Genomic DNA from Forensic Samples

User manual

NucleoSpin® DNA Trace

June 2006/Rev. 04

MACHEREY-NAGEL

Protocol at a glance (Rev. 04)

Genomic DNA from Forensic Samples

Funnel

NucleoSpin® DNA Trace

1	Lyse sample			4-8 ml FLB 50 µl proteinase K 56°C 1h
2	Clarify sample			10 min 5,000 x g
3	Adjust DNA binding conditions			3,5 ml ethanol vortex
4	Bind DNA			Load sample
				3 min 3,000 x g
5	Wash silica membrane		1st wash 2nd wash 3rd wash	2,5 ml BW 5 ml B5 5 ml B5
			1st, 2nd and 3rd wash	3 min 3,000 x g
6	Dry silica membrane			10 min 3,000 x g
7	Elute highly pure DNA			100 µl BE (70°C) RT 2 min
				3 min 3,000 x g

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1 Kit contents

	NucleoSpin® DNA Trace	
	4 preps	**25 preps**
Cat. No.	**740942.4**	**740942.25**
Buffer FLB	50 ml	250 ml
Buffer B5 (concentrate)*	20 ml	80 ml
Buffer BW	15 ml	75 ml
Buffer BE	5 ml	15 ml
Proteinase K*	6 mg	30 mg
Proteinase Buffer	0.8 ml	1.8 ml
NucleoSpin® DNA Trace F columns (plus 50 ml collection tubes)	4	25
0.5 ml elution tubes	4	25
50 ml collecting tubes	4	25
Protocol	1	1

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

2 Product description

2.1 The basic principle

With the **NucleoSpin® DNA Trace** method, DNA can be prepared from cells, tissue and many other sources. Lysis is achieved by incubation of homogenized samples in a solution containing chaotropic ions in the presence of proteinase K. Appropriate conditions for binding of DNA to the silica membrane in the **NucleoSpin® DNA Trace F columns** are created by chaotropic salt itself and added ethanol. The binding process is reversible and specific to nucleic acids. Contaminations are removed by repeated washing with 2 different ethanolic buffers. 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 of DNA from forensic samples using a **NucleoSpin® DNA Tace** 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

- **NucleoSpin® DNA Trace** kit is designed for the preparation of highly pure genomic DNA from small amounts of any tissue, cells and forensic samples e.g. dried blood spots. The **NucleoSpin® DNA Trace F columns** included in the kit are ideally suited for collecting small amounts of nucleic acids from large volumes because these columns are shaped like a funnel combining a large volume capacity with a small diameter of the binding membrane (**F** means funnel). The DNA isolated by **NucleoSpin® DNA Trace F columns** can be used directly for PCR* or other enzymatic reactions.

- Age, storage conditions, quantity and consistency of samples can affect DNA quality, and therefore the protocol may be adapted accordingly (e.g. increasing incubation time). For successful DNA preparation, it is essential that the sample is lysed well and separated afterwards – only clear lysates should be loaded onto **NucleoSpin® DNA Trace F columns** in order to avoid clogging of the silica membrane.

- The **NucleoSpin® DNA Trace** kit allows purification of up to 20 µg of pure genomic DNA with an $A_{260/280}$ ratio of between 1.70 and 1.90. Some samples

* PCR is patented by Roche Diagnostics

(especially forensic samples) may contain only traces of DNA. However, the amount will be sufficient for amplification and detection reactions.

- Additional enzymes, which are not included in the kit, may be necessary for lysis of certain bacteria (e.g. lysozyme, lysostaphine).

- Support protocol for the isolation of genomic DNA from human bones. For this application additional buffers T1, B3, and proteinase K are necessary. Therefore MACHEREY-NAGEL has combined the **NucleoSpin® DNA Trace bones buffer set** (see ordering information). This buffer set is especially designed for completion of the NucleoSpin® DNA Trace kit. It is suited for 25 preparations of genomic DNA from human bones in conjunction with the NucleoSpin® DNA Trace kit (Cat. No. 740942.25).

Kit specifications at a glance	
	DNA Trace **(Funnel)**
Sample size	Forensic samples, which can be extracted with up to 8 ml lysis buffer FLB (in general 10 mg tissue, < 10^5 cells)
Typical Recovery rate	> 70 % for amounts > 10 ng
Typical Sensitivity	traces of DNA, at least 1 ng
Elution volume	100 µl
Purity	ready-to-use for subsequent amplification reactions
Binding capacity	20 µg DNA
Time/prep	60 min (without proteinase K incubation which needs > 1h)
Column type	NucleoSpin® DNA Trace F columns fitting in 50 ml tubes

3 Storage conditions and preparation of working solutions

Attention:

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

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

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

- Before first use of the kit, 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.

