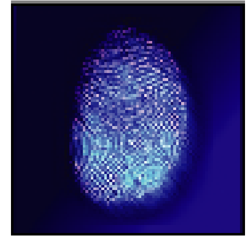

Genomic DNA from Forensic Samples

User manual

NucleoSpin® 8 Trace

November 2003/Rev. 02

MACHEREY-NAGEL

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1 Kit contents

	NucleoSpin® 8 Trace	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740 722.1**	**740 722**
Buffer FLB	100 ml	500 ml
Buffer B5 concentrate	40	200 ml
Proteinase K (lyophilized)[1]	33 mg	5 x 33 mg
Proteinase Buffer	3.6 ml	35 ml
Buffer BE	50 ml	250 ml
NucleoSpin® Trace Binding Strips	12	60
MN Wash Plate (including six paper sheets)	2	10
MN Square-well Blocks	2	10
MN Tube Strips[2]	1	5
Cap Strips	12	60
Self-adhering PE Foil	2	10
Protocol	1	1

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

2 Product description

2.1 The basic principle

With the **NucleoSpin® 8 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 Strips** are 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® 8 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 use with the NucleoVac 96 vacuum manifold (Cat. No. 740681) or similar suitable vacuum manifolds (see section 2.3).

- If using a centrifuge with a swing-out rotor capable to accommodate the **NucleoSpin® Trace Binding Strips**/MN Square-well Block sandwich (bucket height: 85 mm), e.g. Hermle/Macherey-Nagel: NucleoSwing Z513, Qiagen/Sigma 4-15c, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™, Beckman Coulter, Allegra R) no NucleoVac 96 vacuum manifold is necessary.

Kit specifications at a glance	
	NucleoSpin® 8 Trace
Sample type	buccal swabs
Average yield	1-2 µg
Elution volume	50-100 µl
Binding capacity	20 µg
Time/6 strips or one plate	70 min

2.3 Required hardware

The **NucleoSpin® 8 Trace** kit can be used manually with the NucleoVac 96 vacuum manifold (Cat. No. 740681) by using the Starter Set A containing Column Holders A and Dummy Strips (see ordering information).

For automation on laboratory platforms with standard 96-well plate vacuum chambers the use of the Starter Set A is also required.

If automation is desired on a Qiagen BioRobot® 9604/3000 the Starter Kit B is required. Use of the MN Frame (see ordering information) is strongly recommended.

Processing of the **NucleoSpin® 8 Trace** kit under centrifugation is possible by using the Starter Set C (see ordering information), containing Column Holders C, MN Square-well Blocks, Tube Strips. For detailed information refer to the Starter Set C manual.

2.4 Suitability with other common vacuum manifolds

The **NucleoSpin® 8 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 Strips**. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate and Elution Plate, U-Bottom inside the QIAvac 96 in order to ensure best performance.

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

Attention:

Buffer FLB contains 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® 8 Trace** protocol prepare the following:

- **Proteinase K**: Add 3 ml proteinase Buffer per vial to dissolve the lyophilized proteinase K. Store the proteinase K solution at **+4°C for up to 3 months.** Dividing the solution into small aliquots and storage at –20°C is recommended if the solution will not be used up during this period.

- All other components of the **NucleoSpin® 8 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® 8 Trace	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	740 722.1	740 722
Buffer B5 concentrate	40 ml (add 160 ml ethanol to 40 ml buffer B5 concentrate before use)	200 ml (add 800 ml ethanol to 200 ml buffer B5 concentrate before use)

4 General procedure

Step	Procedure	Conditions
1	Pipet 25 µl proteinase K and at least 125 µl buffer FLB to the sample. Incubate several hours or overnight at room temperature.	
	Optional: Separate lysate from sample material. See support protocol for using **NucleoSpin® Trace Filter Plate** (see ordering information).	
2	Add 1 Vol. Isopropanol to 2 Vol. lysate, mix 3 times and transfer to NucleoSpin® Trace Binding Strips.	
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)*
5	**Dry** NucleoSpin® Trace Binding Strips by vacuum or centrifugation. optional: dry the outlets of the NucleoSpin® Trace Binding Strips 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)*
6	**Elute** highly pure genomic DNA	*50-200 µl BE* *(incubate 3 min)* *ca. – 0.6 bar** *or 3 min 5,600 – 6,000 x g*

* Reduction of atmospheric pressure

4.1 Standard protocol for 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 fill up column holder with dummy strips (see ordering information).

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

Incubate several hours or overnight at room temperature.

Optional: Separate lysate from sample material. See support protocol for using **NucleoSpin® Trace Filter Plate** (see ordering information).

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 Strips** inserted in Column Holder A 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 Strips**.

3 Bind genomic DNA to silica membrane

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

4 Wash silica membrane

1st wash

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

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.

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

Remove any residual washing buffer from the **NucleoSpin® Trace Binding Strips**. If necessary, tap the outlets of the **NucleoSpin® Trace Binding Strips** onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the column holder with **NucleoSpin® Trace Binding Strips** 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.

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6 For elution insert spacers “Microtube Rack” into manifold and rest rack with MN Tube Strips on spacers. Insert Column Holder A with **NucleoSpin® Trace Binding Strips** into manifold lid. Pipet 75-200 µl BE directly to the bottom of each well and incubate for 5 minutes at room temperature.

Apply vacuum of -400 mbar for 2 minutes.

Note:
Elution with centrifuge is recommended (see section 4.2).

4.2 Standard protocol for 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 48 samples fill up Column Holder C with Dummy Strips (see ordering information).

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

Incubate several hours or overnight at room temperature.

Optional: Separate lysate from sample material. See support protocol for using **NucleoSpin® Trace Filter Plate** (see ordering information).

2 Mix lysate with isopropanol:

For 150 µl lysate add 75 µl isopropanol, mix 3 times and transfer to **NucleoSpin® Trace Binding Strips**.

Note: Ratio for mixing isopropanol with sample lysates is:
1 vol isopropanol : 2 vol sample lysate

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 Strips**.
Centrifuge at 5,600 – 6,000 × *g* for 2 min. Empty square well block.

2nd wash

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

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

Residual washing buffer from **NucleoSpin® Trace Binding Strips** 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 Column Holder C with **NucleoSpin® Trace Binding Strips** on a rack with MN Tube Strips and pipet 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.

4.3 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 (e.g. buccal swabs) 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 of the general procedure (adding Isopropanol).

5 Appendix

5.1 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 Strips 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-contamina-tion	*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 Strips.*

5.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Trace	740 722.1	12 x 8 preps
NucleoSpin® 8 Trace	740 722	60 x 8 preps
NucleoSpin® Trace Filter Plate	740 677	20
MN Wash Plate	740 675	20
NucleoSwing Z 513	740 610	1
NucleoSwing Z 513 K (refrigerated)	740 610 K	1

5.3 References

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

5.4 Product Use Restriction / Warranty

NucleoSpin® 8 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® 8 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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