MACHEREY-NAGEL

User manual

Bioanalysis

Genomic DNA from blood

- NucleoSpin® 96 Blood
- NucleoSpin® 96 Blood Core Kit

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Blood		
REF	**1 × 96 preps 740665.1**	**4 × 96 preps[1] 740665.4**	**24 × 96 preps[1] 740665.24**
Lysis Buffer BQ1	45 mL	125 mL	6 × 125 mL
Wash Buffer B5 (Concentrate)[2]	100 mL	2 × 100 mL	12 × 100 mL
Wash Buffer BW	100 mL	300 mL	6 × 300 mL
Elution Buffer BE[3]	60 mL	125 mL	6 × 125 mL
Proteinase K (lyophilized)[2]	75 mg	4 × 75 mg	24 × 75 mg
Proteinase Buffer PB	2 × 1.8 mL	15 mL	6 × 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)	8	32	192
User manual	1	1	6

[1] The kit for 24 × 96 preparations (REF 740665.24) consists of 6 x REF 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

Kit contents *continued*

	NucleoSpin® 96 Blood Core Kit
REF	**4 × 96 preps 740456.4**
Lysis Buffer BQ1	125 mL
Wash Buffer B5 (Concentrate)[1]	2 × 100 mL
Wash Buffer BW	300 mL
Elution Buffer BE[2]	125 mL
Proteinase K (lyophilized)[1]	4 × 75 mg
Proteinase Buffer PB	15 mL
NucleoSpin® Blood Binding Plates (red rings)	4
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® 96 Blood Core Kit (reduced kit composition; REF 740456.4), please see section 2.4.

1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also 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 or updated revisions.

[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® 96 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 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® 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 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® 96 Blood** kits allows simultaneous processing of up to 96 samples typically within less than 70 minutes.
- **NucleoSpin® 96 Blood** kits can be processed completely at room temperature.
- **NucleoSpin® 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 2.5 and contact your local distributor or MN directly.
- The **NucleoSpin® 96 Blood** kits allow for the purification of multiples of 96 samples. The kits are supplied with accessory plates for highest convenience. **The NucleoSpin® 96 Blood Core Kit** provides the buffers, Proteinase K and NucleoSpin® Blood Binding Plates 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® 96 Blood (Core)**
Technology	Silica membrane technology
Format	96-well plates
Processing	Manual or automated, optimized for vacuum or positive pressure processing
Sample material	Whole blood treated with EDTA, citrate, heparin, CPDA, human or animal blood
	Up to 200 μL whole blood, 2 × 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	70 min/plate
Binding capacity	20 μg
Use	For research use only

2.3 Required hardware

Vacuum processing

The **NucleoSpin® 96 Blood** kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information). When using **NucleoSpin® 96 Blood** with less than 96 samples, Self adhering PE Foil (see ordering information) should be used in order to close and protect non-used wells of the NucleoSpin® Blood Binding Plate and thus guarantee proper vacuum.

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. This centrifuge must be able to accomodate the NucleoSpin® Blood Binding Plate stacked on a Round- or Square-well Block and reach accelerations of 5,600–6,000 x *g* is required (bucket height: 85 mm).

Regarding waste collection, suitable consumables (e.g., MN Square-well Blocks) are necessary and they are not included in the kit. For the most convenient handling, without the need of emptying and reusing the MN Square-well Blocks, we recommend using six MN Square-well Blocks if two 96-well plates are processed at once (see ordering information). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the amount of MN Square-well Blocks needed.

2.4 Recommended accessories for use of the NucleoSpin® 96 Blood Core Kit

The **NucleoSpin® 96 Blood Core Kit** provides all necessary buffers, enzymes, and NucleoSpin® Binding Plates. 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® 96 Blood Core Kit**, follow the standard protocols (see section 5.1 or 5.2, respectively).

Recommended accessories for use of the **NucleoSpin® 96 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	4 x Lysis Block per 4 × 96 preps or 4 x Round-well Block with Cap Strips per 4 × 96 preps or 4 x Rack of Tube Strips with Cap Strips per 4 × 96 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	4 x MN Wash Plate per 4 × 96 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	4 x Rack of Tubes Strips with Cap Strips per 4 × 96 preps or 4 x Round-well Block with Cap Strips per 4 × 96 preps	Round-well Blocks and Tube Strips can be closed with Cap Strips.

