Bioanalysis

MACHEREY-NAGEL

User manual

Genomic DNA from forensic samples

■ NucleoSpin® DNA Forensic

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1 Components

1.1 Kit contents

	NucleoSpin® DNA Forensic		
REF	**10 preps 740840.10**	**50 preps 740840.50**	**250 preps 740840.250**
NucleoSpin® DNA Forensic Columns	10	50	250
Collection Tubes (2 mL)	20	100	500
Lysis Buffer FOL	13 mL	50 mL	250 mL
Binding Buffer FOB	7 mL	35 mL	2 x 100 mL
Wash Buffer FOW1	20 mL	2 x 20 mL	2 x 80 mL
Wash Buffer FOW2	6 mL	12 mL	50 mL
Elution Buffer FOE	13 mL	13 mL	30 mL
Liquid Proteinase K	2 x 120 µl	1250 µL	6 mL
Reducing Agent TCEP	14 mg	14 mg	2 x 14 mg
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1.2 Reagents, consumables and equipment to be supplied by user

Reagents

- Ethanol (96–100 %) to prepare Buffer FOW2

Consumables

- Disposable pipette tips
- NucleoSpin® Forensic Filters for incubation and lysate separation in one tube (or conventional 1.5 ml microcentrifuge tubes)
- NucleoSpin® DNA Trace Bone Buffer Set (REF 740943.25) and additional Buffer FOB (REF 740841.100) for isolation of genomic DNA from bones or teeth (sufficient for 250 samples)
- 1.5 mL microcentrifuge tubes for DNA elution

Equipment

- Manual pipettors
- Centrifuge for NucleoSpin® Forensic Filters and microcentrifuge tubes
- Vortex mixer
- Suitable homogenization device (e.g., mortar and pestle, rotor-stator)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first time users of the **NucleoSpin® DNA Forensic** kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. All technical literature is available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® DNA Forensic** procedure is based on reversible adsorption of nucleic acids to the silica membrane in the **NucleoSpin® DNA Forensic Columns** under appropriate buffer conditions. Lysis is achieved by incubation of samples with Proteinase K at 56 °C. For the adjustment of binding conditions under which nucleic acids bind to the silica membrane, Buffer FOB is added to the lysate. After binding, the silica membrane is washed three times to remove contaminants and salts using Wash Buffers FOW1 and FOW2. Residual ethanol from previous wash steps is removed by a drying step. Finally, highly purified DNA is eluted with low salt Elution Buffer (FOE) and can directly be used for downstream applications.

2.2 Kit specifications

- **NucleoSpin® DNA Forensic** is designed for the small-scale preparation of highly pure genomic DNA from buccal swabs, small amounts of any tissue, cells or other forensic samples, for example, dried blood spots, 'trace and touch'-samples or cigarette filters.The purified DNA can be used directly as template for PCR, or any kind of enzymatic reaction.
- Age, storage conditions, quantity as well as consistency of sample material 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 Forensic Columns in order to avoid clogging of the silica membrane.
- The kit provides reagents for the purification of up to 7 µg of pure genomic DNA from suitable samples (typical yields for DNA isolation from buccal swabs: 1–3 µg DNA). Depending on the elution volume used, concentrations of 10–30 ng/µL can be obtained.
- Following lysis of samples with Proteinase K at 56 °C (recommended, optional: Proteinase K treatment can be performed at RT) **NucleoSpin® DNA Forensic** can be processed completely at room temperature, however, elution at 56 °C will increase the yield by about 15–20 %.
- Support protocol for the isolation of genomic DNA from human bones. For this application additional Buffer T1, Buffer B3, and Proteinase K are necessary. Therefore MACHEREY-NAGEL offers the NucleoSpin® DNA Trace Bone Buffer Set (see section 7.2, ordering information).
- For research use only.
- The **NucleoSpin® DNA Forensic kit 740840.50** and **740840.250** comply with all ISO 18385 requirements.

The ISO 18385 specifies demands for the manufacturing of products used in the collection, storage, and analysis of biological material for forensic DNA purposes. We implemented the ISO 18385 for the production of kits for forensic applications. Therefore we minimized the risk of human DNA contamination in all our products labeled for isolation of nucleic acids from forensic samples. All consumables used in our forensic product line are treated (post-production) with ethylene oxide (EO), the recommended method to remove amplifiable DNA prior to forensic sampling*.

