Genomic DNA from Forensic Samples

User manual

NucleoSpin® 96 Trace

BIOKÉ
sharing knowledge

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MACHEREY-NAGEL

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1 Kit contents

	NucleoSpin® 96 Trace	
	2 x 96 preps	**4 x 96 preps**
Cat. No.	**740 726.2**	**740 726.4**
Buffer FLB	200 ml	400 ml
Buffer B5 concentrate[1]	100 ml	200 ml
Proteinase K (lyophilized)[1]	2 x 33 mg	4 x 33 mg
Proteinase Buffer	8 ml	15 ml
Buffer BE	100 ml	2 x 100 ml
NucleoSpin® Trace Binding Plate	2	4
MN Wash Plate (including six paper sheets)	2	4
MN Square-well Block	2	4
Rack with MN Tube Strips[2]	2	4
Cap Strips	24	48
Protocol	1	1

[1] For preparation of working solutions and storage conditions see section 3.
[2] Set of 1 rack, 12 strips with 8 tubes each.

2 Product description

2.1 The basic principle

With the **NucleoSpin® 96 Trace** method, genomic DNA is prepared from forensic samples. Lysis is achieved by incubation of samples in a solution containing chaotropic ions in the presence of proteinase K at room temperature. Appropriate conditions for binding of DNA to the silica membrane in the **NucleoSpin® Trace Binding Plate** is created by addition of isopropanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by two washing steps with ethanolic buffer. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® 96 Trace** is designed for the rapid, small-scale preparation of highly pure genomic DNA from forensic samples. The obtained DNA can be used directly as template for PCR.

- Typically yields of 1-2 µg genomic DNA can be purified from buccal swabs.

- The final concentration of eluted DNA is 10-20 ng/µl (depending on elution buffer volume). Typically, the $A_{260/280}$ ratio is 1.8 – 1.9.

- The kit is for using a centrifuge with a swing-out rotor capable to accommodate the **NucleoSpin® Trace Binding Plate**/MN Square-well Block sandwich (bucket height: 85 mm), e.g. Hermle Z513, Qiagen/Sigma 4-15c, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™, Beckman Coulter, Allegra R)

- The NucleoVac 96 vacuum manifold (Cat. No. 740681) or similar suitable vacuum manifolds (see section 2.3) may be used for binding and washing steps.

- In order to achieve high concentrations of the eluted DNA centrifugation is recommended for the elution step.

Kit specifications at a glance	
	NucleoSpin® 96 Trace
Sample type	buccal swabs
Average yield	1-2 µg
Elution volume	50-100 µl
Binding capacity	20 µg
Time/plate	70 min

2.3 Required hardware

NucleoSpin® 96 Trace:

The **NucleoSpin® 96 Trace** kit can be used manually (binding and washing steps) with the NucleoVac 96 vacuum manifold (Cat. No. 740681, see above).

For automation on laboratory platforms with standard 96-well plate vacuum chambers the use of MN Frame is strongly recommended (see ordering information).

Processing of the **NucleoSpin® 96 Trace** kit under centrifugation is possible by using a suitable plate centrifuge (see section 2.2).

2.4 Suitability with other common vacuum manifolds

The **NucleoSpin® 96 Trace** kit can be used with other common vacuum manifolds. For further details see list below.

Vacuum manifold	Suitability	Additional equipment
Qiagen/ QIAvac 96*	yes	MN Frame (see ordering information)
BioRad/ Aurum vacuum manifold	no	
Eppendorf/ Perfect VAC Manifold	no	
Millipore/ MultiScreen	no	

*In general is the QIAvac 96 suitable for the use with the **NucleoSpin® Trace Binding Plate**. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate inside the QIAvac 96 in order to ensure best performance.

2.5 Available programs

- For use of **NucleoSpin® 96 Trace** on laboratory automation workstations such as Genesis RSP/RWS Separation System or Miniprep System (TECAN), MultiPROBE II (Packard BioScience) or Biomek (Beckman Coulter):

Robot Supplier	Robot
Tecan	• Genesis RSP/RWS Separation System series • Genesis MiniPrep series
Packard BioScience	• MultiPROBE II
Beckman-Coulter	• Biomek 2000

Visit MN on the internet at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol. All MN protocols can be downloaded from our website.