	NucleoSpin® DNA Trace	
	4 preps	**25 preps**
Cat. No.	**740942.4**	**740942.25**
Proteinase K	6 mg add 300 µl Proteinase Buffer	30 mg add 1500 µl Proteinase Buffer
Buffer B5 (concentrate)	20 ml add 80 ml ethanol	80 ml add 320 ml ethanol

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

- Upon storage, especially at low temperatures, a white precipitate may form in **buffer FLB**. Such precipitates have to be dissolved by incubating at 45-50°C for 10 min before use.

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® DNA Trace kits contain hazardous contents.

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

Compo-nent	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
BW	guanidine hydrochloride + isopropanol < 25%	Xn*	Flammable. Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16-25
Proteinase K	Proteinase K, lyophilized	Xn Xi*	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 10	Flammable
R 16	Keep away
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 7	Keep container tightly closed
S 16	Keep away from sources of ignition - No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 25	Avoid contact with the eyes
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 Standard protocol for the isolation of genomic DNA from solid samples e.g. small amounts of cells or tissue (forensic samples)

1 Lyse sample

Place the sample in a 15 ml centrifuge tube (not included) and **add 4-8 ml lysis buffer FLB**. The sample should be covered completely with lysis buffer FLB.

Solid samples should be homogenized by commercial tools (pestle and mortar, rotor-stator homogeniser). In general, 10 mg tissue, < 10^5 cells or any DNA-containing solid sample can be used. Forensic samples (dried blood spots, chewing gum, swabs etc.) should be covered completely with lysis buffer.

+ 4-8 ml FLB

Add 50 µl proteinase K stock solution, mix by vortexing, and incubate at **56°C** in a (shaking) water bath until complete lysis is obtained **(1-3 h or overnight**).

Vortexing every 15 min (3-4 times) leads to shorter lysis times if no shaking water bath / incubator is available. Final incubation at 70°C-100°C for 5 min may be recommended for optimal denaturation and lysis of difficult samples (e.g. dried, old or clotted blood samples)

+ 50 µl prot. K

56°C
1 h

2 Clarify sample

Afterwards, any insoluble particles remaining in the sample have to be removed by **centrifugation for 10 min at ≥ 5.000 x g** in order to avoid clogging of the NucleoSpin® DNA Trace membrane.

10 min,

≥ 5,000 x g

3 Adjust DNA binding conditions

Add 3.5 ml ethanol (96-100%) to **4 ml cleared FLB-lysate** and vortex the mixture. Use proportionally up scaled volumes of ethanol, if more FLB-lysate has been prepared in step 1.

+ 3,5 ml ethanol

vortex

4 Bind DNA — **load sample**

Pipette mixture onto the NucleoSpin® DNA Trace F column. Centrifuge for **3 min at 3,000 x *g***. Discard flow-through.

3 min
3,000 x g

5 Wash silica membrane

1st wash — **+ 2,5 ml BW**

Add 2,5 ml buffer BW to the NucleoSpin® DNA Trace F column. Centrifuge for 3 min at 3,000 x *g*. — **3 min, 3,000 x g**

2nd wash — **+ 5ml B5**

Add 5 ml buffer B5 to the NucleoSpin® DNA Trace F column. Centrifuge for 3 min at 3,000 x *g* and discard flow-through. — **3 min, 3,000 x g**

3rd wash — **+ 5ml B5**

Add 5 ml buffer B5 to the NucleoSpin® DNA Trace F column. Centrifuge for 3 min at 3,000 x *g* and discard flow-through. — **3 min, 3,000 x g**

6 Dry silica membrane

Centrifuge additional 10 min at 3,000 x *g* in order to remove buffer B5 completely. — **10 min, 3,000 x g**

7 Elute highly pure DNA

Attach the supplied elution tube with adaptor to the NucleoSpin® DNA Trace F column and insert assembly into a new 50 ml collection tube (not provided). Pipette **100 µl elution buffer BE (preheated to 70°C)** onto the NucleoSpin® membrane and **incubate for 2 min** at room temperature.

+ 100 µl BE

RT
2 min

Centrifuge for **3 min at 3,000 x *g*** to collect the nucleic acid-containing fraction.

3 min,
3,000 x g

Remove the elution tube containing the nucleic acids and keep it for further use.

5.1 Support protocol for the isolation of genomic DNA from human bones

Before starting with the preparation, please read remarks below.

Before starting with the preparation set incubators or water baths to 56°C and 70°C, respectively. Before elution, equilibrate elution buffer BE to 70°C.

Attention:

- The list numbers in this support protocol do not correspond with the list numbers in section 4 and protocol at a glance.

- Additional buffer T1, B3 and proteinase K is necessary. The **NucleoSpin® DNA Trace bones buffer set** (Cat. No. 740943.25) is especially designed for completion of the NucleoSpin® DNA Trace kit. It is suited for 25 preparations of genomic DNA from human bones in conjunction with the NucleoSpin® DNA Trace kit (Cat. No. 740942.25).

