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sharing knowledge

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Genomic DNA from Blood

User Manual

NucleoSpin® 8 Blood
NucleoSpin® 96 Blood
NucleoSpin® 96 Blood Core Kit

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSpin® 8 Blood	
	12 x 8 preps	**60 x 8 preps**
Cat. No.	**740664**	**740664.5**
Lysis Buffer BQ1	40 ml	2 x 100 ml
Wash Buffer B5 (Concentrate)[1]	100 ml	5 x 100 ml
Wash Buffer BW	150 ml	2 x 375 ml
Elution Buffer BE[2]	50 ml	2 x 125 ml
Proteinase K (lyophilized)[1]	75 mg	5 x 75 mg
Proteinase Buffer PB	3.6 ml	18 ml
NucleoSpin® Blood Binding Strips (red rings)	12	60
MN Wash Plates[3]	1	5
Rack of Tube Strips[4]	1	5
Cap Strips	12	60
Tubes (2 ml) for Proteinase K	4	20
Tubes (15 ml) for BioRobot® 9604	8	40
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Material supplied by user: Suitable lysis tubes or plates, e.g. Rack of Tube Strips with Cap Strips (Cat. No. 740477, 4 sets; see ordering information).

[1] For preparation of working solutions and storage conditions see section 3.

[2] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

[3] For use with vacuum only

[4] Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

1.1 Kit contents *continued*

	NucleoSpin® 96 Blood		
	1 x 96 preps	**4 x 96 preps**	**24 x 96 preps**[1]
Cat. No.	**740665.1**	**740665.4**	**740665.24**
Lysis Buffer BQ1	40 ml	125 ml	6 x 125 ml
Wash Buffer B5 (Concentrate)[2]	100 ml	4 x 100 ml	24 x 100 ml
Wash Buffer BW	125 ml	2 x 300 ml	12 x 300 ml
Elution Buffer BE[3]	50 ml	125 ml	6 x 125 ml
Proteinase K (lyophilized)[2]	75 mg	4 x 75 mg	24 x 75 mg
Proteinase Buffer PB	3.6 ml	15 ml	6 x 15 ml
NucleoSpin® Blood Binding Plates (red rings)	1	4	24
MN Wash Plates[4]	1	4	24
Lysis Blocks	1	4	24
Rack of Tube Strips[5]	1	4	24
Tubes (2 ml) for Proteinase K	4	16	96
Tubes (15 ml) for BioRobot® 9604	8	32	192
User Manual	1	1	6

[1] The kit for 24 x 96 preparations (Cat.No. 740665.24) consists of 6 x Cat. No. 740665.4.

[2] For preparation of working solutions and storage conditions see section 3.

[3] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

[4] For use with vacuum only

[5] Sets of 1 rack, 12 strips with 8 tubes each, including Cap Strips

1.1 Kit contents *continued*

	NucleoSpin® 96 Blood Core Kit
	4 x 96 preps
Cat. No.	**740456.4**
Lysis Buffer BQ1	125 ml
Wash Buffer B5 (Concentrate)[1]	4 x 100 ml
Wash Buffer BW	2 x 300 ml
Elution Buffer BE[2]	125 ml
Proteinase K (lyophilized)[1]	4 x 75 mg
Proteinase Buffer PB	15 ml
NucleoSpin® Blood Binding Plates (red rings)	4
User Manual	1

Additional material required (see section 1.3).

1.2 Reagent to be supplied by user

- 96 - 100% ethanol (for preparation of working solutions; see section 3)

[1] For preparation of working solutions and storage conditions see section 3.

[2] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.3 Accessories supplied by user - NucleoSpin® 96 Blood Core Kit

The **NucleoSpin® 96 Blood Core Kit** provides the buffers, Proteinase K, and NucleoSpin® Blood Binding Plates only. Accessory plates (e.g. lysis plates, elution plates) are not provided with the core kits. The user can individually select additional consumables from a variety of suitable accessory plates according to his requirements for highest flexibility.

For usage of **NucleoSpin® 96 Blood Core Kit** follow the standard protocol (see section 5.2 and 5.3).

Recommended accessories for use of the **NucleoSpin® 96 Blood Core Kits** are available from MACHEREY-NAGEL (see ordering information):

Protocol step	Suitable consumables, not supplied with the core kits	Remarks
Lysis	4 x Lysis Block per 4x96 preps or 4 x Round-well Block with Cap Strips per 4x96 preps or 4 x Rack of Tube Strips with Cap Strips per 4x96 preps	Round-well Blocks and Tube Strips can be closed with Cap Strips.
Binding of DNA to the membrane	4 x MN Wash Plate per 4x96 preps 2 x MN Square-well Block	MN Wash Plate minimizes the risk of cross contamination (vacuum processing only). For waste collection during centrifugation (reusable)
Elution	4 x Rack of Tubes Strips with Cap Strips per 4x96 preps or 4 x Round-well Block with Cap Strips per 4x96 preps	Round-well Blocks and Tube Strips can be closed with Cap Strips.

1.4 Required hardware

Vacuum processing

The **NucleoSpin® 8/96 Blood** kits can be used manually with the NucleoVac 96 Vacuum Manifold (see ordering information). Alternatively, other suitable vacuum manifolds may be used.

For processing the 8-well strips the Starter Set A (see ordering information), containing Column Holders A and NucleoSpin® Dummy Strips is required. For automation on laboratory platforms with standard 96-well plate vacuum chambers the use of the Starter Set A is also required.

