GeneProof
Molecular diagnostics for your routine

PACKAGE INSERT

GeneProof PathogenFree DNA Isolation Kit
IDNA050
IDNA250

CE IVD *In vitro* diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

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VERSION: IDNA_04_18_05 Valid from: 11-07-18

CONTENT

1. Kit content

GeneProof PathogenFree DNA Isolation Kit		
	50 preps	**250 preps**
Lysis Buffer B3	15 ml	5 x 15 ml
Wash Buffer B5 (concentrate)*	12 ml	5 x 12 ml
Wash Buffer BW	30 ml	5 x 30 ml
Elution Buffer BE	13 ml	5 x 13 ml
Proteinase K (lyophilized)*	30 mg	5 x 30 mg
Proteinase Buffer	1.8 ml	5 x 1.8 ml
DNA binding columns (plus collection tubes)	50	250 pcs
2 ml collection tubes	3 x 50	15 x 50 pcs

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

2. Product description

2.1. The basic principle

With the **GeneProof DNA isolation** method, genomic DNA is prepared from whole blood, plasma, CSF, urine, sputum, bronchoalveolar lavage, aspirate, sperm, tick, saliva, and swab. 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. Kit specifications

- **GeneProof PathogenFree DNA Isolation Kit** are designed for the rapid isolation of highly pure genomic DNA from whole blood, 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_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.

- This kit allows purification of highly pure genomic DNA with an $A_{260/280}$-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 PCR with GeneProof PCR Kits,

Parameter	GeneProof PathogenFree DNA Isolation Kit
Sample size	up to 200 µl of body fluids
Average yield	4-6 µg
Elution volume	50 µl/100 µl
Binding capacity	60 µg

2.3. Storage of blood samples

Samples for DNA isolation using a **GeneProof PathogenFree DNA Isolation Kit** can be stored at 2-8 °C for a couple of days. DNA yield drops down with the prolonging storage. It is recommended to freeze the samples at -20 °C or less for long term storage to increase DNA yield.

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 (cca. 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 ≥ 8. This will increase DNA stability especially during long term and/or multi use storage at 2 to 8 °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 or less. Repeated 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 2 - 8 °C or room temperature due to shearing of DNA or adsorption to surfaces.

3. Storage conditions and preparation of working solutions

Attention: Buffers B3, and BW contain guanidine hydrochloride! Wear gloves and goggles!

- All kit components can be stored at room temperature (15-25 °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 2 to 8 °C for up to 6 months. Storage at -20 °C or less 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 (15-25 °C) for up to one year.
- Upon storage, especially at low temperatures, a white precipitate may form in buffer B3. Dissolve such precipitates by incubation of the bottle at 70 °C before use.

GeneProof PathogenFree DNA Isolation Kit		
Component	**50 preps**	**250 preps**
Buffer B5 (concentrate)	12 ml add 48 ml ethanol	5 x 12 ml add 48 ml ethanol to each bottle
Proteinase K (lyophilized)	30 mg add 1.35 ml Proteinase Buffer	5 x 30 mg add 1.35 ml to each vial Proteinase Buffer

4. Safety instructions

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	Hazard Phrases	Precaution Phrases
B3	Guanidine hydrochloride 36-50 %	 Warning	302, 319	280, 301+312, 305+351+338, 330, 337+313
BW	Guanidine hydrochloride 36-50 % + isopropanol 20 -50 %	 Warning	226, 302, 319	210, 233, 280, 301+312, 305+351+338, 330, 337+313, 403+235
Proteinase K	Proteinase K, lyophilized	 Danger	334	261, 304+340, 342+311

Hazard phrases

H 226	Flammable liquid and vapour.
H 302	Harmful if swallowed.
H 319	Causes serious eye irritations.
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precaution phrases

P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P 233	Keep container tightly closed.
P 261	Avoid breathing dust.
P 280	Wear protective gloves/eye protection.
P 301 + 312	IF SWALLOWED: Call a POISON CENTER/doctor/…/if you feel unwell.
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P 305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P 330	Rinse mouth.
P 337+313	If eye irritation persists: Get medical advice/attention.
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor/…
P 403+235	Store in a well ventilated place. Keep cool.

5. Protocol for DNA purification from body fluids

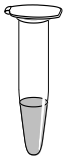
Before starting with the preparation, set incubator or water bath to 70 °C. Preheat the required amount of elution buffer BE to 70 ° C. Prepare buffer B5 and proteinase K solution (section 3).