- Preparation of buffer **B3**: Transfer the total contents of **buffer B1** to **reagent B2**. Mix well. The resulting **buffer B3** is stable for at least 5 months at room temperature in the dark.

- For each prep 2 ml additional buffer has to be prepared (0.5 M EDTA/ 0.25 M PO_4^{3-}, pH 8, not included in the NucleoSpin® DNA Trace bones buffer set)

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1 Prepare sample

Mill 1 g bone to a fine powder.

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2 Pre-Lysis

Add 2 ml buffer (0.5M EDTA/0.25 M PO_4^{3-}, pH 8) and 7 ml **buffer T1** and **100 µl proteinase K solution.** Vortex to mix. Be sure that the samples are completely covered with lysis solution.

If processing several samples, proteinase K and buffer T1 may be premixed directly before use. Do never mix bufer T1 and proteinase K more than 10 – 15 min before addition to the sample: proteinase K tends to self-digestion in buffer T1 without substrate.

Incubate at **56°C** overnight.

Afterwards incubate sample for 48h at 4°C on a shaking incubator.

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3 Lysis

Vortex the samples. Add **8 ml buffer B3,** vortex vigorously and incubate at **70°C** for **10 min.** Vortex briefly.

Centrifuge for 10 min at 5,000 x g and transfer the supernatant to a new microcentrifuge tube.

4 Adjust DNA binding conditions

Add **8.4 ml ethanol** (96-100%) to the sample and vortex vigorously.

5 Bind DNA

For each sample, place one NucleoSpin® DNA Trace F column into a 50 ml collecting tube. Apply the sample successively to the column. Centrifuge for **3 min** at **3,000** × **g**. Discard the flow-through and place the column back into the collecting tube.

6 Wash silica membrane

1st wash

Add **3 ml buffer BW.** Centrifuge for **3 min** at **3,000** × **g.** Discard the flow-through and place the column back into the collecting tube.

2nd wash

Add **3 ml buffer B5** to the column and centrifuge for **3 min** at **3,000** × **g.** Discard the flow-through and place the column back into the collecting tube.

3rd wash

Add **3 ml buffer B5** to the column and centrifuge for **3 min** at **3,000** × **g.** Discard the flow-through and place the column back into the collecting tube.

7 Dry silica membrane

Centrifuge the column for **10 min at 3,000 x g.**

Residual ethanol is removed during this step.

8 Elute highly pure DNA

Attach the supplied elution tube with adaptor to the NucleoSpin® DNA Trace F column and insert assembly into a new 50 ml collection tube (not provided). Add **60 µl elution buffer BE** (preheated to 70°C). **Incubate** at room temperature **for 2 min**.

Centrifuge for **3 min at 3,000 x *g*** to collect the nucleic acid-containing fraction.

Remove the elution tube containing the nucleic acids and keep it for further use.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield, poor DNA quality	*Incomplete lysis of sample* • Sample not thoroughly homogenized and mixed with buffer FLB / proteinase K. The mixture has to be shaken continuously. Alternatively, prolong incubation time with proteinase K. *Reagents not applied properly* • Prepare buffer B5, and proteinase K solutions according to instructions (section 3). Add ethanol to lysates before loading them on NucleoSpin® DNA Trace F columns. *Suboptimal elution of DNA from the column* • Apply buffer BE (70°C) directly onto the center of the silica membrane and incubate for 2 min. Elution efficiencies decrease dramatically, if elution is done with other buffers at pH ≤ 7.0.
Poor DNA quality and/or suboptimal performance of genomic DNA in enzymatic reactions	*RNA in sample* • If RNA-free DNA is desired, add 20 µl of RNase A solution (20 mg/ml) to lysis buffer FLB. *Carryover of ethanol* • Be certain to centrifuge ≥ 5 min at 3,000 x g in order to remove all of ethanolic buffer B5 before eluting the DNA. If for any reason, the level of buffer B5 has reached the column outlet after the second wash, discard flow-through. Place the NucleoSpin® DNA Trace F column back into the collecting tube, and centrifuge again.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® DNA Trace kit	740942.4	4 preps
NucleoSpin® DNA Trace kit	740942.25	25 preps
NucleoSpin® funnel column	740959	30 columns
Wash buffer BW	740922	100 ml
Wash buffer B5 concentrate (for 100 ml)	740921	20 ml
NucleoSpin® DNA Trace bones buffer set	740943.25	1 set
Proteinase K	740506	100 mg
RNase A	740505	100 mg
RNase A	740505.50	50 mg

6.3 Product use restriction / warranty

NucleoSpin® DNA Trace kit components were developed, designed 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® DNA Trace** 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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