Centrifugation

For centrifugation a microtiterplate centrifuge is required which is able to accommodate the NucleoSpin® Blood Binding Strips/Plate stacked on a Round- or Square-well Block and reaches accelerations of 5,600 - 6,000 x *g* (bucket height: 85 mm), e.g. Hermle Z513, Qiagen/Sigma 4-15c, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™, Beckman Coulter, Allegra R25).

For processing the 8-well strips the Starter Set C (see ordering information), containing Column Holders C, NucleoSpin® Dummy Strips, MN Square-well Blocks, Rack of Tube Strips is required.

1.5 Suitable vacuum manifolds

The **NucleoSpin® 8/96 Blood** kits can be used with the NucleoVac 96 Vacuum Manifold or other common vacuum devices. For further details see list below.

Vacuum manifold	Suitability	Additional equipment
NucleoVac 96	Yes	Starter Set A for NucleoSpin® 8 Blood
Qiagen/QIAvac 96[1]	Yes	MN Frame (see ordering information), Starter Set A for NucleoSpin® 8 Blood
Promega/Vac-Man® 96[2]	Yes	NucleoSpin® 96 Blood only!

[1] In general the QIAvac 96 is suitable for the use with the NucleoSpin® Blood Binding Strips/Plate. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate and Elution Plate in order to ensure best performance.

[2] MN Wash Plate cannot be used.

2 Product description

2.1 The basic principle

With the **NucleoSpin® 8/96 Blood** method, genomic DNA is prepared from whole blood, buffy coat, or cultured cells. Lysis is achieved by incubation of whole blood in a lysis buffer containing chaotropic ions in the presence of Proteinase K at room temperature. For optimal lysis a microplate shaker is recommended. Appropriate conditions for binding of DNA to the silica membrane in the **NucleoSpin® Blood Binding Strips or Plate** are created by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by three wash steps with ethanolic buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® 8/96 Blood** kits are designed for the rapid, small-scale preparation of highly pure genomic DNA from whole animal or human blood, serum, plasma, or other body fluids. The obtained DNA can be used directly as template for PCR, blotting, or any kind of enzymatic reactions.

- The kits provide reagents and consumables for purification of up to 20 µg (average 4 - 6 µg) of pure genomic DNA from 200 µl whole blood with an A_{260}/A_{280} ratio between 1.8 and 1.9 and a typical concentration of 20 - 60 ng/µl.

- Fresh and frozen blood and blood treated either with EDTA, citrate, or heparin can be used. The procedure is optimized for a sample volume of 200 µl. Using the **NucleoSpin® 8/96 Blood** kits allows simultaneous processing of up to 96 samples typically within less than 70 minutes.

- **NucleoSpin® 8/96 Blood** kits can be processed completely at room temperature.

- **NucleoSpin® 8/96 Blood** can be processed by vacuum or centrifugation. The kits allow for easy automation on common liquid handling instruments. For more information about the automation process and the availability of ready-to-run scripts for certain platforms please refer to section 2.4 and contact your local distributor or MN directly.

- The **NucleoSpin® 8/96 Blood** kits allow for the purification of multiples of 8 **(NucleoSpin® 8 Blood)** or 96 samples **(NucleoSpin® 96 Blood)**. Both kits are supplied with accessory plates for highest convenience. The **NucleoSpin® 96 Blood Core Kit** provides the buffers, Proteinase K and NucleoSpin® Blood Binding Plate only. Accessory components (e.g. lysis plates, elution plates) are not provided with the core kit but can be individually selected from a variety of suitable accessories (see section 6.2 for further information). This allows highest flexibility for the user.

Table 1: Kit specifications at a glance	
Parameters	**NucleoSpin® 8/96 Blood (Core)**
Sample material	Up to 200 µl
Typical DNA yield	4 - 6 µg
Elution volume	100 µl
DNA binding capacity	20 µg
A_{260}/A_{280}	1.8 - 1.9
Preparation time for 6 strips or 1 plate	35 min

Application data

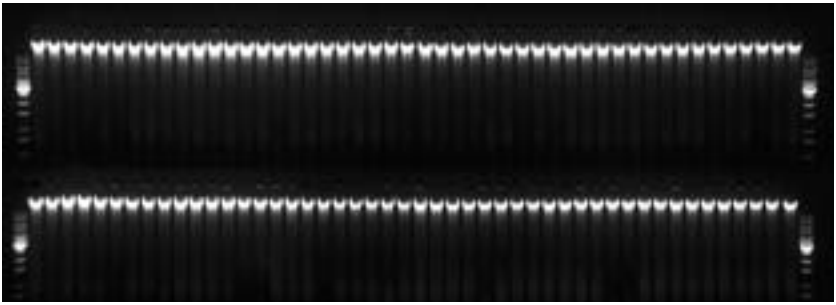


Figure 1: DNA isolation from human blood samples

Genomic DNA from 200 µl of human blood (96 samples) was isolated using **NucleoSpin® 96 Blood** on a Tecan Genesis instrument. Of each eluate (100 µl elution volume) 20 µl were loaded and analyzed on a 0.7% agarose gel. Highly reproducible yields of high-quality genomic DNA were obtained.