1 Sample lysis

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

The need of respecting the order of pipetting in order to depreciate the 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 plasma. If cultured cells are used, resuspend up to 5 × 10^6 cells in a final volume of 200 µl PBS.

25 µl proteinase K



+ 200 µl sample

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

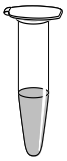
+ 200 µl B3
vortex

Incubate samples at 70 °C for 30 min.

70°C;
30 min

The lysate from blood samples 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. Short spin.

2 Adjust DNA binding conditions

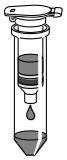


Add 210 µl ethanol (96–100 %) to each sample and vortex again. Short spin.

+ 210 µl
ethanol
vortex

3 Bind DNA

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



load lysate

Maximal volume per one column is 650 µl.

Centrifuge 1 min at 11,000 × *g*.

1 min
11,000 × g

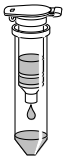
If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000 × g).

Discard collection tube with flow-through.

4 Wash silica membrane

1st wash

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



+ 500 µl BW

1 min
11,000 x g

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

2nd wash

+ 600 µl B5

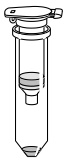
1 min
11,000 x g

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.

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.

1 min
11,000 x g

Residual ethanol is removed during this step

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.

+ 50 µl BE
(70 °C)

Incubate at room temperature for 1 min.

RT
1 min

Centrifuge 1 min at 11,000 x g.

1 min
11,000 x g

2x

Repeat step 6 once again that the final volume is 100 µl.

Resulting elution volume is 100 µl

For alternative elution procedures see section 2.4.

6. Procedure for DNA isolation from smear/swab

Sampling material: Swab or brush

Note: Sampling from smears or swabs for the DNA isolation has to be dry without any medium (unless stated otherwise). Sample transport has to be made as soon as possible after sampling. Sample is stored for 24 hours at 2-8°C or stored frozen for longer time.

1. Cut off a part of the swab/brush with a taken sample, small enough to fit into the 2 ml tube.
2. Insert swab/brush into the 2 ml tube.
3. Add 200 µl Nuclease-free or DNA/RNA free water.
4. Add 200 µl buffer B3.
5. Gently mix (flip the tube or vortex shortly).
6. Incubate for 15 minutes at the room temperature (15 – 25 °C).
7. Vortex gently and then centrifuge briefly.
8. Add 25 µl Proteinase K.
9. Gently mix (flip the tube or vortex shortly).
10. Incubate for 30 minutes at 70 °C.
11. Vortex shortly and then centrifuge briefly.
12. Open the tube and discard the swab/brush (infectious material).
13. Continue according to the Package insert from the section 2; elute into the 50 µl BE.

7. Procedure for DNA isolation from simulated smear/swab

Sampling material: Samples can be supplied by institutions providing EQA panels (the samples do not contain a brush or a swab, but contain the smear imitating material on the bottom of the tube).

***Notice*:** this procedure is identical to the procedure for the DNA isolation from smears or swabs, but this procedure does not contain the manipulation with a brush or a swab).

1. Add 200 µl Nuclease-free or DNA/RNA free water to the tube with the sample.
2. Add 200 µl buffer B3.
3. Lightly mix (flip the tube or vortex shortly).
4. Incubate for 15 minutes at the room temperature.
5. Vortex gently and then centrifuge briefly.
6. Add 25 µl Proteinase K.
7. Gently mix (flip the tube or vortex shortly).
8. Incubate for 30 minutes at 70 °C.
9. Vortex shortly and then centrifuge briefly.
10. Open the tube and discard the swab/brush (infectious material).
11. Continue according to the Package insert from the section 2; elute into the 50 µl BE.

8. Procedure for DNA isolation from urine

Sampling material: urine

1. Transfer 1 ml of urine and centrifuge (11,000 g/15 min).
2. Discard 800 µl of supernatant and use residual 200 µl for following isolation.
3. Continue according to the Package insert from the section 1.

9. Procedure for DNA isolation from sputum

Sampling material: sputum

1. Transfer 100 µl of sputum sample and mix with 100 µl 0.1 % dithiotreitol.
2. Vortex thoroughly and incubate 15 min at 37 °C, continuously vortex several times.
3. After incubation, vortex sample repeatedly.
4. Continue according to the Package insert from the section 1.

