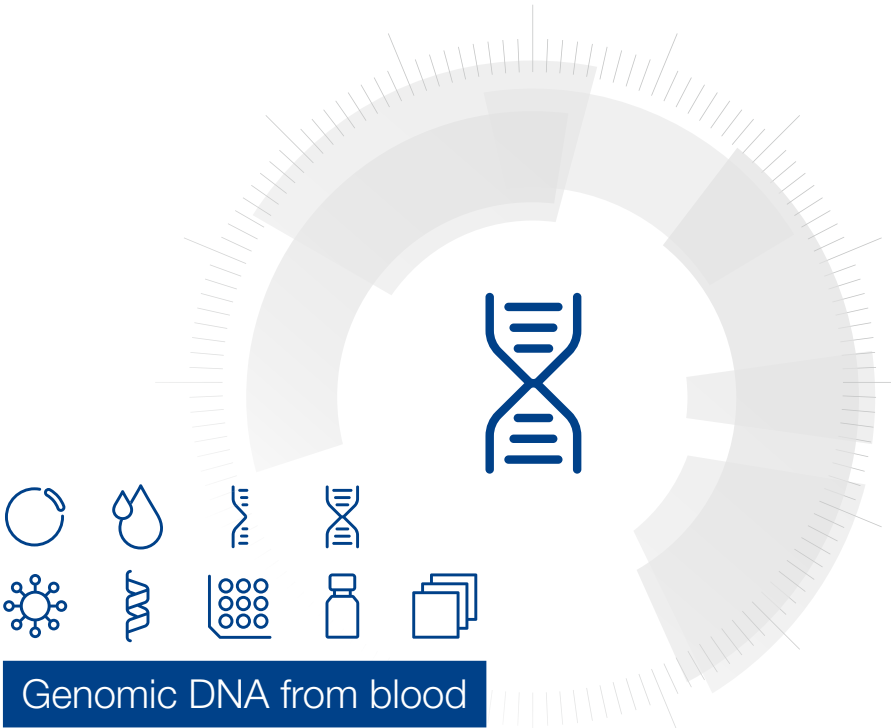


MACHEREY-NAGEL

User manual



■ NucleoSpin® 96 Blood QuickPure

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1 Components

1.1 Kit contents

NucleoSpin® 96 Blood QuickPure			
REF	2 × 96 preps 740667.2	4 × 96 preps 740667.4	24 × 96 preps 740667.24
Lysis Buffer BQ1	60 mL	100 mL	6 × 100 mL
Wash Buffer BQ2 (Concentrate) ¹	50 mL	2 × 50 mL	12 × 50 mL
Elution Buffer BE ²	30 mL	60 mL	6 × 60 mL
Proteinase K (lyophilized) ¹	126 mg	2 × 126 mg	12 × 126 mg
Proteinase Buffer PB	8 mL	15 mL	6 × 15 mL
NucleoSpin® Blood QuickPure Binding Plates (dark red rings)	2	4	24
MN Square-well Blocks	4	6	26
Round-well Block, Low U-bottom	2	4	24
Self adhering Foil	10	20	120
User Manual	1	1	1

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

Reagents

- 96–100 % ethanol (to prepare Wash Buffer BQ2 and to adjust binding conditions)

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

With the **NucleoSpin® 96 Blood QuickPure** 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 and Proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding **NucleoSpin® Blood QuickPure Binding Plates** are created by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminants are efficiently washed away with only one step for washing and drying. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- NucleoSpin® 96 Blood QuickPure is designed for ultrafast small-scale purification of highly pure genomic DNA from whole blood, serum, plasma, or other body fluids.
- Blood treated either with EDTA, citrate, heparin, or CPDA can be used. 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 Na₂HPO₄, and 0.24 g KH₂PO₄ in 800 mL H₂O. Adjust pH to 7.4 with HCl. Add H₂O to 1 liter).
- The kit allows purification of highly pure genomic DNA with an A₂₆₀/A₂₈₀-ratio between 1.6 and 1.9 and a typical concentration of 40–60 ng per µL.
- 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	NucleoSpin® 96 Blood QuickPure
Technology	Silica membrane technology
Format	96-well plates
Processing	Manual, centrifugation
Sample material	200 µL (default volume) 300 µL (support protocol)
Fragment size	300 bp–approx. 50 kbp
Typical DNA yield	4–6 µg
A_{260}/A_{280}	1.6–1.9
Elution volume	100 µL (default volume; 75 µL increases DNA concentration)
Preparation time	60 min/2 plates
Binding capacity	60 µg
Use	For research use only

2.3 Required hardware

Centrifuge: For centrifugation, a microtiterplate centrifuge which is able to accommodate NucleoSpin® 96 Blood QuickPure Binding Plates stacked on an MN Square-well Block or Rack of Tube Strips and which reaches accelerations of 5,600 $\times g$ is required (bucket height: 85 mm).