* Shaw et al, 2008, Comparison of the effects of sterilisation techniques on subsequent DNA profiling. Int J Legal Med 122:29–33

2.3 Elution procedures

In addition to the standard method (recovery rate about 70–90 %), several modifications are possible to increase the yield, concentration, and convenience. Use elution buffer 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 approximately 30 % higher than with standard elution. The yield of eluted nucleic acid will be 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.
- Elution at 70 °C: For certain sample types, heating the elution buffer to 70 °C increases the DNA yield.

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. Note: Elution Buffer BE (5 mM Tris/HCl, pH 8.5) provided with the kit does not contain EDTA.

For optimal performance of isolated DNA in downstream applications, we recommend eluting with the supplied elution buffer and storage, especially long term, at -20 °C. Freeze-thaw cycles will have no effect on most downstream applications. Possible exceptions are detection of trace amounts of DNA or long-range PCR (e.g., > 10 kbp). Multiple freeze-thaw cycles or storing DNA at 4 °C or room temperature may influence detection sensitivities or reaction efficiencies due to DNA shearing or adsorption to surfaces.

3 Storage conditions and preparation of working solutions

Attention: *Buffers FOL, FOB and FOW1 contain chaotropic salt! Wear gloves and goggles!*

CAUTION: Buffers FOB and FOW1 contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Storage conditions:

- All components of the **NucleoSpin® DNA Forensic** kit should be stored at 15–25 °C and are stable until: See package label. Storage at lower temperatures may cause precipitation of salts. If a salt precipitation is observed, incubate the bottle at 30–40 °C for some minutes and mix well until all of the precipitate is redissolved. The performance of the kits is not affected by the salt precipitates

Before starting the **NucleoSpin® DNA Forensic** protocol, prepare the following:

- **Wash Buffer FOW2:** Add the indicated volume of ethanol (96–100 %) to **Buffer FOW2 Concentrate** before use. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer FOW2 at 15–25 °C for up to one year
- **Reducing Agent TCEP**: Add 1 mL of sterile H_2O to the TCEP vial and incubate for several minutes at room temperature. Mix the vial to dissolve the TCEP completely. Store dissolved TCEP at -20 °C.

	NucleoSpin® DNA Forensic		
REF	**10 preps 740840.10**	**50 preps 740840.50**	**250 preps 740840.250**
Wash Buffer FOW2 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol
Reducing Agent TCEP	1 vial (14 mg) Add 1 mL H_2O	1 vials (14 mg) Add 1 mL H_2O	2 vials (14 mg/vial) Add 1 mL H_2O to each vial

4 Safety instructions

When working with the **NucleoSpin® DNA Forensic** kit, wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information, consult the appropriate Material Safety Data Sheets (MSDS available online at ***http://www.mn-net.com/msds***).

Caution: Guanidinium thiocyanate in Buffer FOB and Buffer FOW1 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® DNA Forensic** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment, but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according to local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocol for the isolation of genomic DNA from forensic samples

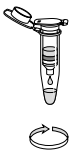
Protocol at a glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 12.

Before starting the preparation:

- Check if Wash Buffer FOW2 and TCEP were prepared according to section 3.

1	**Lyse sample**		**Add 20 µL Liquid Proteinase K, 5 µL TCEP solution (14 mg/mL) and 450 µL Buffer FOL** **Mix** **56 °C, 1 h**
2	**Adjust DNA binding conditions**		**Add 580 µL FOB** **Vortex**
3	**Bind DNA**		**Load 600 µL lysate** **1 min at 11,000 x *g*.**
			**Load remaining lysate** **1 min at 11,000 x *g*.**
4	**Wash with FOW1**		**Add 400 µL FOW1** **1 min at 11,000 x *g*.**
5	**Wash with FOW2** **1st wash**		**Add 400 µL FOW2** **1 min at 11,000 x *g*.**

6	**Wash with FOW2** **2nd wash**		**Add 400 µL FOW2** **1 min at 11,000 x *g*.**
7	**Dry silica membrane**		**2 min at 11,000 x *g*.**
8	**Elute DNA**		**Add 50–100 µL Buffer FOE.** **1 min RT** **1 min at 11,000 x *g*.**

Detailed protocol

- For additional equipment and hardware requirements, refer to section 1.2, respectively.

Before starting the preparation:

- Check if Wash Buffer FOW2 was prepared according to section 3.

Sample collection

Collect the samples with cotton, Dacron, or C.E.P. swabs. Scrape firmly against the inside of each cheek several times and let the swabs air dry.

