



**GeneProof**  
**PathogenFree DNA Isolation Kit**

**User Manual**

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# 1 Components

## 1.1 Kit contents

GeneProof PathogenFree DNA Isolation Kit		
	50 preps	250 preps
Buffer B1	10 ml	50 ml
Reagent B2	2.5 ml	12.5 ml
Buffer B5 (concentrate)*	7 ml	2 x 20 ml
Buffer BW	30 ml	2 x 75 ml
Buffer BE	13 ml	60 ml
Proteinase K (lyophilized)*	30 mg	2 x 75 mg
Proteinase Buffer	1.8 ml	8 ml
DNA binding columns (plus collection tubes)	50	250
2 ml collection tubes	150	750
Label for buffer B3	1	1
Protocol	1	1

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

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## 2 Product description

### 2.1 *The basic principle*

With the **GeneProof DNA isolation** method, genomic DNA is prepared from whole blood, cultured cells, serum, plasma or other body fluids. Lysis is achieved by incubation of the sample 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 **DNA binding columns** are created by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

### 2.2 *About this user manual*

Experienced users who are performing the isolation using a **GeneProof PathogenFree DNA Isolation Kit** may refer to the Protocol-at-a-glance instead of this user manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this user manual.

### 2.3 *Kit specifications*

- **GeneProof PathogenFree DNA Isolation Kit** 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 and bacterial DNA from clinical samples. As viral DNA co-purifies with cellular DNA, we recommend usage of cell-free sample (serum or plasma) to prepare pure viral DNA.
- Blood treated either with EDTA, citrate, or heparin 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)
- This kit allows purification of highly pure genomic DNA with an A<sub>260/280</sub>-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.

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Table 1: Kit specifications	GeneProof DNA Isolation Kit
<b>Parameter</b>	
Sample size	up to 200 µl of body fluids
Average yield	4-6 µg
Elution volume	100 µl
Binding capacity	60 µg
Time/prep	30 min

## ***2.4 Storage of blood samples***

Samples for DNA isolation using a **GeneProof PathogenFree DNA Isolation Kit** can be stored at +4 °C for a couple of days. DNA yield drops down with the prolonging storage. It is recommended to freeze the samples at -20 °C for long term storage to increase DNA yield.

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## 2.5 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 (ca. 130 %). 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 of ambient temperature may be used. This will result in a somewhat lower yield (approximately 20 %) compared to elution with heated 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 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. > 10kb) 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 shearing of DNA or adsorption to surfaces.

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### 3 Storage conditions and preparation of working solutions

#### Attention:

*Buffers B1, B3, and BW contain guanidine hydrochloride! Wear gloves and goggles!*

- All kit components can be stored at room temperature (20-25°C) and are stable up to one year.

Before starting the **GeneProof DNA isolation** protocol, prepare the following:

- Before the first use of the kit, add the indicated volume of Proteinase Buffer to dissolve lyophilized **proteinase K**. Proteinase K solution is stable at +4 °C for up to 6 months. Storage at –20 °C is recommended if the solution will not be used up during this period.
- **Buffer B5:** Add the indicated volume of ethanol (96-100 %) to **buffer B5** concentrate. Store buffer B5 at room temperature (20-25 °C) for up to one year.
- Prepare **buffer B3:** Transfer **buffer B1** to **reagent B2** completely and mix well. The resulting **buffer B3** is stable for up to one year at room temperature.
- Upon storage, especially at low temperatures, a white precipitate may form in buffer B3 or B1. Dissolve such precipitates by incubation of the bottle at 70 °C before use.




GeneProof PathogenFree DNA Isolation Kit		
	50 preps	250 preps
Buffer B5 (concentrate)	7 ml add 28 ml ethanol	2 × 20 ml add to each bottle 80 ml ethanol
Proteinase K (lyophilized)	30 mg add 1.35 ml Proteinase Buffer	2 × 75 mg add to each vial 3.35 ml Proteinase Buffer

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## 4 Safety instructions – risk and safety phrases

The following components of the **GeneProof PathogenFree DNA isolation kit** contains hazardous contents.

