Genomic DNA from blood

User manual

NucleoSpin® 8 Blood

NucleoSpin® 8 Blood Core Kit

April 2014 / Rev. 07

MACHEREY-NAGEL

www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoSpin® 8 Blood	
	12 x 8 preps	**60 x 8 preps**
REF	**740664**	**740664.5**
Lysis Buffer BQ1	40 mL	2 x 100 mL
Wash Buffer B5 (Concentrate)[1]	50 mL	3 x 100 mL
Wash Buffer BW	100 mL	500 mL
Elution Buffer BE[2]	60 mL	250 mL
Proteinase K (lyophilized)[1]	75 mg	5 x 75 mg
Proteinase Buffer PB	8 mL	35 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, see section 2.4.

[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® 8 Blood Core Kit
	48 x 8 preps
REF	**740455.4**
Lysis Buffer BQ1	125 mL
Wash Buffer B5 (Concentrate)[1]	2 x 100 mL
Wash Buffer BW	300 mL
Elution Buffer BE[2]	125 mL
Proteinase K (lyophilized)[1]	4 x 75 mg
Proteinase Buffer PB	15 mL
NucleoSpin® Blood Binding Strips (red rings)	48
User manual	1

1.2 Reagent to be supplied by user

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

For more detailed information regarding special hardware required for centrifuge or vacuum processing, please see section 2.3.

For recommended accessories for use of the flexible NucleoSpin® 8 Blood Core Kit (reduced kit composition; REF 740455.4), please see section 2.4.

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

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

2 Product description

2.1 The basic principle

The **NucleoSpin® 8 Blood** kits are designed for the isolation of genomic DNA 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 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 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 the 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 Blood** kits allows simultaneous processing of up to 48 samples typically within less than 35 minutes.

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

- **NucleoSpin® 8 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 2.5 and contact your local distributor or MN directly.

- The **NucleoSpin® 8 Blood** kits allow for the purification of multiples of 8 samples. The kits are supplied with accessory plates for highest convenience. The **NucleoSpin® 8 Blood Core Kit** provides the buffers, Proteinase K and NucleoSpin® Blood Binding Strips 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 Blood (Core)**
Technology	Silica-membrane technology
Format	8-well strips
Processing	Manual or automated, optimized for vacuum processing
Sample material	Whole blood treated with EDTA, citrate, heparin, CPDA, human or animal blood Up to 200 µL whole blood, 2x 10^6 cultured cells
Fragment size	300 bp–approx. 50 kbp
Typical yield	4–6 µg
A_{260}/A_{280}	1.8–1.9
Elution volume	100 µL
Preparation time	35 min/6 strips
Binding capacity	20 µg

2.3 Required hardware

Vacuum processing

The **NucleoSpin® 8 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.

Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended. Alternatively, adjust the vacuum so that during the purification the sample flows through the column with a rate of 1–2 drops per second. Depending on the amount of sample being used, the vacuum times may need to be increased for complete filtration.

Centrifugation

For centrifugation, a microtiterplate centrifuge is required which is able to accommodate the NucleoSpin® Blood Binding Strips stacked on a Round- or Square-well Block and reaches accelerations of 5,600–6,000 x *g* (bucket height: 85 mm).

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.

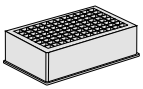
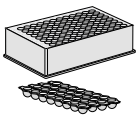
For general consumables and equipment needed, please see section 1.2.

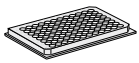
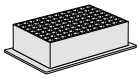
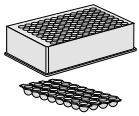
2.4 Recommended accessories for use of the NucleoSpin® 8 Blood Core Kit

The **NucleoSpin® 8 Blood Core Kit** provides all necessary buffers, enzymes, and NucleoSpin® Binding Strips. Accessories (e.g., lysis plates, waste collection plates, elution plates, or tubes) are not provided with the core kit. The reduced kit composition along with a large variety of separately available accessories, allow optimal adjustment of the kit to individual user needs. The user can select additional consumables according to his requirements for highest flexibility.

For use of **NucleoSpin® 8 Blood Core Kit,** follow the standard protocols (see section 5.1 or 5.2, respectively).

Recommended accessories for use of the **NucleoSpin® 8 Blood Core Kit** are available from MACHEREY-NAGEL. For ordering information please refer to section 6.2.