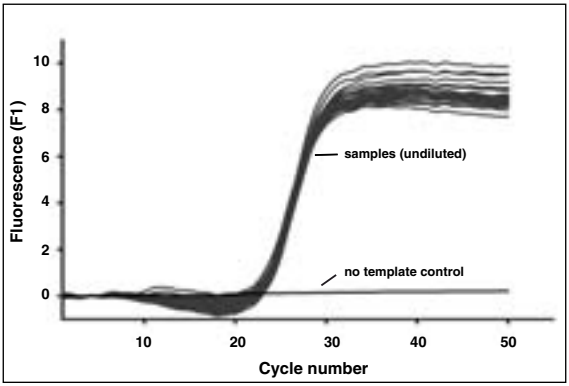


Figure 2: Analysis of purified DNA by real time PCR (LightCycler)

Genomic DNA from 200 µl of blood (96 samples) was isolated using **NucleoSpin® 96 Blood** on a Tecan Genesis instrument. 2 µl of eluate (100 µl elution volume) were amplified with the LightCycler – SYBR Green I kit and 28S primers (0.5 µM). All samples were amplified with homogenous crossing point values. Amplification plots indicate the absence of inhibitors.

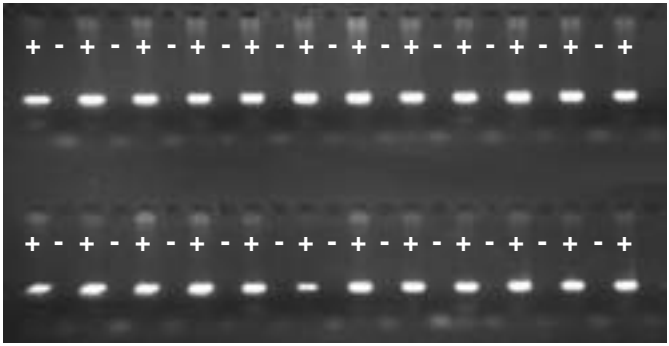


Figure 3: Cross-contamination analysis

Genomic DNA was purified from 200 µl of blood using **NucleoSpin® 96 Blood** on a Biomek 2000 (Beckman Coulter) instrument. Blood samples and PBS buffer were arrayed in a checkerboard pattern. For PCR detection of genomic DNA 2 µl of eluate were amplified on iCycler (BIORAD) with Haemochromatosis-Primer and Taq Polymerase (Life Technologies). 35 cycles were performed. 48 samples were analyzed. No cross-contamination was detected.

+ : samples containing 200 µl of blood

- : control samples without blood (next to a well containing blood)

2.3 Elution procedure

Recovery of gDNA from the membrane depends on the elution volume. Elution volumes of 50 - 200 µl are possible, with an optimum of 100 - 125 µl dispensed volume. The purity is not effected by the elution volume. See table for correlation between dispensed elution buffer volume and typical recoveries following the standard protocol.

Table 2: Recovery volumes in correlation to applied elution volumes					
Dispensed elution volume	40 µl	60 µl	80 µl	100 µl	120 µl
Recovered volume:					
Vacuum	25 µl	45 µl	65 µl	85 µl	105 µl
Centrifuge	38 µl	58 µl	78 µl	98 µl	118 µl

If highest yield is required, prewarming of the elution buffer to 70°C will give about 10 - 15% higher yields by supporting DNA recovery from the membrane.

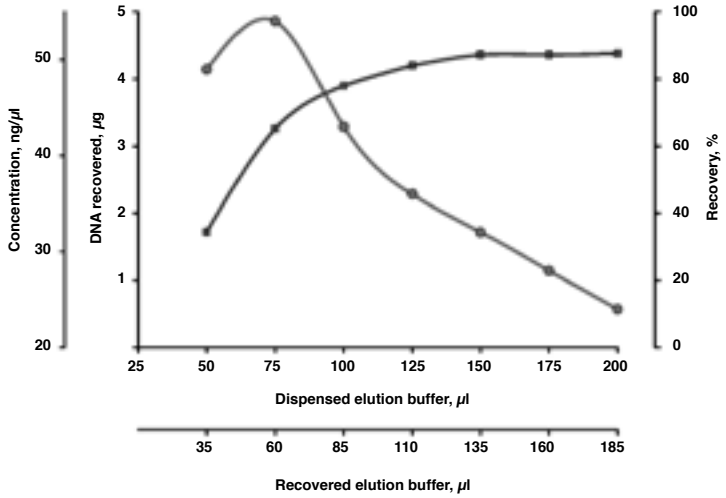


Figure 4: Elution of genomic DNA (vacuum processing)

Total DNA yield, recovery (-■-) and concentration of recovered DNA (-●-) are plotted versus dispensed elution buffer volume. High elution buffer volumes result in high elution efficiency whereas high concentrated DNA solutions can be obtained with smaller elution buffer volumes. The dead volume of the silica membrane under vacuum is approximatively 15 µl.

2.4 Automated processing on robotic platforms

NucleoSpin® 8/96 Blood can be fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8/96 Blood** on a certain workstation please contact MN. Full processing under vacuum enables complete automation without the need for centrifugation steps for drying of the membrane or for elution.

The risk of cross-contamination is reduced by optimized vacuum settings during the elution step and by the improved shape of the outlets of the **NucleoSpin® 8/96 Blood Binding Strips/Plate**.

