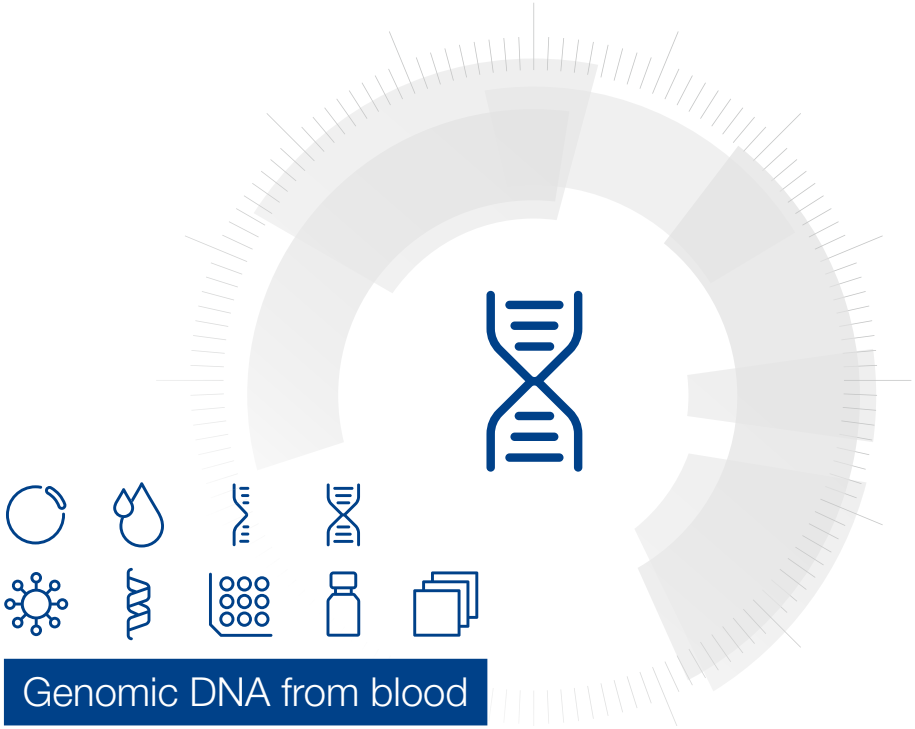
Bioanalysis

MACHEREY-NAGEL

User manual



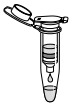
Genomic DNA from blood

- NucleoSpin® Blood
- NucleoSpin® Blood L
- NucleoSpin® Blood XL
- NucleoSpin® Blood QuickPure

March 2026 / Rev. 20

Genomic DNA from blood

Protocol at a glance (Rev. 20)

	Mini **NucleoSpin® Blood**	Midi **NucleoSpin® Blood L**	Maxi **NucleoSpin® Blood XL**	Mini **NucleoSpin® Blood QuickPure**
1 Lyse blood samples	 200 µL blood 25 µL Pro.K 200 µL B3 Mix 70 °C 10 – 15 min	 2 mL blood 150 µL Pro.K 2 mL µL BQ1 Mix 56 °C 10 – 15 min	 10 mL blood 500 µL Pro.K 10 mL BQ1 Mix 56 °C 10 – 15 min	 200 µL blood 25 µL Pro.K 200 µL BQ1 Mix 70 °C 10 – 15 min
2 Adjust DNA binding conditions	210 µL ethanol	2 mL ethanol	10 mL ethanol	200 µL ethanol
3 Bind DNA	 Load all 11,000 x *g* 1 min	 Load 3 mL 4,500 x *g* 3 min	 Load 15 mL 4,000 x *g* 3 min	 Load all 11,000 x *g* 1 min
	—	Load 3 mL of residue 4,500 x *g* 5 min	Load 15 mL of residue 4,000 x *g* 3 min	—
4 Wash silica membrane				
1st wash	500 µL BW 11,000 x *g* 1 min	2 mL BQ2 4,500 x *g* 2 min	7.5 mL BQ2 4,000 x *g* 2 min	350 µL BQ2 11,000 x *g* 3 min
2nd wash	600 µL B5 11,000 x *g* 1 min	2 mL BQ2 4,500 x *g* 10 min	7.5 mL BQ2 4,000 x *g* 10 min	—
5 Dry silica membrane	11,000 x *g* 1 min	Drying is performed during centrifugation of the last washing step	Drying is performed during centrifugation of the last washing step	Drying is performed during centrifugation of the last washing step
6 Elute highly pure DNA	 100 µL BE (70 °C) RT 1 min 11,000 x *g* 1 min	 200 µL BE (70 °C) RT 2 min 4,500 x *g* 2 min	 500 – 2000 µL BE (70 °C) RT 2 min 4,000 x *g* 2 min	 50 µL BE (70 °C) RT 1 min 11,000 x *g* 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® Blood		
REF	**10 preps 740951.10**	**50 preps 740951.50**	**250 preps 740951.250**
Lysis Buffer B3	10 mL	15 mL	60 mL
Wash Buffer BW	6 mL	30 mL	150 mL
Wash Buffer B5 (Concentrate)*	6 mL	12 mL	50 mL
Elution Buffer BE**	13 mL	13 mL	60 mL
Proteinase K (lyophilized)*	6 mg	30 mg	2 × 75 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
NucleoSpin® Blood Columns (red rings – plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	20	100	500
User manual	1	1	1

