



# **Genomic DNA from Blood**

## **User Manual**

NucleoSpin® 8 Blood QuickPure

NucleoSpin® 96 Blood QuickPure

February 2009/ Rev. 02

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# 1 Components

## 1.1 Kit contents

NucleoSpin® 8 Blood QuickPure		
Cat. No.	12 x 8 preps	60 x 8 preps
	740666	740666.5
Lysis Buffer BQ1	25 ml	125 ml
Wash Buffer BQ2 (Concentrate) <sup>1</sup>	20 ml	5 x 50 ml
Elution Buffer BE <sup>2</sup>	15 ml	75 ml
Proteinase K (lyophilized) <sup>1</sup>	60 mg	2 x 126 mg
Proteinase Buffer PB	3.6 ml	15 ml
NucleoSpin® Blood QuickPure Binding Strips (dark red rings)	12	60
Rack of Tube Strips <sup>3</sup>	2	5
Cap Strips	24	60
Self-adhering PE Foil	12	60
User Manual	1	1

<sup>1</sup> For preparation of working solutions and storage conditions see section 3.

<sup>2</sup> Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

<sup>3</sup> Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

## 1.1 Kit contents *continued*

Cat. No.	NucleoSpin® 96 Blood QuickPure		
	2 x 96 preps 740667.2	4 x 96 preps 740667.4	24 x 96 preps 740667.24
Lysis Buffer BQ1	50 ml	100 ml	6 x 100 ml
Wash Buffer BQ2 (Concentrate) <sup>1</sup>	50 ml	2 x 50 ml	12 x 50 ml
Elution Buffer BE <sup>2</sup>	50 ml	75 ml	6 x 75 ml
Proteinase K (lyophilized) <sup>1</sup>	126 mg	2 x 126 mg	12 x 126 mg
Proteinase Buffer PB	8 ml	15 ml	6 x 15 ml
NucleoSpin® Blood QuickPure Binding Plates (dark red rings)	2	4	24
MN Square-well Blocks	4	6	26
Round-well Blocks, Low	2	4	24
Self-adhering Foil	10	20	120
User Manual	1	1	1

## 1.2 Reagent to be supplied by user

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

<sup>1</sup> For preparation of working solutions and storage conditions see section 3.

<sup>2</sup> Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

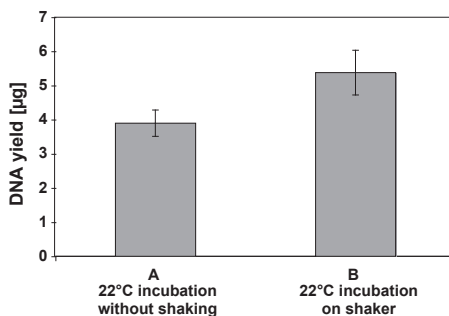
### 1.3 Additional recommended equipment

**Centrifuge:** For centrifugation a microtiterplate centrifuge which is able to accommodate NucleoSpin® 8/96 Blood QuickPure Binding Strips/Plates stacked on an MN Square-well Block or Rack of Tube Strips and which reaches accelerations of 5,600 x *g* is required (e.g. Hermle Z513, Qiagen/Sigma 4-15c, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™, Beckman Coulter, Allegra R25).

**Shaker:** For supreme 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; e.g. *Thermomixer comfort*, Eppendorf; *Teleshake*, Variomag; *Titramax 101*, Heidolph; *Vibrax VXR Basic*, IKA). 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 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.

**Starter Set C** is required for first time users of NucleoSpin® 8 Blood QuickPure kits. The Starter Set C contains Column Holders C, MN Square-well Blocks for waste collection, and Rack of Tube Strips, which serve as support strips during elution.



**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).

## 1.4 About this User Manual

It is strongly recommended reading the detailed protocol sections of this User Manual if the **NucleoSpin® 8/96 Blood QuickPure** kit is used for the first time. 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](http://www.mn-net.com).