Drying of the **NucleoSpin® 8/96 Blood Binding Strips/Plate** under vacuum is sufficient because the bottom of the strips/plate is protected from spraying wash buffer during the washing steps by the MN Wash Plate. Thus, if possible, the MN Wash Plate should be integrated into the automated procedure. The MN Frame (see ordering information) can be used to position the MN Wash Plate inside the vacuum chamber. Thorough cleaning of the vacuum chamber is recommended after each run to prevent forming of gDNA-containing aerosols.

Visit MN on the internet at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instruc-tions and selection of the protocol. Several application notes of the **NucleoSpin® 8/96 Blood** kit on various automation workstations can also be found at *www.mn-net.com* at Bioanalyis/Literature.

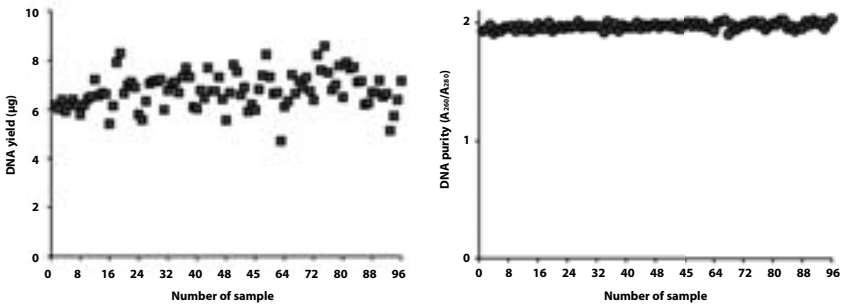


Figure 5: Automated DNA isolation from human blood samples

DNA was purified from 96 aliquots from the same blood using **NucleoSpin® 96 Blood** on a Tecan Genesis 150 instrument (vacuum processing). The average yield is 6.76 µg ± 0.71 with a coefficient of variation (CV) of 10%. The average purity is 1.97 ± 0.02 (CV: 1%).

3 Storage conditions and preparation of working solutions

Attention:

Buffers BQ1 and BW contain guanidinium hydrochloride! Wear gloves and goggles when handling them!

- All components of the **NucleoSpin® 8/96 Blood** kits should be stored at room temperature for a maximum of 1 year. Storage at lower temperatures may cause precipitation of salts. If a salt precipitation is observed, incubate the bottle at 30 - 40°C for some minutes and mix well until all of the precipitate is redissolved.

Before starting with any **NucleoSpin® 8/96 Blood** kit procedure prepare the following:

- **Wash Buffer B5:** Add the indicated volume of 96 - 100% ethanol to the Buffer B5 Concentrate. Mark the label of the bottle to indicate that ethanol is added. Store Wash Buffer B5 at room temperature (20 - 25°C) for up to one year.
- Before first use of the kit, add the indicated volume of Proteinase Buffer to lyophilized Proteinase K. Proteinase K solution is stable at - 20°C for 6 months.

	NucleoSpin® 8 Blood	
	12 x 8 preps	**60 x 8 preps**
Cat. No.	**740664**	**740664.5**
Wash Buffer B5 (Concentrate)	100 ml Add 400 ml ethanol	5 x 100 ml Add 400 ml ethanol to each bottle
Proteinase K (lyophilized)	75 mg Add 3.35 ml Proteinase Buffer	5 x 75 mg Add 3.35 ml Proteinase Buffer to each bottle

	NucleoSpin® 96 Blood		
	1 x 96 preps	**4 x 96 preps**	**24 x 96 preps***
Cat. No.	**740665.1**	**740665.4**	**740665.24**
Wash Buffer B5 (Concentrate)	100 ml Add 400 ml ethanol	4 x 100 ml Add 400 ml ethanol to each bottle	24 x 100 ml Add 400 ml ethanol to each bottle
Proteinase K (lyophilized)	75 mg Add 3.35 ml Proteinase Buffer to each vial	4 x 75 mg Add 3.35 ml Proteinase Buffer to each bottle	24 x 75 mg Add 3.35 Proteinase Buffer to each bottle

	NucleoSpin® 96 Blood Core Kit
	4 x 96 preps
Cat. No.	**740456.4**
Wash Buffer B5 (Concentrate)	4 x 100 ml Add 400 ml ethanol to each bottle
Proteinase K (lyophilized)	4 x 75 mg Add 3.35 ml Proteinase Buffer to each bottle

* The kit of 24 x 96 preparations (Cat.No. 740665.24) consists of 6 x Cat. No. 740665.4.

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 8/96 Blood** kits contain hazardous contents.

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

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
BQ1	Guanidine hydrochloride	Xn*	Harmful if swallowed - Irritating to eyes and skin	R 22-36/38	
BW	Guanidine hydrochloride + isopropanol <25%	Xn*	Flammable - Harmful by if swallowed - Irritating to eyes and skin	R 10-22-36/38	S 7-16-25
Proteinase K	Proteinase K, lyophilized	Xn Xi*	Irritating to eyes, respiratory system and skin - May cause sensitization by inhalation.	R 36/37/38-42	S 22-24-26-36/37

Risk phrases

R 10	Flammable
R 22	Harmful if swallowed
R 36/37/38	Irritating to eyes, respiratory system and skin
R 36/38	Irritating to eyes and skin
R 42	May cause sensitization by inhalation

Safety phrases

S 7	Keep container tightly closed
S 16	Keep away from sources of ignition - No smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 25	Avoid contact with the eyes
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 36/37	Wear suitable protective clothing and gloves

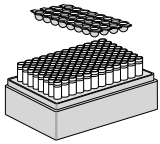
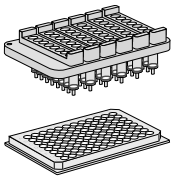
* Hazard labeling not neccessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 General procedure

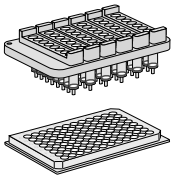
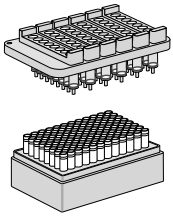
NucleoSpin® 8 Blood, vacuum processing
(For details on each step see section 5.1.)

Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.

1	**Lyse** samples	**200 µl blood (equilibrated to room temperature)** **25 µl Proteinase K** **200 µl BQ1** **Mix 3 times** **Incubate at RT 10 min** **or** **Mix 3 times and shake at 1250 rpm at RT 10 min**	 e.g. Rack of Tube Strips with Cap Strips (not supplied with the kit)
2	**Adjust** DNA binding conditions	**200 µl ethanol** **Mix at least 3 - 5 times** *Note: High-speed pipetting (400 µl/s) should be used for optimized mixing, if possible.*	
3	**Load** samples	**Transfer samples to NucleoSpin® Blood Binding Strips**	 Column Holder A with NucleoSpin® Blood Binding Strips and MN Wash Plate
4	**Overlay** with Buffer B5	**150 µl B5**	
5	**Bind** DNA to silica membrane	**- 0.2 bar*** **5 min**	

* Reduction of atmospheric pressure

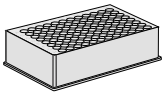
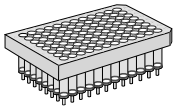
6	**Wash** silica membrane	**600 µl BW** **- 0.2 bar*** **3 min** **900 µl B5** **- 0.2 bar*** **1 min** **900 µl B5** **- 0.2 bar*** **1 min**	 Column Holder A with NucleoSpin® Blood Binding Strips and MN Wash Plate
7	**Dry** silica membrane	**Remove MN Wash Plate** **- 0.6 bar*** **10 min**	
8	**Elute** DNA	**50 - 200 µl BE** **(Incubate 5 min)** **- 0.6 bar*** **1 min**	 Column Holder A with NucleoSpin® Blood Binding Strips on Rack of Tube Strips

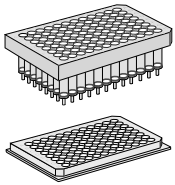
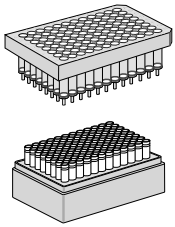
* Reduction of atmospheric pressure

NucleoSpin® 96 Blood, vacuum processing
(For details on each step see section 5.2.)

Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.

1	**Lyse** samples	**200 µl blood (equilibrated to room temperature)** **25 µl Proteinase K** **200 µl BQ1** **Mix 3 times** **Incubate at RT 10 min** **or** **Mix 3 times and shake at 1250 rpm at RT 10 min**	 Lysis Block
2	**Adjust** DNA binding conditions	**200 µl ethanol** **Mix at least 3 - 5 times** *<u>Note</u>: High-speed pipetting (400 µl/s) should be used for optimized mixing, if possible.*	
3	**Load** samples	**Transfer samples to NucleoSpin® Blood Binding Plate**	  NucleoSpin® Blood Binding Plate and MN Wash Plate
4	**Overlay** with Buffer B5	**150 µl B5**	

5	**Bind** DNA to silica membrane	**-0.2 bar*** **5 min**	
6	**Wash** silica membrane	**600 µl BW** **-0.2 bar*** **3 min**	 NucleoSpin® Blood Binding Plate and MN Wash Plate
		900 µl B5 **-0.2 bar*** **1 min**	
		900 µl B5 **-0.2 bar*** **1 min**	
7	**Dry** silica membrane	**Remove MN Wash Plate** **- 0.6 bar*** **10 min**	
8	**Elute** DNA	**50 - 200 µl BE** **(Incubate 5 min)** **-0.6 bar*** **1 min**	 NucleoSpin® Blood Binding Plate on Rack of Tube Strips

* Reduction of atmospheric pressure

5.1 NucleoSpin® 8 Blood, vacuum processing

For processing of **NucleoSpin® 8 Blood** under vacuum the NucleoVac 96 Vacuum Manifold and the Starter Kit A are required (see ordering information). Starter Kit A contains the Column Holders A and the NucleoSpin® Dummy Strips to close unused rows of the Column Holder A. Alternatively, other suitable vacuum manifolds can be used.

The use of NucleoSpin® Blood Binding Strips in a Column Holder A allows the isolation of up to n x 8 samples (n = 1 to 6). Insert as many of the NucleoSpin® Blood Binding Strips as required into the reusable column holder and place it onto an MN Square-well Block.

Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.

Lysis tubes are not supplied with the NucleoSpin® 8 Blood kit. Lysis can be performed in any appropriate microtube or in suitable 96-well plates. We recommend usage of the Lysis Block or Rack of Tube Strips with Cap Strips (see ordering information).

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1 Lyse samples

Dispense **25 µl Proteinase K** and **200 µl blood** (equilibrated to room temperature) to each lysis tube/well.