Protocol step	Suitable consumables, not supplied with the core kits	Remarks
1. Lyse samples	8 x Lysis Block per 48 x 8 preps  or 8 x Round-well Block with Cap Strips per 48 x 8 preps  or 8 x Rack of Tube Strips with Cap Strips per 48 x 8 preps 	Round-well Blocks and Tube Strips can be closed with Cap Strips.

Protocol step	Suitable consumables, not supplied with the core kits	Remarks
3. Transfer samples	8 x MN Wash Plate per 48 x 8 preps 	MN Wash Plate minimizes the risk of cross contamination (vacuum processing).
	2 x MN Square-well Block 	For waste collection during centrifugation (reusable).
8. Elute DNA	8 x Rack of Tubes Strips with Cap Strips per 48 x 8 preps 	Round-well Blocks and Tube Strips can be closed with Cap Strips.
	or 8 x Round-well Block with Cap Strips per 48 x 8 preps 	

2.5 Automated processing on robotic platforms

NucleoSpin® 8 Blood can be fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8 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 Blood Binding Strips.

Drying of the NucleoSpin® Blood Binding Strips under vacuum is sufficient because the bottom of the strips 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 online at ***www.mn-net.com*** or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol. Several application notes of the **NucleoSpin® 8 Blood** kit on various automation workstations can also be found at ***www.mn-net.com*** at Bioanalyis / Literature.

2.6 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 the table below for correlation between dispensed elution buffer volume and typical recoveries following the standard protocol.

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, preheating of the elution buffer to 70 °C will give about 10–15 % higher yields by supporting DNA recovery from the membrane.

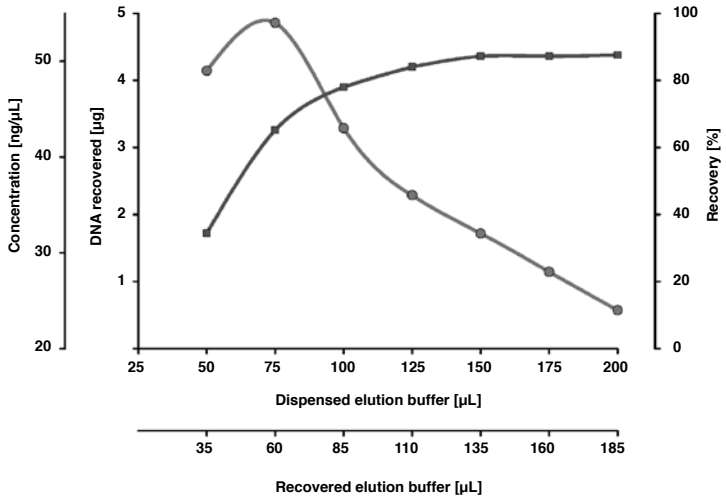


Figure 1: Elution of genomic DNA (vacuum processing)

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.

3 Storage conditions and preparation of working solutions

Attention: Buffers BQ1 and BW contain chaotropic salts! Wear gloves and goggles!

CAUTION: Buffers BQ1 and BW contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components should be stored at room temperature (18–25 °C) and are stable for at least one year. Storage at lower temperatures may cause precipitation of salts. If precipitation occurs, incubate the bottle for several minutes at about 30–40 °C and mix well until the precipitate is dissolved.

Before starting any **NucleoSpin® 8 Blood** protocol, prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96–100 %) to Buffer B5 Concentrate before use. Mark the label of the bottle to indicate that ethanol was added. Buffer B5 is stable at room temperature (18–25 °C) for at least one year.
- **Proteinase K:** Add the indicated volume of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for at least 6 months.

	NucleoSpin® 8 Blood	**NucleoSpin® 8 Blood**	**NucleoSpin® 8 Blood Core Kit**
	12 x 8 preps	**60 x 8 preps**	**48 x 8 preps**
REF	**740664**	**740664.5**	**740455.4**
Wash Buffer B5 (Concentrate)	50 mL Add 200 mL ethanol	3 x 100 mL Add 400 mL ethanol to each bottle	2 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 vial	4 x 75 mg Add 3.35 mL Proteinase Buffer to each vial

4 Safety instructions

The following components of the **NucleoSpin® 8 Blood** and **NucleoSpin® 8 Blood Core** kits contain hazardous contents.

