batch gDNA was isolated in 6 replicates were withdrawn from one blood sample. Average DNA yields per six preparations was 5.2, 4.8, and 5.0 μ g with standard deviations of 0.4, 0.5, and 0.5 μ g, respectively. CV of the average yields of the three batches was 4%.

For the evaluation of reproducibility, DNA was isolated from whole blood samples side-by-side by two operators. The average yield (n=6) was $2.9 \pm 0.3 \mu g$ and $3.0 \pm 0.2 \mu g$, respectively, corresponding to a CV of 9% and 7% per six preparations. The difference of the average yields of both operators was 0.1 μg .

In-vitro diagnostic use of NucleoSpin® Dx Blood is exemplified in the following publications:

Hadzsiev, K. *et al.* (2019) Rubinstein-Taybi syndrome 2 with cerebellar abnormality and neural tube defect. Clin Dysmorphol., 28(3), 137–141.

Komlósi, K. *et al.* (2015) Phenotypic variability in a Hungarian patient with the 4q21 microdeletion syndrome. Mol Cytogenet., 8, 16.

Czakó, M. *et al.* (2019) Possible Phenotypic Consequences of Structural Differences in Idic(15) in a Small Cohort of Patients. Int J Mol Sci., 20(19), 4935.

Szabo, A. *et al.* (2015) Partial tetrasomy of the proximal long arm of chromosome 15 in two patients: the significance of the gene dosage in terms of phenotype. Mol Cytogenet., 8, 41.

2.6 Elution procedures

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

Typically, up to 10 μ L of the eluate can be used as template in a 50 μ L 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.

Storage of nucleic acids

Recommendation:

Short term storage (up to 24 h): 2-8 °C Long term storage (over 24 h): -20 °C

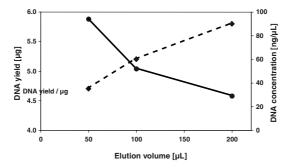


Figure 1

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

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 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 18 – 25 °C until the expiration date.
- Lyophilized Proteinase K can be stored at 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		
REF	50 preps 740899.50	250 preps 740899.250	
Wash Buffer B5 (Concentrate)	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol	
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

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 buffer B3 and 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 Bood** 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 local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

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. 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.
- 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.

5.1 Protocol at a glance

Supplemental protocol-overview:

Carefully read the detailed protocol (section 5.2) before starting the procedure.

Lyse blood	1	25 μL Proteinase K	
samples	2	200 μL blood	
	3	200 µL B3, mix	
	4	RT, 5 min	
	5	70 °C, 10 min, mix	\bigcirc
	6	2,000 x <i>g</i> , 1 s	
Adjust DNA	7	210 μL ethanol, mix	
binding conditions			-
	8	2,000 x <i>g</i> , 1 s	
Bind DNA	9	Load lysate	
	10	11,000 x <i>g</i> , 1 min	
	11	Transfer the NucleoSpin[®] Dx Blood Column to a new Collection Tube	ð
Wash silica	12	500 μL BW	f
membrane	13		
		11,000 x <i>g</i> , 1 min	
	14	11,000 x <i>g</i> , 1 min Transfer the NucleoSpin[®] Dx Blood Column to a new Collection Tube	
		Transfer the NucleoSpin [®] Dx Blood Column	
	14	Transfer the NucleoSpin [®] Dx Blood Column to a new Collection Tube	
Dry silica membrane	14 15	Transfer the NucleoSpin[®] Dx Blood Column to a new Collection Tube 600 μL B5	
	14 15 16	Transfer the NucleoSpin [®] Dx Blood Column to a new Collection Tube $600 \mu L B5$ $11,000 \times g, 1 min$ Transfer the NucleoSpin [®] Dx Blood Column	

Elute DNA	19	Transfer the NucleoSpin® Dx Blood Column to an Elution Tube	
	20	50–200 μL BE	A
	21	11,000 x <i>g</i> , 1 min	\bigcirc

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 for 5 min (± 1 min).
- 5 Incubate the Lysis Tube at 70 °C (\pm 2 °C) for 10 min (\pm 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 pulsevortexing 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 ($15,000-20,800 \times g$ 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.

<u>Note:</u> 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
	 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.
No or poor DNA yield	 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.
	Inhomogeneous blood sample
Clogged DNA binding column	 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).

Problem	Possible cause and suggestions			
	 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			
Poor DNA quality	 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. 			
	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.			
Suboptimal performance of genomic DNA in enzymatic reactions	• 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 μ L from 100 μ L eluate as template in a 20 μ L 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.			
	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. 			

Please contact:

MACHEREY-NAGEL Germany Tel.: +49 (0) 24 21 969 270 e-mail: TECH-BIO@mn-net.com

6.2 Notification requirement

Please note that any serious incident that has occurred in relation to the product shall be reported immediately to the manufacturer and the competent authority of the European member state in which the incident occurred. European vigilance contact points: https://ec.europa.eu/health/md_sector/contact_en

6.3 General Literature

Thiemann F. *et al.* (2006) Leitfaden Molekulare Diagnostik - Grundlagen, Gesetze, Tipps und Tricks, WILEY-VCH, ISBN 3-527-31471-7.

Orzińska A. *et al.* (2015) 14 Years of Polish Experience in Non-Invasive Prenatal Blood Group Diagnosis. Transfus Med Hemother, 42, 361–364.

Papadopoulou A. *et al.* (2014) Calcium sensing receptor in pregnancies complicated by gestational diabetes mellitus. Placenta, 35, 632e638.

Bleda S. *et al.* (2012) Vascular endothelial growth factor polymorphisms are involved in the late vascular complications in Type II diabetic patients. Diabetes & Vascular Disease Research, 9(1), 68–74.

6.4 Ordering information

Product	REF	Pack of
NucleoSpin [®] Dx Blood	740899.50/.250	50/250
NucleoSpin [®] Dx Virus	740895.50	50
NucleoMag [®] Dx Pathogen	744215.4	384

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

6.5 Explanation of symbols

REF	Item number	ł	Permitted storage temperature range
LOT	Batch identification		Use by
	Manufacturer	\triangle	Caution: Further information in user manual
IVD	In-vitro diagnostic products	2	Do not reuse
i	Please read instructions for use		
Σ	Sufficient for < n> tests		

6.6 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.

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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Last updated: April 2022/Rev. 05

Reason for revision:

Addition of analytical and clinical performance data to chapter 2.5. Reference to new languages of the user manual (chapter 1.3).

Trademarks:

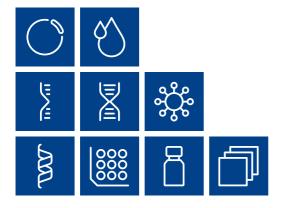
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MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG DE Tel.: +49 24 21 969-0 info@mn-net.com Valencienner Str. 11 52355 Düren · Germany

CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com US Tel.: +1 888 321 62 24 sales-us@mn-net.com



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