3 Storage conditions, preparation of working solutions, safety precautions, and setup of vacuum source

Attention:

Buffers FLB and BW contain guanidinium hydrochloride! Wear gloves and goggles!

- Store lyophilized proteinase K at +4°C. All other kit components are stable at room temperature.

Before starting any **NucleoSpin® 96 Trace** protocol prepare the following:

- **Proteinase K**: Add 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.

- All other components of the **NucleoSpin® 96 Trace** kit should be stored at room temperature (for a maximum of 1 year). Storage at lower temperatures may cause precipitation of salts. If a salt precipitate is observed, incubate the bottle at 30-40°C for some minutes and mix well until all of the precipitation is redissolved.

- Establish a reliable vacuum source for the NucleoVac 96 vacuum manifold. The manifold may be used with vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of 200-400 mbar (pressure difference). Alternatively, adjust vacuum that during the purification the sample flows through the column with a rate of 1-2 drops per second. Depending on the amount of sample used the vacuum times might have to be increased for complete filtration.

- Add the indicated volume of **96-100 % ethanol** to **B5** concentrate.

	NucleoSpin® 96 Trace	
	2 x 96 preps	**4 x 96 preps**
Cat.No.	**740 726.2**	**740 726.4**
Proteinase K	2 x 33 mg add 3 ml Proteinase Buffer to each vial	4 x 33 mg add 3 ml Proteinase Buffer to each vial
Buffer B5 concentrate	100 ml add 400 ml ethanol	200 ml add 800 ml ethanol

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® 96 Trace 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
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
FLB	guanidine hydrochloride < 10%	Substance does not have to be specially labeled as hazardous			

Risk Phrases

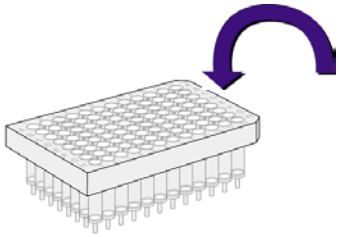
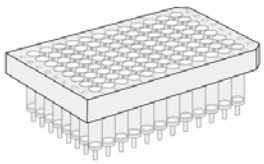
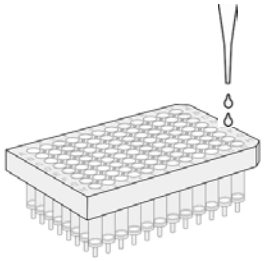
R 22	Harmful if swallowed
R 36/37/38	Irritating to eyes, respiratory system and skin
R 36/38	Irritating to eyes and skin
R 42	May cause sensitisation by inhalation

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

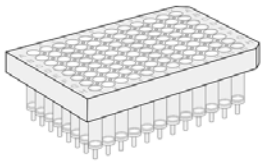
* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 General procedure

1	Pipet 25 µl proteinase K and at least 125 µl buffer FLB to the sample. See support protocol for using **NucleoSpin® Trace Filter Plate** (see ordering information). Incubate several hours or overnight at room temperature.		
	Optional: Separate lysate from sample material.		
2	Add 1 vol Isopropanol to 2 vol lysate, mix 3 times and transfer to NucleoSpin® Trace Binding Plate.		
3	**Bind** DNA to silica membrane by applying vacuum	*ca. – 0.2 bar** *(2 min)*	
4	**Wash** silica membrane	*900 µl B5* *900 µl B5* *ca. – 0.2 bar** *(1 min each step)*	

* Reduction of atmospheric pressure

5	**Remove** MN Wash Plate	
6	**Dry** NucleoSpin® Trace Binding Plate by vacuum or centrifugation. *Optional:* Dry the outlets of the NucleoSpin® Trace Binding Plate before the vacuum step by tapping it to a sheet of paper.	*ca. – 0.6 bar** *or 5,600 – 6,000 x g* *(10 min)*
7	**Elute** highly pure genomic DNA	*50-200 µl BE* *(incubate 3 min at room temperature)* *5,600 – 6,000 x g* *(3 min)*



* Reduction of atmospheric pressure

5.1 Standard protocol for the manual purification of genomic DNA under vacuum

Prepare buffer B5 by adding ethanol. Prepare proteinase K by dissolving one vial of lyophilzed powder in 3 ml Proteinase Buffer (see section 3 for details). If using less than 96 samples seal unused wells of the Trace Binding Plate with a self adhering foil (not supplied with the kit).