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

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

Kit contents *continued*

	NucleoSpin® Blood L	
REF	**20 preps 740954.20**	**100 preps 740954.100**
Lysis Buffer BQ1	45 mL	2 × 125 mL
Wash Buffer BQ2 (Concentrate)*	20 mL	2 × 50 mL
Elution Buffer BE**	13 mL	60 mL
Proteinase K (lyophilized)*	60 mg	5 × 60 mg
Proteinase Buffer PB	8 mL	35 mL
NucleoSpin® Blood L Columns (plus Collection Tubes)	20	100
Collection Tubes (15 mL)	20	100
User manual	1	1

	NucleoSpin® Blood XL	
REF	**10 preps 740950.10**	**50 preps 740950.50**
Lysis Buffer BQ1	125 mL	3 × 200 mL
Wash Buffer BQ2 (Concentrate)*	50 mL	4 × 50 mL
Elution Buffer BE**	30 mL	125 mL
Proteinase K (lyophilized)*	126 mg	5 × 126 mg
Proteinase Buffer PB	8 mL	35 mL
NucleoSpin® Blood XL Columns (plus Collection Tubes)	10	50
Collection Tubes (50 mL)	10	50
User manual	1	1

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

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

Kit contents *continued*

	NucleoSpin® Blood QuickPure		
REF	**10 preps 740569.10**	**50 preps 740569.50**	**250 preps 740569.250**
Lysis Buffer BQ1	13 mL	13 mL	60 mL
Wash Buffer BQ2 (Concentrate)*	7 mL	7 mL	2 × 20 mL
Elution Buffer BE**	13 mL	13 mL	60 mL
Proteinase K (lyophilized)*	6 mg	30 mg	2 × 75 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
NucleoSpin® Blood QuickPure Columns (dark red rings – plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	10	50	250
User manual	1	1	1

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

Reagents

- 96 – 100 % ethanol
- Phosphate-buffered saline (PBS) may be required for some samples

Consumables

- 1.5 mL microcentrifuge tubes (NucleoSpin® Blood / QuickPure), 15 mL (NucleoSpin® Blood L), or 50 mL centrifuge tubes (NucleoSpin® Blood XL), for sample lysis and DNA elution
- Disposable pipette tips

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

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

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes (NucleoSpin® Blood/QuickPure), centrifuge for 15 mL (NucleoSpin® Blood L) or 50 mL (NucleoSpin® Blood XL) centrifuge tubes, with a swing-bucket rotor
- Vortex mixer
- Thermal heating block (NucleoSpin® Blood / QuickPure) or water bath (NucleoSpin® Blood L / XL)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended for the first time users to read the detailed protocol sections of the user manual NucleoSpin® Blood kits before using these products. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes to the current user manual compared with previous revisions.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Blood** method, genomic DNA is prepared from whole blood, cultured cells, serum, plasma, or other body fluids. Lysis is achieved by incubation of whole blood in a solution containing large amounts of chaotropic ions in the presence of Proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding **NucleoSpin® Blood Columns** are achieved by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations. With the **NucleoSpin® Blood QuickPure** kit, contaminations are removed by a single wash step. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® Blood** kits are designed for the rapid isolation of highly pure genomic DNA from whole blood, serum, plasma, or other body fluids. It is also possible to purify viral DNA (e.g., HBV) from blood samples. As viral DNA co-purifies with cellular DNA, we recommend using cell-free samples (serum or plasma) to prepare pure viral DNA.
- The **NucleoSpin® Blood QuickPure** kit is designed for ultra-fast small-scale purification of highly pure genomic DNA from whole blood, serum, plasma, or other body fluids. The number of washing and drying steps is reduced from 3 to 1! Therefore, the hands-on time is less than 10 min.
- DNA can be purified successfully from blood samples treated with EDTA, citrate, or heparin. If leukocyte rich materials like buffy coat are used, apply smaller volumes and dilute the samples with sterile PBS (dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 in 800 mL H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 liter).
- The kits allow purification of highly pure genomic DNA with an A_{260}/A_{280} ratio between 1.60 and 1.90 and a typical concentration of 40 – 60 ng per µL for the **NucleoSpin® Blood** kit, 80 – 120 ng per µL for the **NucleoSpin® Blood QuickPure** kit and 200 – 300 ng per µL for the **NucleoSpin® Blood L / XL** kits.
- The obtained DNA is ready-to-use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reactions.