Shaker: For higher convenience (ease of handling, pipette tip savings) and most consistent results (high DNA yield and purity, high yield uniformity), a 96-well plate shaker with small spinning radius and high mixing frequency is recommended (~ 2–4 mm radius; > 1000 rpm; see Figure 1). Incubators with a larger spinning radius and lower speeds (e.g., bacterial culture incubators with ~ 19–30 mm spinning radius and < 500 rpm) are not recommended due to their inefficient mixing effect.

If no shaker is available, mixing blood, Proteinase K, and Buffer BQ1 by pipetting 3–5 times up and down is highly recommended (lysis step). Pipette tips with small diameter are recommended. Do not use serological pipet tips with large outlet diameter as marginal mixing of the components may lead to incomplete blood lysis and reduced kit performance.

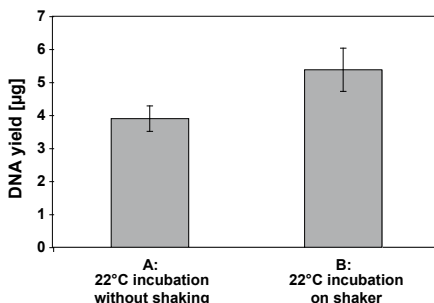


Figure 1: Lysis procedure: Shaking during incubation increases yield

200 µL frozen, human EDTA whole blood was subjected to DNA isolation with NucleoSpin® 96 Blood QuickPure. A: Blood, Proteinase K, and lysis buffer were mixed by pipetting 3x up and down. B: MN Square-well Block containing blood, Proteinase K, and lysis buffer were incubated on a Thermomixer™ Comfort (Eppendorf®). Median yield of DNA and average error are indicated (sample number = 32).

2.4 Storage of blood samples

For the isolation of genomic DNA from blood treated with anticoagulant (heparin, citrate, EDTA, CPDA) using a NucleoSpin® 96 Blood QuickPure kit the blood samples can be stored at room temperature, 4 °C, or frozen.

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

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

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

2.5 Elution procedures

It is possible to adapt the elution method and the volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 70–90 %) several modifications are possible:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. Alternatively, heat the elution buffer to 70 °C before applying it onto the membrane. About 96–100 % of bound nucleic acid can be eluted.
- **High concentration:** Perform one elution step with 75 % of the volume indicated in the individual protocol. Concentration of DNA will be approximately 40 % higher compared to standard elution. Maximal 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 with high concentration.

- **Convenient elution:** For convenience, elution buffer may be used at room temperature (default procedure). This will result in a little lower yield (approximately 20 %) compared to elution with heated elution buffer.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH 8 to 9. This will increase DNA stability, especially during long term storage at 4 °C or ambient temperature, by inhibiting DNases. However, EDTA interferes with certain downstream applications depending on the final concentration. DNA isolated with **NucleoSpin® 96 Blood QuickPure** from human EDTA whole blood, eluted in Elution Buffer BE, has been shown to be stable at 20 – 37 °C for several weeks without observable DNA degradation according to gel electrophoresis.

For optimal performance of isolated DNA in downstream applications we recommend elution with the supplied elution buffer and storage 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 amounts of DNA might be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4 °C or room temperature due to degradation of DNA or adsorption to surfaces.

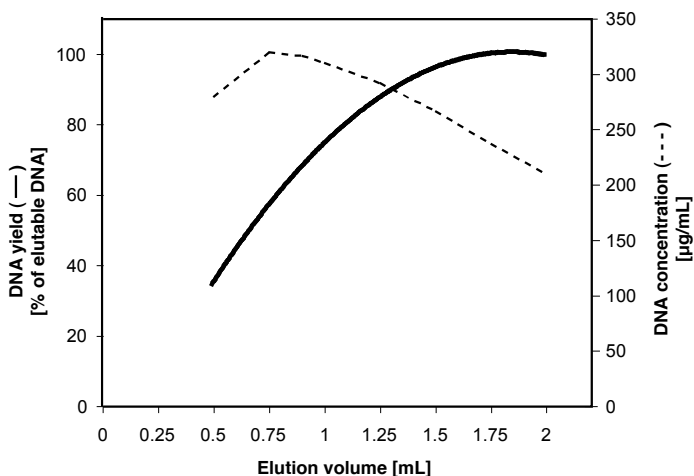


Figure 2: Influence of elution volume on DNA yield and concentration

3 Storage conditions and preparation of working solutions

Attention: Buffer BQ1 contains guanidine hydrochloride! Wear gloves and goggles when handling them!