## 2 Product description

### 2.1 The basic principle

With the **NucleoSpin® 8/96 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/Strips** 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® 8/96 Blood QuickPure** is designed for ultra-fast 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 Na<sub>2</sub>HPO<sub>4</sub>, and 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 800 ml H<sub>2</sub>O. Adjust pH to 7.4 with HCl. Add H<sub>2</sub>O 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.
- 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**

Parameters	NucleoSpin® 8/96 Blood QuickPure
Sample material	200 µl (default volume) 300 µl (support protocol)
Typical DNA yield	4-6 µg
Elution volume	100 µl (default volume; 75 µl increases DNA concentration)
DNA binding capacity	60 µg
$A_{260}/A_{280}$	1.6-1.9
Preparation time	approx. 60 min (for 12 strips or 2 plates)

## 2.3 Storage of blood samples

For the isolation of genomic DNA from blood treated with anticoagulant (heparin, citrate, EDTA, CPDA) using a **NucleoSpin® 8/96 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.

## 2.4 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%) several modifications are possible:

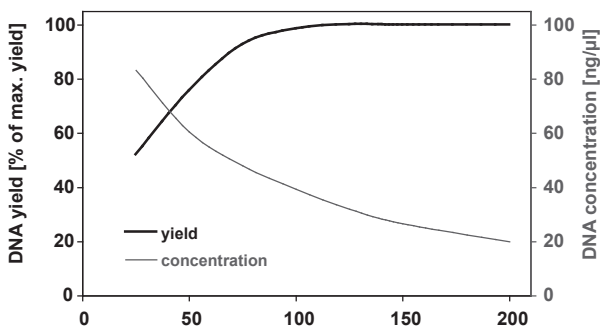
- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. Alternatively, heat the elution buffer to 70°C before applying it onto the membrane. About 95-100% of bound nucleic acid can be eluted.
- **High concentration:** Perform one elution step with 75% of the volume indicated in the individual protocol. Concentration of DNA will be approximately 40% higher than with 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 at a high concentration.
- **Convenient elution:** For convenience, elution buffer may be used at room temperature (default procedure). This will result in 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 omnipresent DNases. However, EDTA interferes with certain downstream applications depending on the final concentration. DNA isolated with **NucleoSpin® 8/96 Blood QuickPure** from human EDTA whole blood, eluted in Elution Buffer BE, has been shown to be stable at 20°C-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 amount 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:

*Buffers BQ1 contains guanidinium hydrochloride! Wear gloves and goggles when handling them!*

- All components of the **NucleoSpin® 8/96 Blood QuickPure** kits should be stored at room temperature and are stable for up to one year.
- 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® 8/96 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 (20-25°C) for up to 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 6 months.



NucleoSpin® 8 Blood QuickPure		
Cat. No.	12 x 8 preps	60 x 8 preps
	740666	740666.5
Wash Buffer BQ2 (Concentrate)	20 ml Add 80 ml ethanol	2 x 50 ml Add 200 ml ethanol to each bottle
Proteinase K (lyophilized)	60 mg Add 2.7 ml Proteinase Buffer	2 x 126 mg Add 6.6 ml Proteinase Buffer to each vial

NucleoSpin® 96 Blood QuickPure			
	<b>2 x 96 preps</b>	<b>4 x 96 preps</b>	<b>24 x 96 preps</b>
<b>Cat. No.</b>	<b>740667.2</b>	<b>740667.4</b>	<b>740667.24</b>
Wash Buffer BQ2 (Concentrate)	50 ml Add 200 ml ethanol	2 x 50 ml Add 200 ml ethanol to each bottle	12 x 50 ml Add 200 ml ethanol to each bottle
Proteinase K (lyophilized)	126 mg Add 5.75 ml Proteinase Buffer	2 x 126 mg Add 5.75 ml Proteinase Buffer to each vial	12 x 126 mg Add 5.75 Proteinase Buffer to each vial

## 4 Safety instructions – risk and safety phrases

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

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

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

### Risk phrases

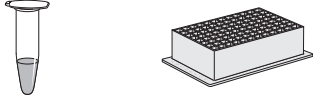
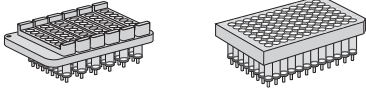
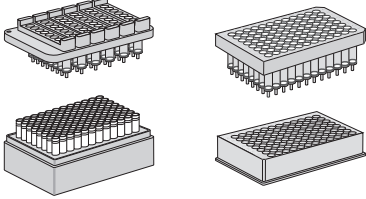
R 22	Harmful if swallowed
R 36/37/38	Irritating to eyes, respiratory system and skin
R 36/38	Irritating to eyes and skin
R 42	May cause sensitization by inhalation