Add **200 µl Buffer BQ1** to each lysis tube/well, **mix 3 times** by pipetting up and down and incubate samples at least **10 min at room temperature**.

or:

Add **200 µl Buffer BQ1** to each tube/well. **Mix 3 times** by pipetting up and down and shake samples during incubation. Recommended are **10 min at 1250 rpm**. Shake at room temperature.

Prepare the NucleoVac 96 Vacuum Manifold

Place waste tray into vacuum manifold base. Insert spacers labeled “MTP/ Multi-96 plate” notched side up and rest the MN Wash Plate on them. Close the manifold with the manifold lid.

Insert desired number of NucleoSpin® Blood Binding Strips in the Column Holder A. Use NucleoSpin® Dummy Strips to close unused openings in the column holder.

Place Column Holder A with inserted NucleoSpin® Blood Binding Strips on top of the manifold.

2 Adjust DNA binding conditions

Add **200 µl 96 - 100% ethanol** to each lysis tube/well, mix **at least 3 times**.

High-speed pipetting (400 µl/sec) should be used for optimal mixing.

3 Load samples

Transfer the samples to the NucleoSpin® Blood Binding Strips.

Note: Do not moisten the rims of the individual wells while dispensing the samples, as this may lead to cross-contamination.

4 Overlay with Buffer B5

Overlay crude lysate on the NucleoSpin® Blood Binding Strips slowly (50 µl/s) with **150 µl Buffer B5**.

5 Bind DNA to silica membrane

Apply vacuum until all lysates have passed through the wells of the NucleoSpin® Blood Binding Strips **(- 0.2 bar*; 5 min)**. Release the vacuum.

6 Wash silica membrane

1st wash

Add **600 µl Buffer BW** to each well of the NucleoSpin® Blood Binding Strips. Apply vacuum **(- 0.2 bar*; 3 min)** until all buffer has passed through the wells of the NucleoSpin® Blood Binding Strips. Release the vacuum.

2nd wash

Add **900 µl Buffer Buffer B5** to each well of the NucleoSpin® Blood Binding Strips. Apply vacuum **(- 0.2 bar*; 1 min)** until all buffer has passed through the wells of the NucleoSpin® Blood Binding Strips. Release the vacuum.

* Reduction of atmospheric pressure

3rd wash

Add **900 µl Buffer Buffer B5** to each well of the NucleoSpin® Blood Binding Strips. Apply vacuum **(-0.2 bar*; 1 min**) until all buffer has passed through the wells of the NucleoSpin® Blood Binding Strips. Release the vacuum.

7 Remove MN Wash Plate

After the final washing step close the valve, release the vacuum and remove the Column Holder A with inserted NucleoSpin® Blood Binding Strips from the vacuum manifold. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry silica membrane

Remove any residual washing buffer from the outlets of the NucleoSpin® Blood Binding Strips. If necessary, tap the outlets onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the Column Holder A with the NucleoSpin® Blood Binding Strips again into the lid and close the manifold. Apply maximum vacuum **(at least -0.6 bar*)** for **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

8 Elute DNA

Insert spacers “Microtube rack” into the NucleoVac 96 Vacuum Manifold‘s short sides. Place a Rack of Tube Strips onto the spacer. Close the vacuum manifold and place the Column Holder A with the NucleoSpin® Blood Binding Strips on top. Dispense **50-200 µl Buffer BE** directly to the bottom of each well. **Incubate** for **5 min at room temperature**. Apply vacuum for elution **(-0.6 bar*; 1 min)**. Dismantle the vacuum manifold and close Tube Strips with Cap Strips for storage.

Optional: Preheat Buffer BE to 70°C to increase yield.

* Reduction of atmospheric pressure

5.2 NucleoSpin® 96 Blood, vacuum processing

For processing of **NucleoSpin® 96 Blood** under vacuum the NucleoVac 96 Vacuum Manifold is required (see ordering information). Alternatively other suitable vacuum manifolds may be used.

Before starting the preparation prepare Buffer B5, and Proteinase K solution (see section 3 for details).

Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.

1 Lyse samples

Dispense **25 µl Proteinase K** and **200 µl blood** (equilibrated to room temperature) to each well of the Lysis Block.

Add **200 µl Buffer BQ1** to each well, mix **3 times** by pipetting up and down and incubate samples **at least 10 min at room temperature**.

or:

Add **200 µl Buffer BQ1** to each well. **Mix 3 times** by pipetting up and down and shake samples during incubation. Recommended are **10 min at 1250 rpm**. Shake at room temperature.

Prepare the NucleoVac 96 Vacuum Manifold

Place waste tray into vacuum manifold base. Insert spacers labeled “MTP/ Multi-96 plate” notched side up and rest the MN Wash Plate on them. Close the manifold with the manifold lid.

Place a NucleoSpin® Blood Binding Plate on top of the manifold.

2 Adjust DNA binding conditions

Add **200 µl 96-100% ethanol** to each well of the Lysis Block, **mix at least 3 times**.

High-speed pipetting (400 µl/s) should be used for optimal mixing.

3 Load samples

Transfer the samples from the Lysis Block to the NucleoSpin® Blood Binding Plate.

Note: Do not moisten the rims of the individual wells while dispensing the samples, as this might lead to cross-contamination.

4 Overlay with Buffer B5

Overlay crude lysate on the NucleoSpin® Blood Binding Plate slowly (50 µl/s) with **150 µl Buffer B5**.

5 Bind DNA to silica membrane

Apply vacuum until all lysates have passed through the wells of the NucleoSpin® Blood Binding Plate **(-0.2 bar*; 5 min)**. Release the vacuum.