Table 1: Kit specifications at a glance

Parameter	Blood	Blood L	Blood XL	Blood QuickPure
Sample material	Up to 200 µL / 5×10^6 cells	Up to 2 mL / 2×10^7 cells	Up to 10 mL / 1×10^8 cells	Up to 200 µL / 5×10^6 cells
Typical yield	4 – 6 µg	40 – 60 µg	200 – 300 µg	4 – 6 µg
Elution volume	60 – 200 µL	120 – 200 µL	600 – 2000 µL	30 – 50 µL
Binding capacity	60 µg	250 µg	700 µg	50 µg
Preparation time	30 min/prep	1 h/prep	1 h/prep	< 10 min/prep
Format	Mini spin column	Midi spin column	Maxi spin column	Mini spin column
Use	For research use only			

2.3 Storage of blood samples

For the isolation of genomic DNA from blood treated with anticoagulants (heparin, citrate, or EDTA) using a **NucleoSpin® Blood** kit the blood samples can be stored at room temperature, +4 °C, or frozen.

Blood samples stored at room temperature or +4 °C for up to several days or weeks, respectively, will still allow DNA isolation. However, DNA yield and quality will slowly decrease due to prolonged storage of blood samples under these conditions.

Blood stored frozen for years is well suited for DNA isolation.

Highest yields and quality of DNA are obtained from fresh blood.

2.4 Elution procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 70 – 90 %) there are several modifications possible. Use elution buffer preheated to 70 °C for one of the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90 – 100 % of bound nucleic acid can be eluted.
- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution (**NucleoSpin® Blood**: ca. 130 %; **NucleoSpin® Blood QuickPure**: ca. 150 ; **NucleoSpin® Blood L**: ca. 140 %; **NucleoSpin® Blood XL**: ca. 115 %). Maximum yield of bound nucleic acid is about 80 %.
- **High yield and high concentration:** Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply

a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85 – 100 % of bound nucleic acid is eluted in the standard elution volume at a high concentration.

- **Convenient elution:** For convenience, elution buffer of ambient temperature may be used. This will result in a lower yield (approximately 20 %) compared to elution with preheated elution buffer.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and / or multi use storage at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in subsequent downstream applications we recommend elution with the supplied elution buffer and storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g., > 10 kbp) or detection sensitivity of trace amount of DNA species might be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at +4 °C or room temperature due to DNA shearing or adsorption to surfaces.

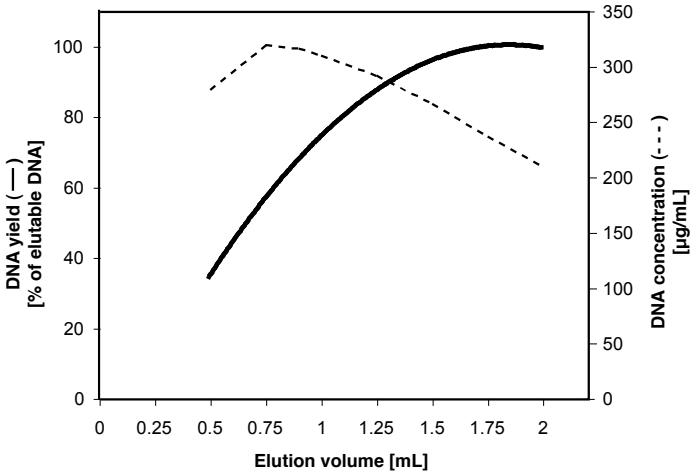


Figure 1: Dependence of DNA yield (solid line) and concentration (dashed line) on elution volume.
Genomic DNA was purified from 10 mL whole blood and eluted using different elution volumes as indicated. Highest DNA yield is obtained with 1.5 – 2.0 mL elution volume. Highest DNA concentration is obtained with approximately 0.75 mL elution volume. Furthermore, yield and concentration may vary as they depend on the kind of sample (blood, serum, plasma), type of blood sample (human or animal), and quality of the samples (fresh, old, frozen, clotted, etc.).