CAUTION: Buffers BQ1 contains 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:

- All components of the **NucleoSpin® 96 Blood QuickPure** kit should be stored at room temperature and are stable until: see package label.
- Upon storage at lower temperature a white precipitate may form in Buffer BQ1. Dissolve such precipitates by incubation of the bottle at 70 °C before use.

Before starting with any **NucleoSpin® 96 Blood QuickPure** kit procedure, prepare the following:

- **Wash Buffer BQ2:** Add the indicated volume of 96–100 % ethanol to the **Buffer BQ2 Concentrate**. Mark the label of the bottle to indicate that ethanol is added. Store Wash Buffer BQ2 at room temperature (15–25 °C) for at least one year.
- Before first use of the kit, add the indicated volume of **Proteinase Buffer PB** to lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for at least 6 months.

NucleoSpin® 96 Blood QuickPure			
REF	2 × 96 preps 740667.2	4 × 96 preps 740667.4	24 × 96 preps 740667.24
Wash Buffer BQ2 (Concentrate)	50 mL Add 200 mL ethanol	2 × 50 mL Add 200 mL ethanol to each bottle	12 × 50 mL Add 200 mL ethanol to each bottle
Proteinase K (lyophilized)	126 mg Add 5.75 mL Proteinase Buffer to each vial	2 × 126 mg Add 5.75 mL Proteinase Buffer to each vial	12 × 126 mg Add 5.75 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoSpin® 96 Blood QuickPure** 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 www.mn-net.com/msds).



Caution: Guanidine hydrochloride 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 QuickPure** 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

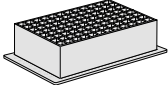
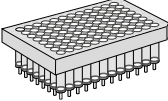
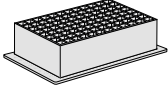
5.1 NucleoSpin® 96 Blood QuickPure – standard procedure

- For hardware requirements refer to section 2.3.
- For detailed information on each step see page 13.

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.

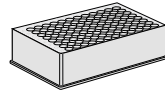
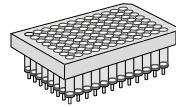
Protocol at a glance

1 Lyse samples	200 µL blood 25 µL Proteinase K 200 µL BQ1 Incubate on shaker (1.200–1.500 rpm) RT, 10 min	 e.g., MN Square-well Block
2 Adjust DNA binding conditions	200 µL ethanol Mix	
3 Bind DNA to silica membrane	Transfer samples to NucleoSpin® Blood QuickPure Binding Plate 5,600 x g, 3 min	
4 Wash and dry silica membrane	500 µL BQ2 5,600 x g, 5 min	 NucleoSpin® Blood QuickPure Binding Plate on MN Square-well Block

5 Elute DNA

100 μ L BE

**5,600 x *g*,
2 min**



NucleoSpin[®] Blood
QuickPure Binding Plate
on Round-well Block

Detailed protocol

- For hardware requirements refer to section 2.3.

Before starting the preparation:

- Check if Wash Buffer BQ2 and Proteinase K were prepared according to section 3.
-

1 Lyse samples

Pipette **25 µL Proteinase K** and **200 µL blood**, buffy coat, or body fluid sample (equilibrated to room temperature) into an MN Square-well Block. Take care to dispense the solution near the bottom of the wells. Do not moisten the rims of the wells.

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.

Add **200 µL Buffer BQ1** to each sample. Cover the MN Square-well Block with Self adhering Foil.

Optional: If the solutions have not been properly dispersed to the bottom of the wells, perform a quick centrifugation to collect all liquid at the bottom of the wells.

Incubate MN Square-well Block at ambient temperature (15–25 °C) for **10 min** on a **shaker** at high shaking speed (1,200–1,500 rpm).

See section 2.3 for recommended shaker. Depending on shaker model and applied mixing frequency, affix lysate plate onto the shaker (adhesive tape) to avoid the plate hopping off the shaker. If no shaker is available, pipette the lysate up and down 3–5 times to ensure thorough mixing of the solution (also read recommendations in section 2.3). The lysate will turn brownish during incubation with Buffer BQ1 and Proteinase K. Increase incubation time with Proteinase K (up to 30 min) if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Remove Self adhering Foil and add **200 µL ethanol (96–100 %)** to each well of the MN Square-well Block. Mix the lysate by pipetting up and down three times.