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

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
B1	guanidine hydrochloride	 Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	S 22
BW	guanidine hydrochloride	 Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	S 22
Proteinase K	Proteinase K, lyophilized	 Xn*	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 sensitisation 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

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\* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

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## 5 Protocol for DNA purification from body fluids

Before starting with the preparation, set incubator or water bath to 70 °C. Equilibrate buffer BE to 70 °C. Prepare buffer B3, B5, and proteinase K solution (section 3).

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### 1 Lyse samples

**Pipette 25 µl proteinase K and up to 200 µl body fluid sample into 1.5 ml microcentrifuge tubes.**

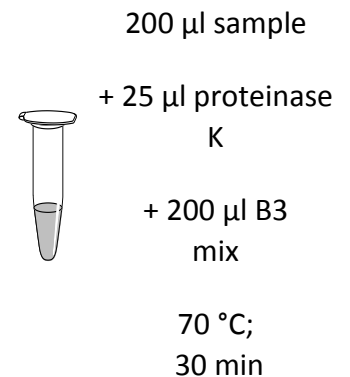
*For sample volumes less than 200 µl, add PBS to adjust the volume to 200 µl. If purifying DNA viruses, we recommend starting with 200 µl serum or plasma. If cultured cells are used, resuspend up to  $5 \times 10^6$  cells in a final volume of 200 µl PBS.*

**Add 200 µl lysis buffer B3 to the samples and vortex the mixture vigorously (10-20 s).**

**Incubate samples at 70 °C for 30 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.*

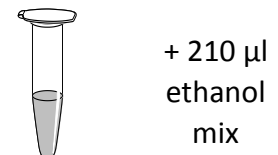
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### 2 Adjust DNA binding conditions

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

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### 3 Bind DNA

**For each preparation, take one DNA binding column placed in a 2 ml centrifuge tube and load the sample.**

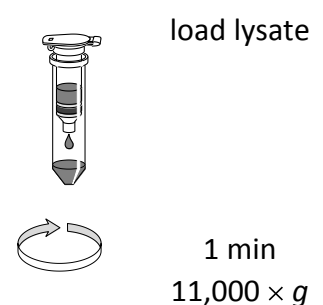
*Maximal volume per one column is 650 µl.*

**Centrifuge 1 min at 11,000 × g.**

*If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000 × g).*

**Discard collection tube with flow-through.**

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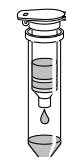
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#### 4 Wash silica membrane

##### 1<sup>st</sup> wash

Place the DNA binding column into a new 2 ml collection tube and add 500 µl buffer BW.

Centrifuge 1 min at 11,000 x g. Discard the collection tube with the flow-through.



+ 500 µl BW

1 min  
11,000 x g

##### 2<sup>nd</sup> wash

Place the DNA binding column into a new 2 ml collection tube and add 600 µl buffer B5.

Centrifuge 1 min at 11,000 x g.

Discard the collection tube with the flow-through.



+ 600 µl B5

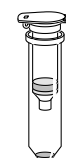
1 min  
11,000 x g

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#### 5 Dry silica membrane

Place the DNA binding column into a new 2 ml collection tube and centrifuge 1 min at 11,000 x g.

*Residual ethanol is removed during this step*



1 min  
11,000 x g



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#### 6 Elute highly pure DNA

Place the DNA binding column in a 1.5 ml microcentrifuge tube and add 50 µl prewarmed elution buffer BE (70°C).

*Dispense buffer directly onto the silica membrane without touching the membrane with the tip.*

Incubate at room temperature for 1 min.

Centrifuge 1 min at 11,000 x g.

Add additional 50 µl prewarmed elution buffer BE (70°C).

*Dispense buffer directly onto the silica membrane without touching the membrane with the tip.*

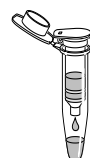
Incubate at room temperature for 1 min.

Centrifuge 1 min at 11,000 x g.