3 Storage conditions and preparation of working solutions

Attention: Buffers B3, BQ1, and BW contain chaotropic salt. Wear gloves and goggles!

CAUTION: Buffer B3, 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.

- Blood L and XL columns need to be stored at 4 °C. All other kit components can be stored at 15 – 25 °C and are stable until: see package label.
- During storage, especially at low temperatures, a white precipitate may form in Buffer T1, B3, or BQ1. Such precipitates can be easily dissolved by incubating the bottle at 70 °C before use.

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

- **Wash Buffer B5** (NucleoSpin® Blood): Add the indicated volume of ethanol (96 – 100 %) to **Wash Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer B5 at 15 – 25 °C for up to one year.
- **Wash Buffer BQ2** (NucleoSpin® Blood L / XL / QuickPure): Add the indicated volume of ethanol (96 – 100 %) to **Wash Buffer BQ2 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer BQ2 at 15 – 25 °C for up to 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 up to 6 months.

	NucleoSpin® Blood		
REF	**10 preps 740951.10**	**50 preps 740951.50**	**250 preps 740951.250**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol
Proteinase K	6 mg Add 260 µL Proteinase Buffer	30 mg Add 1.35 mL Proteinase Buffer	2 × 75 mg Add 3.35 mL Proteinase Buffer to each vial

	NucleoSpin® Blood L	NucleoSpin® Blood XL	NucleoSpin® Blood XL
REF	**20 preps 740954.20**	**10 preps 740950.10**	**50 preps 740950.50**
Wash Buffer BQ2 (Concentrate)	20 mL Add 80 mL ethanol	50 mL Add 200 mL ethanol	4 × 50 mL Add 200 mL ethanol to each bottle
Proteinase K	60 mg Add 3.15 mL Proteinase Buffer	126 mg Add 5.75 mL Proteinase Buffer	5 × 126 mg Add 5.75 mL Proteinase Buffer to each vial

	NucleoSpin® Blood QuickPure		
REF	**10 preps 740569.10**	**50 preps 740569.50**	**250 preps 740569.250**
Wash Buffer BQ2 (Concentrate)	7 mL Add 28 mL ethanol	7 mL Add 28 mL ethanol	2 × 20 mL Add 80 mL ethanol to each bottle
Proteinase K	6 mg Add 260 µL Proteinase Buffer	30 mg Add 1.35 mL Proteinase Buffer	2 × 75 mg Add 3.35 mL Proteinase Buffer to each vial

4 Safety instructions

When working with one of the **NucleoSpin® Blood** kits 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 ***www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer B3, BQ1 and buffer BW can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with one of the **NucleoSpin® Blood** kits 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 for DNA purification from whole blood

5.1 Genomic DNA purification with NucleoSpin® Blood

Before starting the preparation:

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

1 Lyse blood sample

Pipette **25 µL Proteinase K** and up to **200 µL blood**, body fluid sample, or buffy coat from 1 mL blood (equilibrated to room temperature) into 1.5 mL microcentrifuge tubes (not provided).

200 µL blood

+ 25 µL Proteinase K

For sample volumes less than 200 µL, add PBS to adjust the volume to 200 µL. If purifying DNA viruses, we recommend starting with 200 µL serum or plasma. If cultured cells are used, resuspend up to 5 × 10^6 cells in a final volume of 200 µL PBS.

+ 200 µL B3

Mix

Add **200 µL Buffer B3** to the samples and vortex the mixture vigorously (10 – 20 s).

70 °C
10 – 15 min

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

Incubate samples at **70 °C** for **10 – 15 min**.

The lysate should become brownish during incubation with Buffer B3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **210 µL ethanol (96 – 100 %)** to each sample and vortex again.

+ 210 µL ethanol

Mix

3 Bind DNA

For each preparation, take one **NucleoSpin® Blood Column** placed in a Collection Tube and load the sample. Centrifuge **1 min** at **11,000 x *g***. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000 x *g*). Discard Collection Tube with flow-through.