The respective individual should not have consumed food or drink within 30 min before collection of the sample.

Samples should be processed immediately or stored at 4 °C.

1 Lyse samples

Add 20 µL of Liquid **Proteinase K, 5 µL TCEP (14 mg/mL)** and **450 µL Lysis Buffer FOL** to a 1.5ml microcentrifuge tubes (optional: NucleoSpin® Forensic Filter tube) containing the sample. **Mix** well by vigorous shaking or by vortexing. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tube.

Note: Buccal swab heads should be submerged into the lysis solution. Therefore, depending on type or size of buccal swab used, the FOL buffer volume has to be increased. Increasing volume of Proteinase K or TCEP is not required.

Incubate at **56 °C** for **60 min** with shaking. Make sure that the tubes are securely closed. When using 1.5ml microcentrifuge tubes for lysis of e.g. buccal swabs, remove buccal swab and squeeze out to obtain **400 µL lysate**. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tube.

Alternatively: Spin the NucleoSpin® Forensic Filter tube 1 min > 10,000 x *g* to separate carrier e.g. buccal swabs and collect lysate in the collection tube. Discard the filtering cartridge including the carrier material and proceed with the lysate in the collection tube.

2 Adjust DNA binding conditions

Add **580 µL of Binding Buffer FOB** to each sample and vortex the mixture

3 Bind DNA to NucleoSpin® DNA Forensic Column

For each sample, place one NucleoSpin® DNA Forensic Column into a Collection Tube. Apply the 600 µL of the mixture to the column. Centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection Tube.

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

Apply the remaining volume of the mixture to the column. Centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column into a new Collection Tube (provided).

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

4 Wash with FOW1

Add **400 µL Buffer FOW1** to the column and centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection Tube.

5 Wash with FOW2 (1st)

Add **400 µL Buffer FOW2** to the column and centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection Tube.

6 Wash with FOW2 (2nd)

Add **400 µL Buffer FOW2** to the column and centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection Tube.

7 Dry silica membrane

Centrifuge the column for 2 min at 11,000 x *g*.

Residual ethanol is removed during this step.

8 Elute DNA

Place the NucleoSpin® DNA Forensic Column into a 1.5 mL microcentrifuge tube (not provided) and add **70 µL Buffer FOE** (preheated to 70 °C) onto the center of the NucleoSpin® membrane and incubate for 1 min at room temperature.

Centrifuge for **1 min** at **11,000 x g**.

For alternative elution procedures see section 2.3.

6 Protocol for the isolation of genomic DNA from bone or teeth samples

Detailed protocol

- For lysis of bone and teeth samples the NucleoSpin® DNA Trace Bone Buffer Set (REF: 740943.25) and additional Buffer FOB (REF 740841.100) are required.
- For additional equipment and hardware requirements, refer to section 1.2, respectively.

Before starting the preparation:

- Check if Wash Buffer FOW2 and TCEP were prepared according to section 3.
- Additional Buffer T1, Buffer B3 and Proteinase K is necessary. The NucleoSpin® DNA Trace Bone Buffer Set (REF 740943.25) is compatible to the NucleoSpin® DNA Forensic kit. It is suited for approx. 250 preparations of genomic DNA from human bones in conjunction with the NucleoSpin® DNA Forensic kit (REF 740840.250).
- For each prep, 200 µL additional buffer is necessary (0.5 M EDTA / 0.25 M PO_4^{3-}, pH 8, not included in the NucleoSpin® DNA Trace Bone Buffer Set).

Sample preparation

Mill up to 150 mg of bone or teeth to a fine powder.

Transfer up to 150 mg of bone powder into a 2 mL microcentrifuge tube.

1 Pre-lyse samples

Add **200 µL phosphate buffer** (0.5 M EDTA / 0.25 M PO43-, pH 8), **700 µL Buffer T1** and **20 µL Proteinase K. Mix** well by vigorous vortexing and incubate at 56 °C on a shaking incubator overnight.

Afterwards incubate for 48 h at 2–8 °C on a shaking incubator.

Note: Make sure the sample is completely submerged in the lysis solution and does not agglomerate at the bottom of the tube.

2 Lyse samples

Vortex the samples. Add **800 µL Buffer B3** and mix well by vigorous vortexing. Incubate for **10 min** at **70 °C**. Vortex briefly.

Centrifuge for **10 min** at **5,000 x g**. Transfer 800 µL of the supernatant to a new 2 mL microcentrifuge tube.