2.5 Automated processing on robotic platforms

NucleoSpin® 96 Blood can be fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 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® 96 Blood Binding Plate.

Drying of the NucleoSpin® Blood Binding Plate under vacuum is sufficient because the bottom of the 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 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® 96 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.

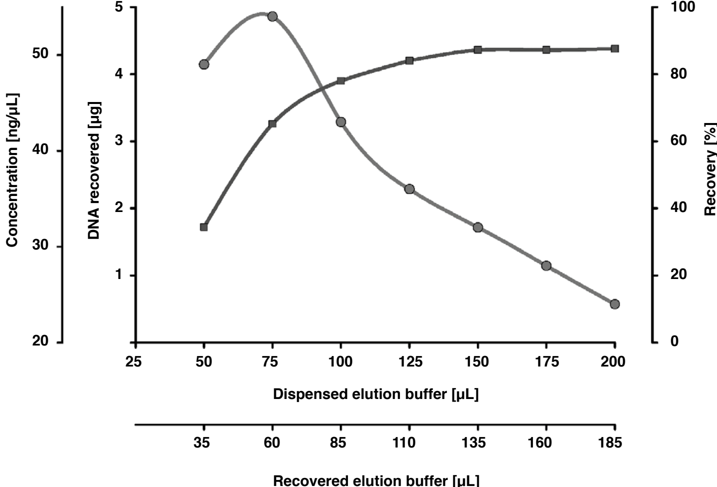


Figure 1 Elution of genomic DNA (vacuum processing)
Total DNA yield, recovery (- ▪ -) and concentration of recovered DNA (- • -) are plotted versus dispensed elution buffer volume. High elution buffer volumes result in high elution efficiency whereas high concentrated DNA solutions can be obtained with 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.

Storage conditions:

- Store NucleoSpin® Blood Binding Plates at 4 °C upon arrival.
- All other kit components should be stored at room temperature (15 – 25 °C) and are stable until: see package label. 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® 96 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 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® 96 Blood	**NucleoSpin® 96 Blood**	**NucleoSpin® 96 Blood Core Kit**
	1 × 96 preps	**4 × 96 preps**	**4 × 96 preps**
REF	**740665.1**	**740665.4**	**740456.4**
Wash Buffer B5 (Concentrate)	100 mL Add 400 mL ethanol	4 × 100 mL Add 400 mL ethanol to each bottle	4 × 100 mL Add 400 mL ethanol to each bottle
Proteinase K (lyophilized)	75 mg Add 3.35 mL Proteinase Buffer	4 × 75 mg Add 3.35 mL Proteinase Buffer to each vial	4 × 75 mg Add 3.35 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoSpin®96 Blood** and **NucleoSpin®96 Blood Core** 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 BW, and guanidinium thiocyanate in buffer BQ1 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®96 Blood** and **NucleoSpin®96 Blood Core** 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 Protocols

5.1 NucleoSpin® 96 Blood – vacuum processing

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 15.
- For detailed information on each step, see page 15.
- For use of the NucleoSpin® 96 Blood Core Kit (REF 740456.4), 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.
- Preheat Elution Buffer BE to 70 °C.

Protocol at a glance

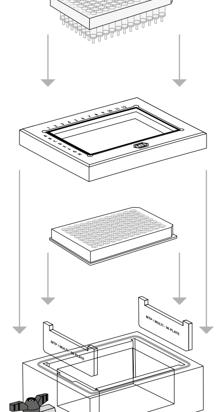
1	**Lyse** samples	**200 µL blood (equilibrated to room temperature)** **25 µL Proteinase K** **200 µL BQ1** **Mix 3 times** **Incubate at RT, 10 min** **or** **Mix 3 times and shake at 1250 rpm at RT, 10 min**
2	**Adjust** DNA binding conditions	**200 µL ethanol** **Mix at least 3 – 5 times** *Note: 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 Plate	
4	**Overlay** samples with Buffer B5	**150 µL B5**
5	**Bind** DNA to silica membrane of the NucleoSpin® Blood Binding Plate	**- 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

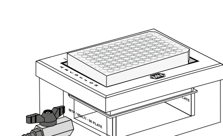


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

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

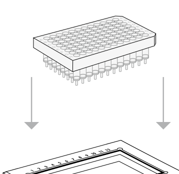
Step 2:
Place the MN Wash Plate in the manifold.