Load lysate

11,000 x *g*
1 min

4 Wash silica membrane

1st wash

Place the NucleoSpin® Blood Column into a new Collection Tube (2 mL) and add **500 µL Buffer BW**. Centrifuge **1 min** at **11,000 x *g***. Discard Collection Tube with flow-through.

+ 500 µL BW

11,000 x *g*
1 min

2nd wash

Place the NucleoSpin® Blood Column into a new Collection Tube (2 mL) and add **600 µL Buffer B5**. Centrifuge **1 min** at **11,000 x *g***. Discard flow-through and reuse Collection Tube.

+ 600 µL B5

11,000 x *g*
1 min

5 Dry silica membrane

Place the NucleoSpin® Blood Column back into the Collection Tube and centrifuge **1 min** at **11,000 x *g***.

Residual ethanol is removed during this step.

11,000 x *g*

1 min

6 Elute highly pure DNA

Place the NucleoSpin® Blood Column in a 1.5 mL microcentrifuge tube (not provided) and add **100 µL preheated Buffer BE (70 °C)**. Dispense buffer directly onto the silica membrane. Incubate at **room temperature** for **1 min**. Centrifuge **1 min** at **11,000 x *g***.

For alternative elution procedures see section 2.4.

+ 100 µL BE (70 °C)

RT
1 min

11,000 x *g*
1 min

5.2 Genomic DNA purification with NucleoSpin® Blood L

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.
- Preheat Elution Buffer BE to 70 °C.
- For centrifugation, a centrifuge with a **swing-out rotor** and appropriate buckets capable of reaching 4,000 – 4,500 x *g* is required.

1 Lyse blood sample

2 mL blood

+ 150 µL Proteinase K

Pipette up to **2 mL blood** (or body fluid) sample (equilibrated to room temperature) and **150 µL Proteinase K** into a 15 mL tube (not provided).

If processing buffy coat, do not use more than 1 mL and add PBS to adjust the volume to 2 mL.

If cultured cells are used, resuspend up to 2 × 10^7 cells in a final volume of 2 mL PBS.

If old or clotted blood samples are processed, see section 6.1 *for recommendations.*

+ 2 mL BQ1

Mix

Add **2 mL Buffer BQ1** (if processing less than 2 mL blood, add one volume of Buffer BQ1) to the samples and vortex the mixture vigorously for 10 s.

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

56 °C
15 min

Incubate samples at **56 °C** for **15 min**.

Let the samples cool down to room temperature before proceeding with addition of ethanol.

The lysate should become brownish during incubation with Buffer BQ1. Increase incubation time with Proteinase K (up to 20 min) and vortex once or twice during incubation if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **2 mL ethanol (96 – 100 %)** (if processing less than 2 mL blood, add 1 volume of ethanol) to each sample and mix by inverting the tube 10 times.

+ 2 mL ethanol

Mix

Note: High local ethanol concentration must be avoided by immediate mixing after addition.

Be sure that the lysate has cooled down to room temperature before loading it onto the column. Loading of hot lysate may lead to diminished yields.

3 Bind DNA

For each preparation, take one **NucleoSpin® Blood L Column** placed in a Collection Tube and load **3 mL of lysate**. Do not moisten the rims of the columns. Close the tubes with screw caps and centrifuge **3 min** at **4,500 x *g***.

Load 3 mL

4,500 x *g*
3 min

Usually the lysate will start to flow-through the columns even before centrifugation. This will not adversely affect DNA yield or purity. Keep NucleoSpin® Blood L Column in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.

Load **all of the remaining lysate** in a second step to the respective NucleoSpin® Blood L Column, avoiding moistening the rim. Centrifuge **5 min** at **4,500 x *g***. Discard the flow-through and place the column back into the Collection Tube.

Load residue

4,500 x *g*
5 min

Remove the Collection Tube with the column carefully from the rotor to avoid that the flow-through comes in contact with the column outlet. Be sure to wipe off any spilled lysate from the Collection Tube before placing the column back.

4 Wash silica membrane

1st wash

Add **2 mL Buffer BQ2**. Centrifuge **2 min** at **4,500 x *g***.

It is not necessary to discard the flow-through after the first washing step.

+ 2 mL BQ2

4,500 x *g*
2 min

2nd wash

Add **2 mL Buffer BQ2**. Centrifuge **10 min** at **4,500 x *g***. Remove the column carefully from the rotor in order to avoid that the flow-through comes in contact with the column outlet.

By prolonged centrifugation during this second washing step, residual ethanolic washing Buffer BQ2 is removed from the silica membrane of the NucleoSpin® Blood L Column.