1 Premix 25 µl proteinase K and at least 125 µl buffer FLB and pipet to the sample. (Suitable lysis vessels have to be supplied by the user)

See support protocol for using NucleoSpin® Trace Filter Plate (see ordering information).

Incubate several hours or overnight at room temperature.

Optional:

Separate lysate from sample material

Prepare the NucleoVac 96 vacuum manifold:

Insert spacers “MTP/Multi 96 plate” into the NucleoVac 96 vacuum manifold's short sides. Place the waste container inside the vacuum manifold and insert a MN Wash Plate into the notches of the spacers. Close the manifold with the lid.

Place a NucleoSpin® Trace Binding Plate into the rubber seal of the vacuum manifold's lid and apply the samples to the wells of the plate.

2 Add 1 vol (e.g. 330 µl) Isopropanol to 2 vol (e.g. 660 µl) lysate, mix 3 times and transfer to NucleoSpin® Trace Binding Plate.

3 **Bind** genomic DNA to silica membrane

Apply vacuum until all lysates have passed through the columns (-200 mbar; 2 min; -600 mbar 10 s). Ventilate the vacuum manifold.

4 **Wash** silica membrane

1st wash

Add **900 µl B5** to each well of the NucleoSpin® Trace Binding Plate. Apply vacuum (-200 mbar; 1 min) until all buffer has passed through the columns. Ventilate the vacuum manifold.

2nd wash

Add **900 µl B5** to each well of the NucleoSpin® Trace Binding Plate. Apply vacuum (-200 mbar; 1 min) until all buffer has passed through the columns. Ventilate the vacuum manifold.

5 Remove MN Wash Plate

After the final washing step close the valve, ventilate the vacuum manifold and remove the wash plate and waste container from the vacuum manifold.

6 **Dry** NucleoSpin® Trace Binding Plate.

Remove any residual washing buffer from the NucleoSpin® Trace Binding Plate. If necessary, tap the outlets of the NucleoSpin® Trace Binding Plate onto a clean paper sheets (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the NucleoSpin® Trace Binding Plate into the lid and close the manifold. Apply maximum vacuum ca. -600 mbar (pressure difference) for at least 10 min to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note:
The ethanol in buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, close the valve and ventilate the vacuum manifold.

7 **Elution** with centrifuge is recommended (see section 5.2, step 6).

5.2 Standard protocol for the manual purification of genomic DNA under centrifugation

Prepare buffer B5 by adding ethanol. Prepare proteinase K by dissolving one vial of lyophilzed powder in 3 ml Proteinase Buffer (see section 3 for details. If using less than 96 samples seal unused wells with a self adhering foil.

1 Premix 25 µl proteinase K and at least 125 µl buffer FLB and pipet to the sample.

See support protocol for using NucleoSpin® Trace Filter Plate (see ordering information).

Incubate several hours or overnight at room temperature.

Optional:

Separate lysate from sample material

2 Add 1 vol (e.g. 330 µl) Isopropanol to 2 vol (e.g. 660 µl) lysate, mix 3 times and transfer to NucleoSpin® Trace Binding Plate.

3 **Bind** genomic DNA to silica membrane

Centrifuge at 5,600 – 6,000 × *g* for 3 min.

4 **Wash** silica membrane

1st wash

Add **900 µl B5** to each well of the NucleoSpin® Trace Binding Plate. Centrifuge at 5,600 – 6,000 × *g* for 2 min. Empty square well block.

2ndtwash

Add **900 µl B5** to each well of the NucleoSpin® Trace Binding Plate. Centrifuge at 5,600 – 6,000 × *g* for 10 min.

5 **Dry** NucleoSpin® Trace Binding Plate.

Residual washing buffer from NucleoSpin® Trace Binding Plate is removed by the prolonged centrifugation time of 10 min after adding the wash buffer B5 as described in step 4. This prolonged time is necessary to eliminate traces of ethanol.

Note:
The ethanol in buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

6 **Elute** highly pure genomic DNA

For elution place the NucleoSpin® Trace Binding Plate on Rack with MN Tube Strips and pipette 50 -200 µl BE directly to the bottom of each well. Incubate 5 min at room temperature and centrifuge at 5,600 – 6,000 × *g* for 3 min. Close MN Tube Strips with Cap Strips for storage.