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



Final setup

Elution step

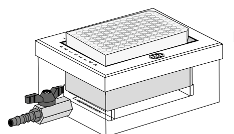


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

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 detailed information regarding the vacuum manifold setup, see page 15.
- For use of the NucleoSpin® 96 Blood Core Kit (REF 740456.4), 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.
- *Preheat Elution Buffer BE to 70 °C (optional).*

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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 lysis 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 place the MN Wash Plate on them. Close the manifold with the manifold lid.

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

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2 Adjust DNA binding conditions

Add **200 µL 96 – 100 % ethanol** to each well of the Lysis Block. 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**. Spin briefly (10 s; 1,500 x *g*) to collect any sample from the lid.

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

---

3 Transfer lysates

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.

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4 Overlay samples 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.

Remove MN Wash Plate

After the final washing step, close the valve, release the vacuum, and remove the NucleoSpin® Blood Binding Plate 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 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 drops come out. Insert the NucleoSpin® Blood Binding Plate 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 NucleoSpin® Blood Binding Plate 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® 96 Blood – centrifuge processing

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

Follow the standard protocol as described in section 5.1. 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 a 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.

5.3 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	Predispense **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 Plate with Self adhering PE-Foil (see ordering information).

A	**Harvest cells** Harvest cells (maximum starting amount 2 × 106) 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

<table>
<tr><th>Problem</th><th>Possible cause and suggestions</th></tr>
<tr><td>Poor DNA quality or yield</td><td>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.</td></tr>
</table>

<table>
<tr><th>Problem</th><th>Possible cause and suggestions</th></tr>
<tr><td>Clogging of NucleoSpin® Blood Binding Strip</td><td>Clogging of the NucleoSpin® Blood Binding Plate
If blood samples are too old and clotting occurs, clogging of the NucleoSpin® Blood Binding Plate may appear. Check for blockage of NucleoSpin® Blood Binding 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.
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.</td></tr>
<tr><td>Contamination of genomic DNA with RNA</td><td>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.</td></tr>
<tr><td>Suboptimal performance of DNA in downstream experiments</td><td>Carry-over of ethanol
Be sure to remove all traces of Buffer B5 after the final washing step. Dry the NucleoSpin® Blood Binding Plate for at least 10 min with maximum vacuum.
Following the final wash step, place NucleoSpin® Blood Binding Plate in an incubator for 10 min at 70 °C to evaporate ethanol.</td></tr>
<tr><td>Cross-contamination</td><td>Splattering of eluate
If eluting with vacuum, be sure that the distance between the outlets of the NucleoSpin® Blood Binding Plate and the Tube Strips is minimized.
Sample transfer
Be sure that no liquid drops out of the tips while moving the tips.</td></tr>
</table>

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 Blood	740665.1 740665.4 740665.24	1 × 96 preps 4 × 96 preps 24 × 96 preps
NucleoSpin® 96 Blood Core Kit	740456.4	4 × 96 preps
NucleoSpin® 8 Blood	740664 740664.5	12 × 8 preps 60 × 8 preps
NucleoSpin® 8 Blood Core Kit	740455.4	48 × 8 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
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

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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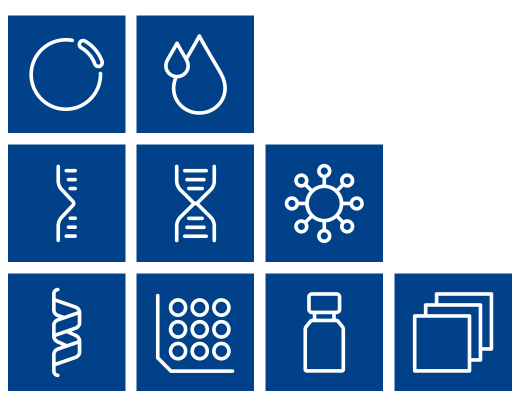

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