3 Adjust binding conditions

Add **1160 µL Binding Buffer FOB** to the lysate and vortex briefly.

4 Bind DNA to NucleoSpin® DNA Forensic Column

Place the NucleoSpin® DNA Forensic Binding column in a Collection Tube (provided). Load 600 µL lysate to the column. Centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back in the Collection Tube.

5 **Repeat binding step.** Load 600 µL lysate to the column. Centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back in the Collection Tube.

6 **Load the remaining lysate to the column.** Centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column in a new Collection Tube (provided).

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

7 **Wash with FOW1**

Add **400 µL Buffer FOW1** to the column and centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection Tube.

8 **Wash with FOW2 (1st)**

Add **400 µL Buffer FOW2** to the column and centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection tube.

9 **Wash with FOW2 (2nd)**

Add **400 µL Buffer FOW2** to the column and centrifuge for 1 min at 11,000 x *g*. Discard the flowthrough and place the column back into the Collection tube.

10 **Dry silica membrane**

Centrifuge the column for 2 min at 11,000 x *g*.

Residual ethanol is removed during this step.

11 **Elute DNA**

Place the NucleoSpin® DNA Forensic Column into a 1.5 mL microcentrifuge tube (not provided) and add **100 µL Buffer FOE** (preheated to 70 °C) onto the center of the NucleoSpin® membrane and incubate for 1 min at room temperature.

Centrifuge for **1 min at 11,000 x *g***.

For alternative elution procedures see section 2.3.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield and quality	*Incomplete sample lysis* • Sample was not thoroughly homogenized and mixed with Lysis buffer, Proteinase K and TCEP. The mixture has to be shaken continuously. Alternatively, prolong incubation time with Proteinase K. *Reagents not prepared properly* • Prepare Buffer FOW2 and TCEP according to the instructions (section 3). *Suboptimal elution of DNA from the column* • For certain sample types, preheat Buffer BE to 70 °C before elution. Apply Buffer FOE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically, if elution is done with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer FOE (pH 8.5). • Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL Buffer FOE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol or salts from wash buffers* • Make sure to centrifuge ≥ 1 min at 11,000 x *g* in order to remove all of ethanolic Buffer FOW2 before eluting the DNA. If, for any reason, the level of Buffer FOW2 has reached the column outlet after drying, repeat the centrifugation. *Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer FOE.

7.2 Ordering information

Product	REF	Pack of
NucleoMag® DNA Forensic	744660.1 744660.4	1 x 96 preps 4 x 96 preps
NucleoSpin® DNA Forensic	740840.10 740840.50 740840.250	1 x 10 preps 1 x 50 preps 1 x 250 preps
NucleoSpin® Forensic Filters (Bulk)	740988.50B 740988.250B 740988.1000B	50 x 96 pieces 250 x 96 pieces 1000 x 96 pieces
NucleoSpin® DNA Trace Bone Buffer Set	740943.25	1 set
Buffer FOB	740841.100	100 mL

Visit www.mn-net.com for more detailed product information.

7.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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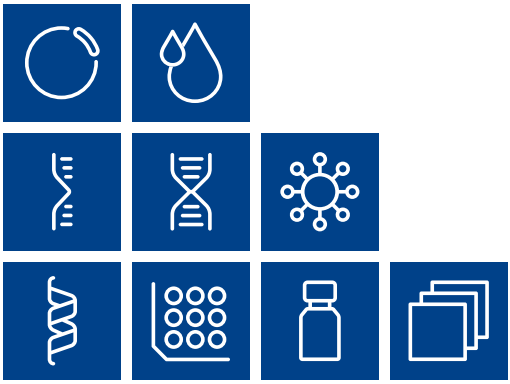
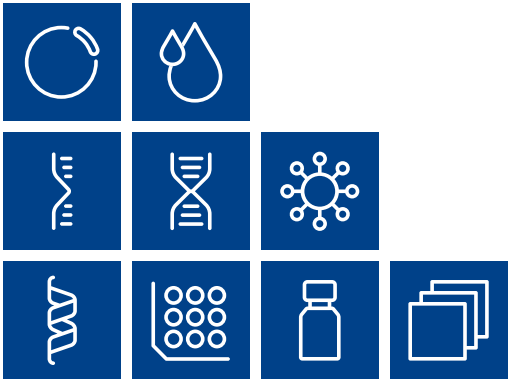


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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