Be sure that all of the water gets into contact with the silica membrane: no water drops should stick to the walls of the columns.

5.3 Standard protocol for automated purification of genomic DNA using common laboratory automation workstations

Prepare buffer B5 by adding ethanol. Prepare proteinase K by dissolving one vial of lyophilzed powder in 3 ml Proteinase Buffer (see section 3 for details). If using less than 96 samples seal unused wells of the NucleoSpin Trace Binding Plate.

1 Place the plastic equipment like plates and the assembled vacuum manifold at the locations as specified in the individual robotic programs.

2 Add sufficient buffer to the reservoirs or place the buffer bottles at the corresponding positions on the robot worktable.

Calculate the needed buffer volumes and pour an additional amount of 10% into the reservoirs. Buffers are delivered in sufficient, but limited amounts and should not be wasted. Do not fill back unused buffer into the flask.

3 Select method for genomic DNA purification and start the run.

Use disposable tips with filter for the transfer of lysates to NucleoSpin® Trace Binding Plate. All other pipetting steps can be performed with needles.

4 Elute highly pure genomic DNA

For elution place the NucleoSpin® Trace Binding Plate on a rack with MN Tube Strips and pipette 50 -200 µl BE directly to the bottom of each well. Incubate 5 min at room temperature and centrifuge at 5,600 – 6,000 × *g* for 3 min. Close MN Tube Strips with Cap Strips for storage.

Be sure that all of the water gets into contact with the silica membrane: no water drops should stick to the walls of the columns.

5.4 Support protocol for using NucleoSpin® Trace Filter Plate

Prepare buffer B5 by adding ethanol. Prepare proteinase K by dissolving one vial of lyophilzed powder in 3 ml Proteinase Buffer (see section 3 for details).

1 Put NucleoSpin® Trace Filter Plate onto a square well block. Add forensic material to the wells of the NucleoSpin® Trace Filter Plate. Premix 25 µl proteinase K and the minimum volume of buffer FLB necessary to soak the material completely to the sample. Incubate several hours or overnight at room temperature.

2 After incubation separate the lysate containing DNA from the forensic material by centrifugation (5 min, 5,600-6,000 x *g*).

Proceed with step 2, section 5.1 of the general procedure (adding Isopropanol).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA quality or yield	*Reagents not applied or restored properly* • *Reagents not properly restored. Add the indicated volume of proteinase buffer to the proteinase K vial and 96 – 100% ethanol to buffer concentrate B5 and mix.* *Kit storage* • *Store aliquots of the reconstituted proteinase K at 4°C.* • *Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.* • *Keep bottles tightly closed in order to prevent evaporation or contamination.* *Suboptimal elution* • *Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7.0. Use slightly alkaline elution buffer like BE (pH 8.5).* • *Be sure that all of the elution buffer gets into contact with the silica membrane. No drops should stick to the walls of the columns*
Suboptimal performance of DNA in downstream experiments	*Carryover of ethanol* • *Be sure to remove all of ethanol buffer B5 after the final washing step. Dry the NucleoSpin® Trace Binding Plate for at least 10 min with maximum vacuum.*
Vacuum manifold	*Vacuum pressure is not sufficient* • *Check if the vacuum manifold lid fits tightly to the manifold base if vacuum is turned on.*

Problem	Possible cause and suggestions
Buffers	*Buffer volumes are not enough* • *Buffers are delivered in sufficient, but limited amounts. Calculate the needed buffer volumes and pour an additional amount of 10% into the reservoirs.* • *Do not fill back unused buffer from reservoir to the flask to avoid contaminations. Ask technical service for extended buffer volumes.*
Cross-contamination	*Cross-contamination during transfer of lysate.* • *Be sure that no liquid drops out of the tips while moving the tips with samples above the NucleoSpin® Trace Binding Plate.*

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 96 Trace	740 726.2	2 x 96 preps
NucleoSpin® 96 Trace	740 726.4	4 x 96 preps
NucleoSpin® Trace Filter Plate	Contact technical support	20
MN Wash Plate	740 675	20
MN Frame	740 680	1

6.3 References

Vogelstein B., and D. Gillespie. 1979. Proc. Natl. Acad. Sci. USA **76:** 615-619.

6.4 Product Use Restriction / Warranty

NucleoSpin® 96 Trace kits components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

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

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