10. Procedure for DNA isolation from sperm

Sampling material: sperm

1. Transfer 350 µl of sperm sample and mix with 350 µl PBS.
2. Centrifuge for 10 minutes at 11,000 g.
3. Remove supernatant 500 µl and remaining 200 µl use for isolation.
4. Continue according to the Package insert from the section 1.

11. Procedure for DNA isolation from tick

Sampling material: tick

1. Insert a tick into a tube and add 250 µl of Nuclease-free water.
2. Use sharp scalpel to disrupt the tick surface.
3. Transfer 200 µl and continue according to the Package insert from the section 1.

12. 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 × *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 not optimal. Follow the order of added components, as specified in the Protocol for isolation in Step 1. Incubate for 15 - 20 min at 70 °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 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 incubation step especially when working with old or clotted blood samples.

Poor DNA quality	*Reagents not applied properly* • Prepare buffers and proteinase K solution according to instructions (section 3). Add ethanol to lysates and mix ore 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 not optimal. Follow the order of added components, as specified in the Protocol for isolation in Step 1. Incubate for at least 30 min at 70 °C. *RNA in sample* • If DNA free of RNA is desired, add 20 µl of an RNase A solution (20 mg/ml) before addition of lysis buffer. *Old or clotted blood samples processed* • For isolation of DNA from older or clotted blood samples, we recommend extension of incubation (see Step 1) and vortexing several times during this step.
Suboptimal performance of genomic DNA in enzymatic reactions	*Carryover of ethanol* • 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. *Contamination of DNA with inhibitory substances* • If DNA has been eluted with Tris/EDTA-buffer (TE), make sure that ETDA does not interfere with downstream applications or repurify DNA and elute in BE buffer. • If preparing DNA from older or clotted blood samples, extend proteinase K incubation at 70 °C to 30 min and vortex once or twice during this step. • If the $A_{260/280}$-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.

13. Product Variants

Product name	Cat. No.	Technology	Package
GeneProof PathogenFree DNA Isolation Kit	IDNA050	Isolation	50 isolations
GeneProof PathogenFree DNA Isolation Kit	IDNA250	Isolation	250 isolations

WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. It is recommended that only demonstrably trained persons with a focus on working with molecular biology techniques and the handling of potentially infectious biological material can work with the GeneProof PathogenFree DNA Isolation Kit.

When handling GeneProof PathogenFree DNA Isolation Kit wear protective gear (protective clothing, gloves). While working with the product do not drink, eat or smoke. Wash your hands thoroughly after handling the product. If swallowed, rinse your mouth. If in contact with the skin, gently wash with plenty of soap and water. In case of contact with eyes, rinse with water for several minutes.

It is recommended to dispose the kit after use in accordance with current legislation. Handling the kit must comply with safety information. The packaging materials are made of paper and polypropylene. In case of any query please contact Customer Service.

14. Customer Service

We appreciate all our customers and besides high-quality products we provided above-standard customer service including the following:

- Provision of free demonstration PCR kits
- Express deliveries
- Quick solution of problems related to the supplied products – service guaranteed within 24 hours from the time of report
- Consultations concerning technological and clinical interpretations

To assure the quickest possible solution of any problem we always require the GeneProof PCR Kit users to provide the following information:

- Kit name
- Problem definition
- Kit lot - specified on the kit package
- Used device
- File with the examination log from the used device

15. Contact

Support and customer care

Phone: +420 543 211 679
Fax: +420 516 770 824
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Orders

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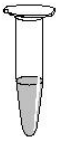
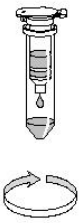
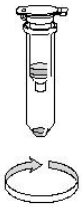
16. Notes

17. Shortened protocol

1	Sample lysis		25 µl proteinase K + 200 µl sample + 200 µl B3 vortex 70 °C; 30 min
2	Adjust DNA binding conditions		+ 210 µl ethanol vortex
3	Bind DNA		load lysate 1 min 11 000 x g
4	Wash silica membrane		+ 500 µl BW 1 min 11 000 x g + 600 µl B5 1 min 11 000 x g
5	Dry silica membrane		1 min 11 000 x g
6	Elute highly pure DNA		+ 50 µl BE (70 °C) RT 1 min 1 min 11 000 x g } 2x