6 Wash silica membrane

1st wash

Add **600 µl Buffer BW** to each well of the NucleoSpin® Blood Binding Plate. Apply vacuum **(-0.2 bar*; 3 min)** until all buffer has passed through the wells of the NucleoSpin® Blood Binding Plate. Release the vacuum.

2nd wash

Add **900 µl Buffer Buffer B5** to each well of the NucleoSpin® Blood Binding Plate. Apply vacuum **(-0.2 bar*; 1 min)** until all buffer has passed through the wells of the NucleoSpin® Blood Binding Plate. Release the vacuum.

3rd wash

Add **900 µl Buffer Buffer B5** to each well of the NucleoSpin® Blood Binding Plate. Apply vacuum **(-0.2 bar*; 1 min)** until all buffer has passed through the wells of the NucleoSpin® Blood Binding Plate. Release the vacuum.

7 Remove MN Wash Plate

After the final washing step close the valve, release the vacuum and remove the NucleoSpin® Blood Binding Plate. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

* Reduction of atmospheric pressure

Dry silica membrane

Remove any residual washing buffer from the outlets of the NucleoSpin® Blood Binding Plate. If necessary, tap the outlets onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no further drops come out. Insert the NucleoSpin® Blood Binding Plate into the lid and close the manifold. Apply maximum vacuum **(at least -0.6 bar*)** for **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

8 Elute DNA

Insert spacers “Microtube rack” into the NucleoVac Vacuum Manifold’s short sides. Place a Rack of Tube Strips onto the spacer. Close the vacuum manifold and place the NucleoSpin® Blood Binding Plate on top. Dispense **50-200 µl Buffer BE** onto the membrane. Incubate for **5 min** at **room temperature**. Apply vacuum for elution **(-0.6 bar*; 1 min)**. Dismantle the vacuum manifold and close Tube Strips with Cap Strips for storage.

Optional: Preheat Buffer BE to 70°C to increase yield.

* Reduction of atmospheric pressure

5.3 NucleoSpin® 8/96 Blood, support protocol for centrifuge processing

Although the NucleoSpin® 8/96 Blood kit is designed primarily for vacuum processing, centrifuge processing is also possible.

NucleoSpin® 8 Blood

For processing under centrifugation the Starter Kit C and a suitable centrifuge are required (see section 1.4). For handling of the 8-well strips and the column holders refer to the protocol of the Starter Kit C.

The use of NucleoSpin® Blood Binding Strips in a Column Holder C allows the isolation of up to n x 8 samples (n = 1 to 6). Insert as many of the NucleoSpin® Blood Binding Strips as required into the same positions of each one of the two reusable column holders and place column holders onto MN Square-well Block (see ordering information). Label the column holders or 8-well strips for later identification. Always use 2 Column Holders C containing identical numbers of NucleoSpin® Blood Binding Strips for centrifugation. This avoids the need to balance the centrifuge, and allows multiples of 16 samples to be processed in parallel. We recommend inserting the NucleoSpin® Blood Binding Strips around the center of the column holder.

Follow the standard protocol as described in section 5.1. The vacuum steps are substituted by centrifugation of the Column Holder C with the NucleoSpin® 8 Blood Strips at 5,600-6,000 x *g* for 3 min.

Drying of the silica membrane is achieved by centrifugation for 10 min after the second Buffer B5 washing step. A separate drying step is not required.

During all centrifugation steps the Column Holder C with the NucleoSpin® 8 Blood Strips should be placed on an MN Square-well Block (see ordering information) to collect the waste.

During the elution step the Column Holder C with the NucleoSpin® 8 Blood Strips are placed on top of a Rack of Tube Strips.

NucleoSpin® 96 Blood

Follow the standard protocol as described in section 5.2. The vacuum steps are substituted by centrifugation of the NucleoSpin® Blood Binding Plate at 5,600 - 6,000 x *g* for 3 min.

Drying of the silica membrane is achieved by centrifugation for 10 min after the second Buffer B5 washing step. A separate drying step is not required.

During all centrifugation steps the NucleoSpin® Blood Binding Plate should be placed on an MN Square-well Block (see ordering information) to collect the waste.

During the elution step the NucleoSpin® Blood Binding Plate is placed on top of a Rack of Tube Strips.

5.4 Support protocol for modified lysis of blood samples

This modified lysis procedure may be used to increase the yield on some liquid handling instruments e.g. instruments with 4 channel pipetting system or if the recommended mixing speed of 400 µl/s for the addition of ethanol to adjust binding conditions can not be achieved.

A	Pre-dispense **25 µl of Proteinase K** solution to each well of the Lysis Block.
B	Transfer **200 µl blood** (equilibrated to room temperature) to the Lysis Block. Do not moisten the rims of the well.
C	Add **75 µl Buffer BQ1** to each sample, pipette up and down **3 times** and mix by shaking **(15 min)** at room temperature. Alternatively, pipette up and down **10 times** and incubate **15 min** at room temperature.
D	Add **400 µl Buffer BQ1/ethanol-mix** (1:1, v/v) to each well of the Lysis Block, mix at least 2 times and transfer lysate (total volume 700 µl) to the NucleoSpin® Blood Binding Plate.
E	Overlay crude lysate on the NucleoSpin® Blood Binding Plate slowly (~ 50 µl/s) with **150 µl Buffer B5**. Wait for **1 min** before applying vacuum for binding.
	Proceed with DNA binding step.