*Resulting elution volume is 100 µl*

*For alternative elution procedures see section 2.5.*

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+ 50 µl BE  
(70°C)

RT  
1 min



1 min  
11,000 x g

## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	<p data-bbox="424 510 976 544"><i>Low concentration of leukocytes in sample</i></p> <ul data-bbox="424 566 1374 723" style="list-style-type: none"><li>• Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature (<math>3,300 \times g</math>; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (= buffy coat).</li></ul> <p data-bbox="424 763 687 797"><i>Incomplete cell lysis</i></p> <ul data-bbox="424 819 1374 1043" style="list-style-type: none"><li>• Sample not thoroughly mixed with lysis buffer / proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer.</li><li>• Proteinase K digestion not optimal. Never add proteinase K directly to lysis buffer. Incubate for 15 - 20 min at <math>70^{\circ}\text{C}</math> / <math>56^{\circ}\text{C}</math>.</li></ul> <p data-bbox="424 1084 820 1120"><i>Reagents not applied properly</i></p> <ul data-bbox="424 1142 1350 1220" style="list-style-type: none"><li>• Prepare buffers and proteinase K solution according to instructions (section 3). Add ethanol to lysates before loading them on columns.</li></ul> <p data-bbox="424 1261 995 1294"><i>Suboptimal elution of DNA from the column</i></p> <ul data-bbox="424 1317 1374 1659" style="list-style-type: none"><li>• Preheat buffer BE to <math>70^{\circ}\text{C}</math> before elution. Apply BE directly onto the center of the silica membrane.</li><li>• Elution efficiencies decrease dramatically if elution is performed with buffers of <math>\text{pH} &lt; 7.0</math>. Use slightly alkaline elution buffer like buffer BE (<math>\text{pH} 8.5</math>).</li><li>• Mix vigorously once during the <math>70^{\circ}\text{C}</math>/ <math>56^{\circ}\text{C}</math> incubation step especially when working with old or clotted blood samples.</li></ul>
Poor DNA quality	<p data-bbox="424 1715 820 1749"><i>Reagents not applied properly</i></p> <ul data-bbox="424 1771 1342 1883" style="list-style-type: none"><li>• Prepare buffers and proteinase K solution according to instructions (section 3). Add ethanol to lysates and mix ore loading them on columns.</li></ul>

<p>Poor DNA quality (continued)</p>	<p><i>Incomplete cell lysis</i></p> <ul style="list-style-type: none"> <li>• Sample not thoroughly mixed with lysis buffer / proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer.</li> <li>• Proteinase K digestion not optimal. Do not add proteinase K directly to lysis buffer. Incubate for at least 15 - 20 min at 56°C/ 70°C.</li> </ul> <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 extension of proteinase K incubation to 30 min and vortexing several times during this step.</li> </ul>
<p>Suboptimal performance of genomic DNA in enzymatic reactions</p>	<p><i>Carryover of ethanol</i></p> <ul style="list-style-type: none"> <li>• Be sure to remove all of ethanolic buffer B5 before eluting the DNA. If the level of B5 after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the collecting tube, 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 BE buffer.</li> <li>• If preparing DNA from older or clotted blood samples, extend proteinase K incubation to 30 min and vortex once or twice during this step.</li> <li>• If the <math>A_{260/280}</math>-ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of buffer B3 plus 1 volume ethanol to the eluate, load on DNA binding column, and proceed with step 3 of the corresponding protocol.</li> </ul>

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## 6.2 Ordering information

	Cat. No.	Package
PathogenFree DNA Isolation Kit	IDNA050	50 isolations
PathogenFree DNA Isolation Kit	IDNA250	250 isolations

### **GeneProof a.s.**

Vinicni 235, Brno, Czech Republic

E-mail: [info@geneproof.com](mailto:info@geneproof.com)

Phone/Fax: +420 543 211 679

Orders: [sales@geneproof.com](mailto:sales@geneproof.com)

Customer service: [support@geneproof.com](mailto:support@geneproof.com)