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

GHS classification

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

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

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
BQ1	Guanidine hydrochloride 50–66 % *Guanidinhydrochlorid 50–66 %*	 Warning *Achtung*	302, 315, 319	280, 301+312, 302+352, 305+351+338, 330, 332+313, 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*	317, 334	261, 280, 302+352, 304+340, 333+313, 342+311, 363

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 315	Causes skin irritation. *Verursacht Hautreizungen.*
H 317	May cause an allergic skin reaction. *Verursacht Hautreizungen.*
H 319	Causes serious eye irritation. *Kann allergische Reaktionen verursachen*
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, hot surfaces, sparks, open flames and other ignition sources. No smoking. *Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten 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/ doctor/…/if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 302+352	IF ON SKIN: Wash with plenty of water/… *BEI KONTAKT MIT DER HAUT: Mit viel Wasser/… waschen.*
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. *BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
P 305+351+338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. *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 332+313	If skin irritation occurs: Get medical advice / attention. *Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 333+313	If skin irritation or rash occurs: Get medical advice / attention. *Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 337+313	Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/… *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/… anrufen.*
P 403+233	Store in a well ventilated place. Keep container tightly closed. *Behälter dicht geschlossen an einem gut belüfteten Ort aufbewahren.*
P 363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*

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

5 Protocols

5.1 NucleoSpin® 8 Blood – vacuum processing

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 17.
- For detailed information on each step, see page 18.
- For use of the NucleoSpin® 8 Blood Core Kit (REF 740455.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56 °C.
- Preheat Elution Buffer BE to 70 °C.

Protocol-at-a-glance

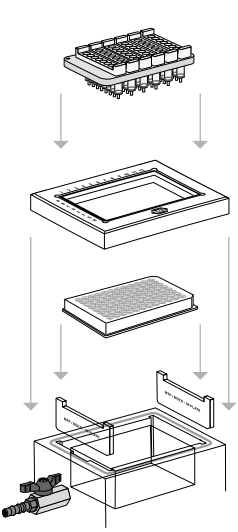
1	**Lyse** samples	**200 µL blood (equilibrated to room temperature)** **25 µL Proteinase K** **200 µL BQ1** **Mix 3 times** **Incubate at RT, 10 min** **or** **Mix 3 times and shake at 1250 rpm at RT, 10 min**
2	**Adjust** DNA binding conditions	**200 µL ethanol** **Mix at least 3–5 times** *Note: High-speed pipetting (400 µL/s) should be used for optimized mixing.* **Prepare the NucleoVac 96 Vacuum Manifold**
3	**Transfer** lysates to NucleoSpin® Blood Binding Strips	

4	**Overlay** samples with Buffer B5	**150 µL B5**
5	**Bind** DNA to silica membrane of the NucleoSpin® Blood Binding Strips	**- 0.2 bar***, **5 min**
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**
		Remove MN Wash Plate
7	**Dry** silica membrane	**- 0.6 bar***, **10 min**
8	**Elute** DNA	**50–200 µL BE** **Incubate 5 min at RT** **- 0.6 bar***, **1 min**

* Reduction of atmospheric pressure

Setup of vacuum manifold:

Binding / Washing steps

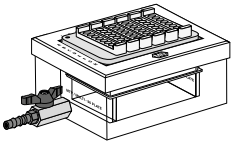


Step 4:
Place the NucleoSpin® Binding Strips inserted the Column Holder A on top of the manifold lid. Unused rows have to be filled with NucleoSpin® Dummy Strips.

Step 3:
Place the manifold lid on top of the manifold base.

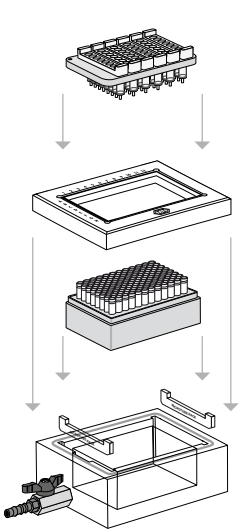
Step 2:
Place the MN Wash Plate in the manifold.

Step 1:
Insert spacers ‘MTP/MULTI-96 PLATE‘ and waste container in the manifold base.



Final setup

Elution step

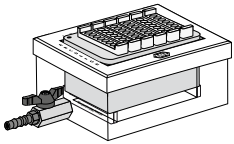


Step 4:
Place the NucleoSpin® Binding Strips inserted the Column Holder A on top of the manifold lid. Unused rows have to be filled with NucleoSpin® Dummy Strips.

Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the Rack of Tube Strips in the manifold.

Step 1:
Insert spacers ‘MICROTUBE RACK‘ in the manifold base.