+ 2 mL BQ2

4,500 x *g*
10 min

5 Dry silica membrane

The drying of the NucleoSpin® Blood L Column is performed by prolonged centrifugation time (10 min) in the 2nd wash step.

6 Elute highly pure DNA

Insert the column into a new Collection Tube (15 mL) and apply **200 µL preheated Buffer BE (70 °C)** directly to the center of the silica membrane. Incubate at **room temperature** for **2 min**. Centrifuge at **4,500 x *g*** for **2 min**.

For alternative elution procedures see section 2.4.

+ 200 µL BE (70 °C)

RT
1 min

4,500 x *g*
2 min

5.3 Genomic DNA purification with NucleoSpin® Blood XL

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.
- Preheat Elution Buffer BE to 70 °C.
- For centrifugation, a centrifuge with a **swing-out rotor** and appropriate buckets capable of reaching 4,000 – 4,500 x *g* is required.

1 Lyse blood sample

Pipette up to **10 mL blood** (or body fluid) sample (equilibrated to room temperature) and **500 µL Proteinase K** into a 50 mL tube (not provided).

10 mL blood

+ 500 µL Proteinase K

If you process ≤ 5 mL blood, sample loading with a single centrifugation step is possible (step 3).

If processing buffy coat, do not use more than 2 mL and add PBS to adjust the volume to 10 mL.

If cultured cells are used, resuspend up to 1 × 10^8 cells in a final volume of 10 mL PBS.

+ 10 mL BQ1

Mix

If old or clotted blood samples are processed, see section 6.1 *for recommendations*.

56 °C
15 min

Add **10 mL Buffer BQ1** (if processing less than 10 mL blood, add one volume of Buffer BQ1) to the samples and vortex the mixture vigorously for 10 s.

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

Incubate samples at **56 °C** for **15 min**.

Let the lysate cool down to room temperature before proceeding with addition of ethanol.

The lysate should become brownish during incubation with Buffer BQ1. Increase incubation time with Proteinase K (up to 20 min) and vortex once or twice during incubation if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **10 mL ethanol (96 – 100 %)** (if processing less than 10 mL blood, add one volume of ethanol) to each sample and mix by inverting the tube 10 times.

+ 10 mL ethanol

Mix

Note: High local ethanol concentration must be avoided by immediate mixing after addition.

Be sure that the lysate has cooled down to room temperature (about 5 min) before loading it onto the columns. Loading of hot lysate may lead to diminished yields.

3 Bind DNA

For each preparation, take one **NucleoSpin® Blood XL Column** placed in a Collection Tube and load **15 mL of lysate**. Do not moisten the rim of the column. Close the tubes with screw caps and centrifuge **3 min** at **4,000 x *g***. Discard flow-through.

Load 15 mL

4,000 x *g*
3 min

Discarding the flow-through may be omitted. Be careful after the second loading step during removal of the tube from the centrifuge and the removal of the column from the tube: keep tube with column upright to avoid contact of flow-through with the column outlet.

Load residue

Usually the lysate will start to flow through the column even before centrifugation. This will not adversely affect DNA yield or purity. Keep NucleoSpin® Blood XL Column in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.

4,000 x *g*
3 min

Load **15 mL of the remaining lysate** to the respective NucleoSpin® Blood XL Column. Again, avoid moistening the rim. Centrifuge **3 min** at **4,000 x *g***. Discard the flow-through and place the column back into the Collection Tube.

Remove the Collection Tube with the column carefully from the rotor and avoid that the flow-through comes in contact with the column outlet.

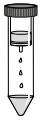
If you process ≤ 5 mL blood no loading of remaining lysate is necessary.

4 Wash silica membrane

1st wash

Add **7.5 mL Buffer BQ2** to the NucleoSpin® Blood XL Column. Centrifuge **2 min** at **4,000 x *g***.

It is not necessary to discard the flow-through after the first washing step.



+ 7.5 mL BQ2

4,000 x *g*
2 min

2nd wash

Add **7.5 mL Buffer BQ2**. Centrifuge **10 min** at **4,000 x *g***. Remove the column carefully from the rotor to avoid that flow-through gets in contact with the column outlet.

By prolonged centrifugation during this second washing step, residual ethanolic Buffer BQ2 is removed from the silica membrane of the NucleoSpin® Blood XL Column.

+ 7.5 mL BQ2

4,000 x *g*
10 min

5 Dry silica membrane

The drying of the NucleoSpin® Blood XL Column is performed by prolonged centrifugation time (10 min) in the 2nd wash step.