### Safety phrases

S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 36/37	Wear suitable protective clothing and gloves

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

## 5 General procedure NucleoSpin® 8/96 Blood QuickPure

		NucleoSpin® 8 Blood QuickPure	NucleoSpin® 96 Blood QuickPure
1	<b>Lyse samples</b>	<p>200 µl blood 25 µl Proteinase K 200 µl BQ1</p> <p>Incubate on shaker (1.200 - 1.500 rpm) RT 10 min</p>	
2	<b>Adjust DNA binding conditions</b>	<p>200 µl ethanol</p> <p>Mix</p>	
3	<b>Bind DNA to silica membrane</b>	<p>Transfer samples to NucleoSpin® Blood QuickPure Binding Strips/Plate</p> <p>5,600 x g 3 min</p>	
4	<b>Wash and dry silica membrane</b>	<p>500 µl BQ2</p> <p>5,600 x g 5 min</p>	
5	<b>Elute DNA</b>	<p>100 µl BE</p> <p>5,600 x g 2 min</p>	

## 5.1 Genomic DNA purification with NucleoSpin® 8 Blood QuickPure

### Before starting the preparation:

- Check if Wash Buffer BQ2 and Proteinase K are 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 a 1.5 or 2.0 ml reaction tube (not supplied) or MN Square-well Block (not supplied).

*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  $5 \times 10^6$  cells in a final volume of 200 µl PBS.*

Add **200 µl Buffer BQ1** to each sample. Close reaction tubes.

Incubate reaction tubes at ambient temperature (18°C-25°C) for **10 min** on a **shaker** at high shaking speed (1.200 - 1.500 rpm).

*See section 1.3 for recommended 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 1.3). 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

Add **200 µl ethanol (96-100%)** to each reaction tube. Mix solution by pipetting up and down three times.

*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.*

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#### 3 Bind DNA to silica membrane

Insert an appropriate number of **NucleoSpin® Blood QuickPure Binding Strips** into the Column Holder C and place the assembly onto an MN Square-well Block.

**Transfer lysates** into the wells of the NucleoSpin® Blood QuickPure Binding Strips. Cover all strips with Self-adhering PE Foil.

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

*Column Holder C and reusable MM Square-well Blocks are provided in the Starter Set C. If the samples are not drawn through the matrix completely, repeat the centrifugation for a 10 min period. It is not necessary to discard flow-through.*

---

#### **4 Wash and dry silica membrane**

Remove Self-adhering PE Foil and add **500 µl Buffer BQ2** to each well of the strips. Cover all strips with new Self-adhering PE Foil.

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

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

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

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#### **5 Elute DNA**

Place NucleoSpin® Blood QuickPure assembly onto a Rack of Tube Strips. The rack must be completely filled with 8-well strips to ensure stable support of the column holder.

Remove Self-adhering PE Foil and add **100 µl Buffer BE** to each well. Cover all strips with new Self-adhering PE Foil.

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

Seal Tube Strips with Cap Strips 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.*

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## 5.2 Genomic DNA purification with NucleoSpin® 96 Blood QuickPure

### Before starting the preparation:

- Check if Wash Buffer BQ2 and Proteinase K are 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  $5 \times 10^6$  cells in a final volume of 200 µl PBS.*

Add **200 µl Buffer BQ1** to each sample. Cover 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 (18 - 25°C) for **10 min** on a **shaker** at high shaking speed (1.200 - 1.500 rpm).

*See section 1.3 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 1.3). 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.*

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#### 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.

*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!*

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### 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 flow-through.*

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### 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 if especially difficult blood samples (old or clot-  
ted) are processed or if extremely demanding downstream applications are per-  
formed. Make sure to remove flow-through of the first washing step if applying  
this optional washing step!