5.5 Support protocol for cultured animal or human cells

Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
- Seal unused wells of NucleoSpin® Blood Binding Strips with Self-adhering PE-Foil (see ordering information).

A Harvest cells

Harvest cells (maximum starting amount 2 x 10^6) and pellet them in the lysis vessel by centrifugation **(300 x *g*, 4 min)**. Remove supernatant and resuspend cell pellets in **200 µl PBS**.

B Lyse cells

Add **25 µl Proteinase K** and **200 µl Buffer BQ1** to each well and shake lysis vessel **at least 10 min at room temperature**. Complete lysis is important for optimal yields.

*Optional: Add **10 µl RNase** (25 mg/ml, not supplied with the kit, see ordering information) to each well after incubation if genomic DNA has to be free of RNA.*

Proceed with step 2 (Adjust binding conditions) of the standard protocol.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA quality or yield	*Low concentration of leukocytes in the whole blood sample* • Prepare buffy coat from the blood sample. *Incomplete cell lysis* • Sample not thoroughly mixed with Buffer BQ1/Proteinase K. Use of a shaker is recommended for optimal results. • Proteinase K digestion not optimal. Do not add Proteinase K directly to Buffer BQ1. • Increase incubation time. Incubate for at least 10 min at RT. *Reagents not applied or restored properly* • Reagents not properly restored. Add the indicated volume of Proteinase Buffer PB to the Proteinase K vial and 96 - 100% ethanol to Buffer B5 Concentrate and mix. *Kit storage* • Store aliquots of the reconstituted Proteinase K at - 20°C. • Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. *Suboptimal elution* • Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7.0. Use slightly alkaline elution buffer like Buffer BE (pH 8.5). • Be sure that all of the elution buffer gets into contact with the silica membrane. No drops should stick to the walls of the columns.

Clogging of binding strip/plate	*Clogging of the NucleoSpin® Blood Binding Strip/Plate* • If blood samples are too old and clotting occurs clogging of the NucleoSpin® Blood Binding Strip/Plate may appear. Check for blockage of NucleoSpin® Blood Binding Strip/Plate visually or automatically and remove supernatant. Increase time and strength for vacuum processing. Whole blood can be stored for several weeks at 4°C. Freeze samples at -20°C if blood should be stored for a longer periods.
Contamination of genomic DNA with RNA	*RNA carryover* • Add 10 µl (25 mg/ml) RNase A to the sample after the incubation of step 2, as recommended for working with fresh, unfrozen cells.
Suboptimal performance of DNA in downstream experiments	*Carryover of ethanol* • Be sure to remove all traces of Buffer B5 after the final washing step. Dry the NucleoSpin® Blood Binding Strip/Plate for at least 10 min with maximum vacuum. • Following the final wash step place NucleoSpin® Blood Binding Strip/Plate in an incubator for 10 min at 70°C to evaporate ethanol.
Vacuum manifold	*Vacuum pressure is not sufficient* • Check if the vacuum manifold lid fits tightly on the manifold base if vacuum is turned on. • Make sure that pump works properly and that any in-line filters are not blocked.
Buffers	*Buffer volumes are not enough* • Buffers are delivered in sufficient, but limited amounts. Calculate the needed buffer volumes and pour an additional amount of 10% into the reservoirs. • Do not fill back unused buffer from reservoir to the flask to avoid contaminations. Ask MACHEREY-NAGEL technical service for larger buffer volumes.

Cross-contamination	*Splattering of eluate* • If eluting with vacuum be sure that the distance between the outlets of the NucleoSpin® Blood Binding Strip/Plate and the Tube Strips is minimized. *Sample transfer* • Be sure that no liquid drops out of the tips while moving the tips.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Blood	740644	12 x 8 preps
NucleoSpin® 8 Blood	740644.5	60 x 8 preps
NucleoSpin® 96 Blood	740665.1	1 x 96 preps
NucleoSpin® 96 Blood	740665.4	4 x 96 preps
NucleoSpin® 96 Blood	740665.24	24 x 96 preps
NucleoSpin® 96 Blood Core Kit	740456.4	4 x 96 preps
Buffer BQ1	740923.1	1 l
Buffer B5 Concentrate	740921.100	100 ml
Buffer BW	740922.500	500 ml
Proteinase K	740506	100 mg
RNase A	740505	100 mg
Lysis Block	740484	4

Product	Cat. No.	Pack of
Rack of Tube Strips with Cap Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Round-well Block with Cap Strips (set consists of 1 Round-well Block 12 Cap Strips)	740475 740475.24	4 sets 24 sets
MN Wash Plate	740479 740479.24	4 24
MN Square-well Block	740476 740476.24	4 24
Starter Set A (for use of 8-well strips on the NucleoVac 96 and automation platforms)	740682	1 set
Starter Set C (for use of 8-well strips under centrifugation)	740684	1 set
MN Frame (for optimized handling of 96-well plates with vacuum manifold on BioRobot® 9600, 9604, and 3000 (Qiagen), MultiPROBE II (PerkinElmer), Biomek 2000, and FX (Beckman Coulter)	740680	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Self-adhering PE Foil	740676	50

6.3 Reference

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

6.4 Product use restriction / warranty

NucleoSpin® 8/96 Blood (Core) kit components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoSpin® 8/96 Blood (Core)** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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 an extra copy.

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