Note: Upon addition of ethanol to the lysate, mix them immediately (within approximately 1 min). Do not let the ethanol float on top of the lysate for an extended time (> 1 min). Extended ethanol floating before mixing can reduce DNA yield and purity.

Short incubation on a shaker is usually not sufficient for thorough mixing of the less dense and thus floating ethanol into the blood lysate. Always mix solutions by pipetting up and down!

3 Bind DNA to silica membrane

Place a **NucleoSpin® Blood QuickPure Binding Plate** onto an empty MN Square-well Block.

Transfer lysates into the NucleoSpin® Blood QuickPure Binding Plate.

Cover plate with a new Self adhering Foil.

Centrifuge **3 min** at **5,600 x g**.

If the samples are not drawn through the matrix completely, repeat the centrifugation for a 10 min period. It is not necessary to discard the flowthrough.

4 Wash and dry silica membrane

Remove Self adhering Foil and add **500 µL Buffer BQ2** to each well of the NucleoSpin® Blood QuickPure Binding Plate. Cover it with a new Self adhering Foil.

Centrifuge **5 min** at **5,600 x g**.

Optional: Repeat washing step, especially if difficult blood samples (old or clotted) are processed or if extremely demanding downstream applications are performed. Make sure to remove flowthrough of the first washing step if applying this optional washing step!

The membrane is washed and residual ethanol is removed during this step. Discard flowthrough, clean, and sterilize MN Square-well Block for reuse as flowthrough collecting device.

5 Elute DNA

Place NucleoSpin® Blood QuickPure Binding Plate onto a Round-well Block, (Low).

Remove Self adhering Foil and add **100 µL Buffer BE** to each well. Cover it with a new Self adhering Foil.

Centrifuge **2 min** at **5,600 x g**.

Seal Round-well Block (Low) with Self adhering Foil for DNA storage.

For alternative elution procedures see section 2.4. If elution in small volume tubes is desired, place a 96 PCR plate (not supplied) on top of the Rack of Tube Strips and elute into the PCR plate.

5.2 NucleoSpin® 96 Blood QuickPure – protocol for 300 µL samples

This support protocol allows DNA purification from 300 µL samples without additional loading steps.

- For hardware requirements refer to section 2.3.

Before starting the preparation:

- Check if Wash Buffer BQ2 and Proteinase K were prepared according to section 3.
 - Additional Buffer BQ1 and Proteinase K is necessary. Please see ordering information.
-

1 Lyse samples

NucleoSpin® 96 Blood QuickPure:

Pipette **37 µL Proteinase K** and **300 µL blood**, buffy coat or body fluid sample (equilibrated to room temperature) into an MN Square-well Block (supplied).

Add **300 µL Buffer BQ1** to each sample. Cover MN Square-well Block with Self adhering PE Foil, close reaction tube (whatever applicable).

Incubate MN Square-well Block or reaction tubes at ambient temperature (15–25 °C) for **10 min** on a **shaker** at high shaking speed.

See section 2.3 for recommended shaker and for further information if no shaker is available. The lysate will turn brownish during incubation with Buffer BQ1 and Proteinase K. Increase incubation time with Proteinase K (up to 30 min) if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **300 µL ethanol (96–100 %)** to each well of the MN Square-well Block or to each 1.5 or 2.0 mL reaction tube. Mix solution by pipetting up and down 3 times.

Note: Upon addition of ethanol to the lysate, mix them immediately (within approximately 1 min). Do not let the ethanol float on top of the lysate for an extended time (> 1 min). Extended ethanol floating before mixing can reduce DNA yield and purity.

Short incubation on a shaker is usually not sufficient for thorough mixing of the less dense and thus floating ethanol into the blood lysate. Always mix solutions by pipetting up and down.

- 3** Continue procedure with step 3 (Bind DNA to silica membrane; see section 5.1) of the NucleoSpin® 96 Blood QuickPure protocol. The lysate can be loaded in one step.