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

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### 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.*

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### 5.3 Genomic DNA purification from 300 µl samples

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

#### 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® 8 Blood QuickPure:**

Pipette **37 µl Proteinase K** and **300 µl blood** or body fluid sample (equilibrated to room temperature) into a 1.5 or 2.0 ml reaction tube (not supplied) or MN Square well Block (not supplied).

##### **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 (18-25°C) for **10 min** on a **shaker** at high shaking speed.

*See section 1.3 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.*

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#### 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.

*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) of the appropriate NucleoSpin® 8 or 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.*

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## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	<i>Low concentration of leukocytes in sample</i> <ul style="list-style-type: none"><li>• 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).</li></ul>
	<i>Incomplete cell lysis</i> <ul style="list-style-type: none"><li>• Sample not thoroughly mixed with lysis buffer/Proteinase K. The three components have to be mixed vigorously after addition of lysis buffer.</li><li>• Proteinase K digestion not optimal. Never add Proteinase K directly to lysis buffer. Increase incubation time to 30 min.</li></ul>
	<i>Reagents not applied properly</i> <ul style="list-style-type: none"><li>• Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysate before loading the columns.</li></ul>
	<i>Suboptimal elution of DNA from the column</i> <ul style="list-style-type: none"><li>• Elution efficiencies decrease dramatically if elution is performed with buffers of pH &lt; 7.0. Use slightly alkaline elution buffer like Buffer BE (pH 8.5).</li></ul>
Poor DNA quality	<i>Reagents not applied properly</i> <ul style="list-style-type: none"><li>• Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysates and mix thoroughly before loading them on columns.</li></ul>
	<i>Incomplete cell lysis</i> <ul style="list-style-type: none"><li>• Sample not thoroughly mixed with lysis buffer/Proteinase K. The three components have to be mixed vigorously after addition of lysis buffer.</li><li>• Proteinase K digestion not optimal. Do not add Proteinase K directly to lysis buffer. Increase incubation time to 30 min.</li></ul>

Poor DNA quality (continued)	<p><i>RNA in sample</i></p> <ul style="list-style-type: none"> <li>If DNA free of RNA is desired, add 20 µl of an RNase A solution (20 mg/ml) before addition of lysis buffer.</li> </ul> <p><i>Old or clotted blood samples processed</i></p> <ul style="list-style-type: none"> <li>For isolation of DNA from older or clotted blood samples, we recommend extending the Proteinase K incubation to 30 min.</li> </ul>
Suboptimal performance of genomic DNA in enzymatic reactions	<p><i>Carryover of ethanol</i></p> <ul style="list-style-type: none"> <li>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 flow-through, place the NucleoSpin® 96 QuickPure Binding Plate or reusable column holder with NucleoSpin® 8 QuickPure Binding Strips back onto the collecting MN Square-well Block, and centrifuge again.</li> </ul> <p><i>Contamination of DNA with inhibitory substances</i></p> <ul style="list-style-type: none"> <li>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.</li> <li>If preparing DNA from older or clotted blood samples, extend Proteinase K incubation to 30 min.</li> <li>If the <math>A_{260}/A_{280}</math>-ratio of the eluate is below 1.6, repeat the purification procedure or apply the optional second wash step next time.</li> </ul>
Large yield variation	<p><i>Possible reasons</i></p> <ul style="list-style-type: none"> <li>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.</li> <li>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.</li> <li>Few single wells used as global blank for a complete 96-well plate measurement will likely cause larger <u>measured</u> yield variation than well to well blank correction measurements.</li> </ul>

## 6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Blood QuickPure	740666 740666.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 96 Blood QuickPure	740667.2 740667.4 740667.24	2 x 96 preps 4 x 96 preps 24 x 96 preps
Buffer BQ1	740923	125 ml
Proteinase K	740506	100 mg
RNase A	740505	100 mg
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477	4 sets
MN Square-well Block	740476 740678	4 20
Round-well Block, Low	740482 740482.20	4 20
Starter Set C (for use of 8-well strips under centrifugation)	740684	1 set
Self-adhering PE Foil	740676	50
NucleoSave	740403.10 740403.100	10 cards 100 cards

## 6.3 Reference

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

## 6.4 Product use restriction/warranty

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

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

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