6 Elute highly pure DNA

Insert the column into a new Collection Tube (50 mL) and apply **1000 µL of preheated Buffer BE (70 °C)** directly to the center of the silica membrane. Incubate at **room temperature** for **2 min**. Centrifuge at **4,000 x *g*** for **2 min**.

For alternative elution procedures see section 2.4.

+ 1000 µL BE (70 °C)

RT
2 min

4,000 x *g*
2 min

5.4 Genomic DNA purification with NucleoSpin® Blood QuickPure

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 70 °C.
- Preheat Elution Buffer BE to 70 °C.

1 Lyse blood sample

Pipette **25 µL Proteinase K** and up to **200 µL blood**, buffy coat or body fluid sample (equilibrated to room temperature) into 1.5 mL microcentrifuge tubes (not provided).

200 µL blood

+ 25 µL Proteinase K

For sample volumes less than 200 µL, add PBS to adjust the volume to 200 µL. If cultured cells are used, resuspend up to 5 × 10^6 cells in a final volume of 200 µL PBS.

+ 200 µL BQ1

Add **200 µL Lysis Buffer BQ1** to the samples and vortex the mixture vigorously (10 – 20 s).

Mix

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

70 °C
10 – 15 min

Incubate samples at **70 °C** for **10 – 15 min**.

The lysate should become brownish during incubation with Buffer BQ1. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **200 µL ethanol (96 – 100 %)** to each sample and vortex again.

+ 200 µL ethanol

Mix

3 Bind DNA

Apply the samples to the **NucleoSpin® Blood QuickPure Columns** placed in a Collection Tube and centrifuge **1 min** at **11,000 x *g***. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher *g*-force (up to 15,000 x *g*). Discard Collection Tube with flow-through.

Load lysate

11,000 x *g*
1 min

4 Wash silica membrane

+ 350 µL BQ2

Place the NucleoSpin® Blood QuickPure Column into a new Collection Tube (2 mL) and add **350 µL Buffer BQ2**. Centrifuge **3 min** at **11,000 x *g***. Discard Collection Tube with flow-through.

11,000 x *g*
3 min

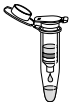
<u>Optional:</u> Place the NucleoSpin® Blood QuickPure Column into a new Collection Tube (2 mL; not provided) and add 200 µL Buffer BQ2. Centrifuge 3 min at 11,000 x g. Discard flow-through and Collection Tube and proceed to step 6.

This additional washing step is only recommended if the DNA is intended for use as a template in especially critical PCRs. In the vast majority of cases, you can save time by omitting this step.

5 Dry silica membrane

The drying of the NucleoSpin® Blood QuickPure Column is performed by the 3 min centrifugation in step 4.

6 Elute highly pure DNA



+ 50 µL BE (70 °C)

Place the NucleoSpin® Blood QuickPure Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL prewarmed Buffer BE (70 °C)**. Dispense buffer directly onto the silica membrane. Incubate at **room temperature** for 1 min. Centrifuge **1 min** at **11,000 x *g***.

RT
1 min

11,000 x *g*
1 min

For alternative elution procedures see section 2.4

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Low concentration of leukocytes in sample* • Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature (3,300 x *g*; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (= buffy coat). *Incomplete cell lysis* • Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer. • Proteinase K digestion is not optimal. Never add Proteinase K directly to lysis buffer. Incubate for 15 – 20 min at 70 °C / 56 °C. *Reagents not applied properly* • Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysates before loading them on columns. *Suboptimal elution of DNA from the column* • Preheat Buffer BE to 70 °C before elution. Apply Buffer BE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically if elution is performed with buffers of pH < 7.0. Use slightly alkaline elution buffer like Buffer BE (pH 8.5). • Mix vigorously once during the 70 °C / 56 °C incubation step especially when working with older or clotted blood samples.