Due to the superior properties of the binding matrix and effective lysis 300 µL samples may be routinely processed with very low risk of membrane clogging compared to competitor products.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
	<i>Low concentration of leukocytes in sample</i> <ul style="list-style-type: none">• Prepare buffy coat from the blood sample: Centrifuge whole blood containing anticoagulant 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).
	<i>Incomplete cell lysis</i> <ul style="list-style-type: none">• Inhomogeneous blood sample or blood clots within the sample: Make sure that blood samples are collected following the instructions of the manufacturer of the blood collection tube. Make sure that only blood is used as sample material which can easily be transferred by pipetting. If necessary, homogenize the blood sample before use.• Sample not thoroughly mixed with lysis buffer/Proteinase K. The three components have to be mixed vigorously after addition of lysis buffer.• Proteinase K digestion not optimal. Never add Proteinase K directly to lysis buffer. Increase incubation time to 30 min.
No or poor DNA yield	<i>Reagents not properly applied</i> <ul style="list-style-type: none">• Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysate before loading the columns. <i>Suboptimal elution of DNA from the column</i> <ul style="list-style-type: none">• Elution efficiencies decrease significantly if elution is performed with buffers of pH < 7. Use slightly alkaline elution buffer like Buffer BE (pH 8.5). <i>Too long resting time of ethanol floating on top of the lysate</i> <ul style="list-style-type: none">• Upon addition of ethanol to the lysate, mix immediately. Do not let the ethanol float on top of the lysate for longer than ~ 1 min.

Problem	Possible cause and suggestions
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Poor DNA quality	<p><i>Reagents not properly applied</i></p> <ul style="list-style-type: none"> • Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysate and mix thoroughly before loading onto the columns. <p><i>Incomplete cell lysis</i></p> <ul style="list-style-type: none"> • Sample not thoroughly mixed with lysis buffer/Proteinase K. The three components have to be mixed vigorously after addition of lysis buffer. • Proteinase K digestion not optimal. Do not add Proteinase K directly to lysis buffer. Increase incubation time to 30 min. <p><i>RNA in sample</i></p> <ul style="list-style-type: none"> • If DNA free of RNA is desired, add 20 µL of an RNase A solution (20 mg/mL) before adding the lysis buffer. <p><i>Old or clotted blood samples processed</i></p> <ul style="list-style-type: none"> • For isolation of DNA from older or clotted blood samples, we recommend extending the Proteinase K incubation to 30 min.
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Suboptimal performance of genomic DNA in enzymatic reactions	<p><i>Carry-over of ethanol</i></p> <ul style="list-style-type: none"> • Be sure to remove all ethanolic Buffer BQ2 before eluting the DNA. If the level of Buffer BQ2 after the wash has reached the column outlet for any reason, discard flowthrough, place the NucleoSpin® 96 QuickPure Binding Plate back onto the collecting MN Square-well Block, and centrifuge again. <p><i>Contamination of DNA with inhibitory substances</i></p> <ul style="list-style-type: none"> • 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. • If the A_{260}/A_{280}-ratio of the eluate is below 1.6, repeat the purification procedure or apply the optional second wash step next time.
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Problem **Possible cause and suggestions**

*Possible reasons*High variation
in yield

- DNA-isolation from 96 different and independent samples (e.g., blood samples from 96 different persons) will result in higher yield variation (measured and actual) than from 96 aliquots of one blood sample.
 - Elution with smaller volumes (e.g., 50 µL vs. 150 µL) will result in larger (measured and actual) yield variation compared to elution in larger volumes.
 - If only a few single wells are used as global blank for a complete 96-well plate measurement this will likely cause larger measured yield variation than well to well blank correction measurements.
-

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 8 Blood QuickPure	740666	12 × 8 preps
	740666.5	60 × 8 preps
NucleoSpin® 96 Blood QuickPure	740667.2	2 × 96 preps
	740667.4	4 × 96 preps
	740667.24	24 × 96 preps
Buffer BQ1	740923	125 mL
Proteinase K	740506	100 mg
RNase A	740505.50	50 mg
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477	4 sets
	740477.24	24 sets
MN Square-well Block	740476	4 sets
	740476.24	24 sets
Round-well Block, Low U-bottom (incl. Self adhering PE Foil)	740487	4
	740487.24	24
Starter Set C (for use of 8-well strips under centrifugation)	740684	1 set
Self adhering PE Foil	740676	50
NucleoCard®	40403.10	10 cards
	740403.100	100 cards

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

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 customer 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.

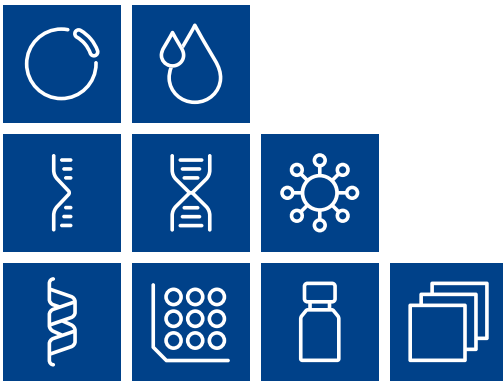
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