Final setup

Detailed protocol

- For hardware requirements, refer to section 2.3.

  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 NucleoSpin® Dummy Strips to seal unused rows.

  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 NucleoSpin® Blood Binding Strips as required into the reusable column holder. Seal unused wells of NucleoSpin® Blood Binding Strips with Self-adhering PE-Foil and close unused wells with NucleoSpin® Dummy Strips. Place the Column Holder on the NucleoVac 96 manifold.
- For detailed information on each step, see page 25.
- For use of the NucleoSpin® 8 Blood Core Kit (REF 740455.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56 °C.
- *Preheat Elution Buffer BE to 70 °C (optional).*

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 place 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 seal unused positions 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 sample. Again, take care not to moisten the rims of the individual wells while dispensing the buffer. **Mix** by pipetting up and down **at least 3–5 times**.

Note: High-speed pipetting (400 µL/s) should be used for optimal mixing, if possible.

3 Transfer lysates

Transfer the lysates resulting from step 2 carefully into the wells of the NucleoSpin® Blood Binding Strips. When using the Rack of Tube Strips, remove the first Cap Strip and transfer lysates before removing the next Cap Strip. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination.

4 Overlay samples 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.

* Reduction of atmospheric pressure

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.

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.

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.

7 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.

* Reduction of atmospheric pressure

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)**. Release vacuum. For alternative elution procedures see section 2.3.

Finally, close Tube Strips with Cap Strips for storage.

Centrifuge the Rack of Tube Strips shortly to collect all sample at the bottom of the Tube Strips.

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

* Reduction of atmospheric pressure

5.2 NucleoSpin® 8 Blood – centrifuge processing

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

For processing under centrifugation, the Starter Kit C and a suitable centrifuge are required (see section 2.3). 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.

5.3 Modified lysis of blood samples

This modified lysis procedure may be used to increase the yield on some liquid handling instruments, for example, 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 step 5 (Bind DNA) of the standard procedure (see section 5.1).

5.4 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 procedure (section 5.1).

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 has not thoroughly been mixed with Buffer BQ1/Proteinase K. Use of a shaker is recommended for optimal results. • Proteinase K digestion was 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 were 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.

Problem	Possible cause and suggestions
Clogging of NucleoSpin® Blood Binding Strip	*Clogging of the NucleoSpin® Blood Binding Strips* • If blood samples are too old and clotting occurs, clogging of the NucleoSpin® Blood Binding Strips may appear. Check for blockage of NucleoSpin® Blood Binding Strips 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. *Insufficient vacuum pressure* • 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.
Contamination of genomic DNA with RNA	*RNA carry-over* • 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	*Carry-over of ethanol* • Be sure to remove all traces of Buffer B5 after the final washing step. Dry the NucleoSpin® Blood Binding Strips for at least 10 min with maximum vacuum. • Following the final wash step, place NucleoSpin® Blood Binding Strips in an incubator for 10 min at 70 °C to evaporate ethanol.
Cross-contamination	*Splattering of eluate* • If eluting with vacuum, be sure that the distance between the outlets of the NucleoSpin® Blood Binding Strips 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	REF	Pack of
NucleoSpin® 8 Blood	740664 740664.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 8 Blood Core Kit	740455.4	48 x 8 preps
NucleoSpin® 96 Blood	740665.1 740665.4 740665.24	1 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® 96 Blood Core Kit	740456.4	4 x 96 preps
Buffer BQ1	740923	125 mL
Buffer B5 Concentrate (for 500 mL Buffer B5)	740921.100	100 mL
Buffer BW	740922.500	500 mL
Proteinase K	740506	100 mg
RNase A (lyophilized)	740505	100 mg
Lysis Block	740484	4
MN Square-well Block	740476 740476 .24	4 24
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Round-well Block (1 set consists of 1 Round-well Block and 12 Cap Strips)	740475 740475.24	4 sets 24 sets
MN Wash Plate	740479 740479.24	4 24
Cap Strips	740478 740478.24	48 288
Starter Set A (for processing NucleoSpin® 8-well strips on NucleoVac 96 Vacuum Manifold)	740682	1

Product	REF	Pack of
Starter Set C (for processing NucleoSpin® 8-well strips under centrifugation)	740684	1
MN Frame	740680	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Self-adhering PE Foil	740676	50

6.3 Product use restriction/warranty

NucleoSpin® 8 Blood (Core Kit) components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the

DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

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