Problem	Possible cause and suggestions
Poor DNA quality	*Reagents not applied properly* • Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysates and mix before loading them on columns. *Incomplete cell lysis* • Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer. • Proteinase K digestion is not optimal. Do not add Proteinase K directly to lysis buffer. Incubate for at least 15 – 20 min at 56 °C / 70 °C. *RNA in sample* • If RNA-free DNA is desired, add 20 µL RNase A solution per 200 µL blood sample before addition of lysis buffer. This would be 240 Units RNase A per 200 µL blood sample (1200 Units RNase A per 1 mL blood sample). For liquid RNase A (REF 740397, 100 mg/mL, 120 U/mg) you can directly use 20 µL RNase A solution per 200 µL blood sample (1000 µL for 10 mL blood). For lyophilized RNase A (REF 740505, 120 U/mg) please use 10 mg RNase A per 1 mL blood. *Old or clotted blood samples processed* • For isolation of DNA from older or clotted blood samples, we recommend prolonging Proteinase K incubation to 30 min and vortexing several times during this step. Especially for NucleoSpin® Blood L / XL with troublesome blood samples performance can be improved by the following steps: First incubate the lysate for 10 – 15 min at room temperature. Incubate for 15 min at the recommended 56 °C afterwards. Clear lysate before addition of ethanol. It is recommended performing a short centrifugation step of about 30 – 60 s after the lysis of the sample material (before addition of ethanol) in order to pellet non-lysed clumps. In case of difficult blood samples it might happen that the washing steps with ethanolic Buffer BQ2 are not sufficient to remove all contamination. An additional wash step with a buffer including chaotropic salt is recommended, for example water / BQ1 / ethanol mix (1:1:1). Afterwards, the washing step with ethanolic Buffer BQ2 should be performed to completely remove the chaotropic salt of the wash buffer.

Problem	Possible cause and suggestions
Suboptimal performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol* • Be sure to remove all of ethanolic Buffer B5 / BQ2 before eluting the DNA. If the level of B5 / BQ2 after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the Collection Tube, and centrifuge again. *Contamination of DNA with inhibitory substances* • If DNA has been eluted with Tris/EDTA-buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in Buffer BE. If preparing DNA from older or clotted blood samples, extend Proteinase K incubation to 30 min and vortex once or twice during this step. • If the A_{260}/A_{280} ratio of the eluate is below 1.6, repeat the purification procedure: **For NucleoSpin® Blood:** Add 1 volume of Buffer B3 plus 1 volume ethanol to the eluate, load on NucleoSpin® Blood Column, and proceed with step 3 of the corresponding protocol. **For NucleoSpin® Blood QuickPure:** Add 1 volume of Buffer BQ1 plus 1 volume ethanol to the eluate, load on NucleoSpin® Blood QuickPure Column, and proceed with step 3 of the corresponding protocol For **NucleoSpin® Blood L / XL**: Add 1 volume of Buffer BQ1 plus 1 volume ethanol to the eluate, load on NucleoSpin® Blood L / XL Column, and proceed with step 3 of the corresponding protocol.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Blood	740951.10/.50/.250	10/50/250
NucleoSpin® Blood L	740954.20	20
NucleoSpin® Blood XL	740950.10/.50	10/50
NucleoSpin® Blood QuickPure	740569.10/.50/.250	10/50/250
Buffer BQ1	740923	125 mL
Buffer B3	740920	100 mL
Buffer B5 Concentrate (for 125 mL Buffer B5)	740921	25 mL
Bufer BW	740922	100 mL
Proteinase K	740506	100 mg
RNase A	740505.50 740505	50 mg 100 mg
Collection Tubes (2 mL)	740600	1000

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

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

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.

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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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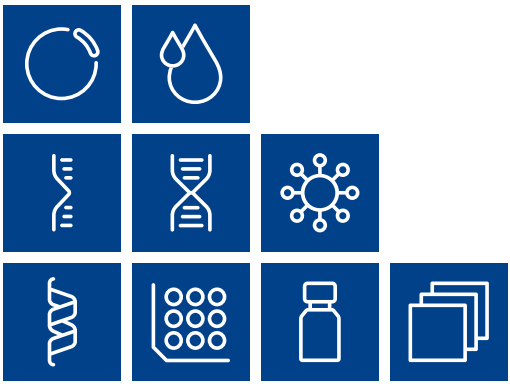
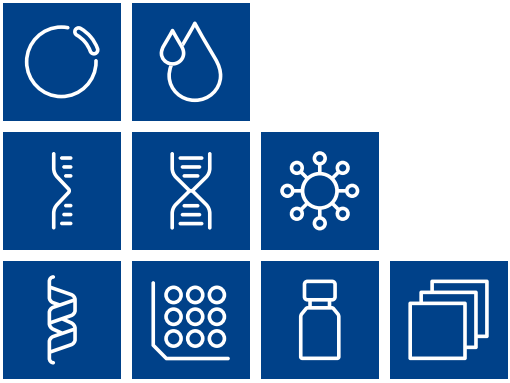


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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