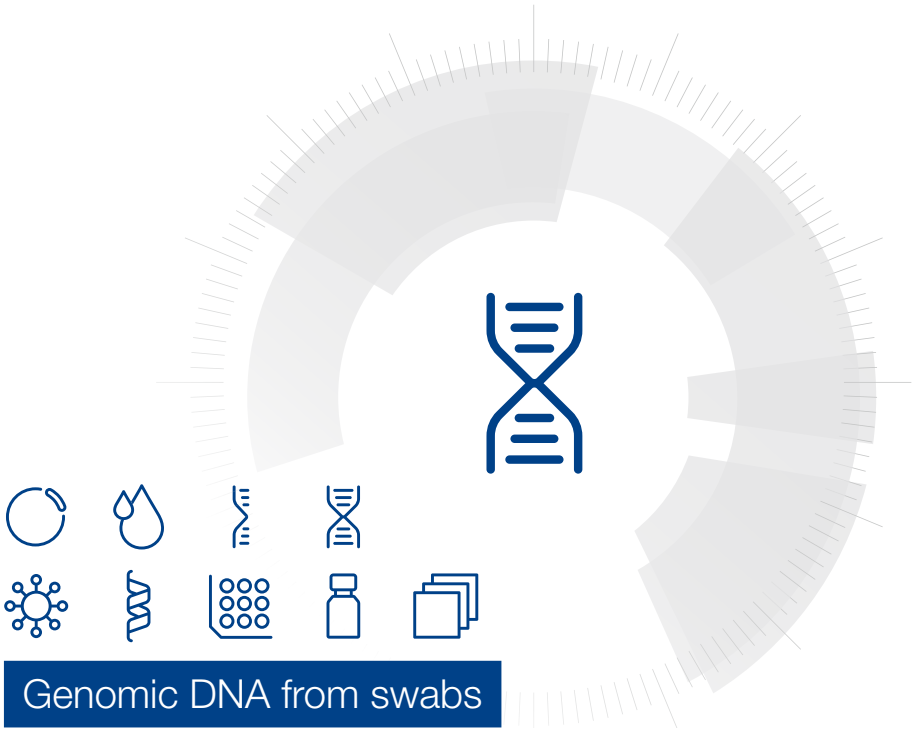
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from swabs

- NucleoMag® DNA Swab

September 2024 / Rev. 02

Contact MN

Germany and international

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
E-mail: info@mn-net.com

Technical Support Bioanalysis

Tel.: +49 24 21 969-333
E-mail: support@mn-net.com

USA

MACHEREY-NAGEL Inc.
924 Marcon Blvd. · Suite 102 · Allentown PA, 18109 · USA
Toll-free: 888 321 6224 (MACH)
E-mail: sales-us@mn-net.com

France

MACHEREY-NAGEL SAS
1, rue Gutenberg – BP135 · 67720 Hoerdt Cedex · France
Tel.: +33 388 68 22 68
E-mail: sales-fr@mn-net.com

MACHEREY-NAGEL SAS (Société par Actions Simplifiée) au capital de 186600 €
Siret 379 859 531 00020 · RCS Strasbourg B379859531 · N° intracommunautaire FR04 379 859 531

Switzerland

MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
E-mail: sales-ch@mn-net.com

www.mn-net.com

Table of contents

1 Components 4
1.1 Kit contents 4
1.2 Consumables and equipment to be supplied by user 4
1.3 About this user manual 5
1.4 Automation support 5
2 Product description 6
2.1 The basic principle 6
2.2 Kit specifications 6
2.3 Magnetic separation systems 7
2.4 Adjusting the shaker settings 8
2.5 Handling of beads 8
2.6 Elution procedures 9
3 Storage conditions and preparation of working solutions 10
4 Safety instructions 11
4.1 Disposal 11
5 Protocols for the isolation of genomic DNA from swabs 12
5.1 Protocol at a glance 12
5.2 Detailed protocol 15
5.3 Detailed protocol for KingFisher® Flex 18
6 Appendix 21
6.1 Troubleshooting 21
6.2 Ordering information 23
6.3 Product use restriction / warranty 24

1 Components

1.1 Kit contents

	NucleoMag® DNA Swab		
REF	**1x 96 preps 744601.1**	**4 x 96 preps 744601.4**	**24 x 96 preps 744601.24**
NucleoMag® B-Beads	1.5 mL	4 x 1.5 mL	42 mL
Lysis Buffer FLB	50 mL	125 mL	3 x 250 mL
Binding Buffer MBL2	2 x 40 mL	2 x 110 mL	2 x 500 mL
Wash Buffer MB3	75 mL	300 mL	2 x 900 mL
Wash Buffer MB4	75 mL	300 mL	2 x 900 mL
Elution Buffer MB6	30 mL	60 mL	300 mL
Liquid Proteinase K	4 x 0.8 mL	3 x 4.5 mL	3 x 30 mL
User manual	1	1	1

1.2 Consumables and equipment to be supplied by user

Reagents

- 80 % ethanol

Product	**REF**	**Pack of**
Magnet for magnetic beads separation NucleoMag® SEP (suitable for 96-(deep)well plates)	744900	1
NucleoMag® SEP Mini (suitable for 1.5–2 mL tubes)	744901	1
NucleoMag® SEP Maxi (suitable for 50 mL tubes)	744902	1
NucleoMag® SEP 24 (suitable for 24-deep-well plates)	744903	1
Separation plate for magnetic beads separation, e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
Liquid Proteinase K	740396	5 mL
Rack of Tube Strips e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Elution plate for collecting purified nucleic acids, e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486.24	24

Product	REF	Pack of
For use of kit on KingFisher® instruments: e.g., KingFisher® 96 Accessory Kit A (Square-well Blocks, Deep-well tip combs, Plates for 4 x 96 NucleoMag® DNA Swab Water preps using KingFisher® 96 / Flex platform)	744950	1 set

1.3 About this user manual

It is strongly recommended that first time users of the **NucleoMag® DNA Swab** 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 as a tool for quick referencing while performing the purification procedure.

All technical literature is available at ***www.mn-net.com***.

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

1.4 Automation support

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

MN extraction kits are designed for streamlined automation, offering compatibility with a range of leading open robotic systems. Whether you're using magnetic rod systems or liquid handlers like Hamilton, Tecan, Eppendorf, or other platforms, our kits ensure efficient and reliable extraction processes. Reach out to us for comprehensive support and tailored automation solutions, making your extraction experience seamless and effortless.

Questions about MACHEREY-NAGEL's scripting support or automation service?

Please contact us for personal assistance:
Phone: +49 2421 969-333
E-mail: support@mn-net.com

2 Product description

2.1 The basic principle

The **NucleoMag® DNA Swab** kit is designed for the isolation of gDNA (human and / or microbial) from swab specimens. The kit is compatible with cotton swabs as well as synthetic swabs (e.g., flocked swabs). The procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Lysis is achieved by incubation of samples with lysis buffer and Proteinase K at 56 °C. For the adjustment of binding conditions under which nucleic acids bind to the paramagnetic beads, Buffer MBL2 and the NucleoMag® B-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed twice to remove contaminants and salts using Wash Buffers MB3 and MB4. Residual ethanol from previous wash steps is removed by a drying step. Finally, highly purified DNA is eluted with low salt Elution Buffer (MB6) and can directly be used for downstream applications. The **NucleoMag® DNA Swab** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit ***www.mn-net.com/automation***.

2.2 Kit specifications

- **NucleoMag® DNA Swab** is designed for rapid manual and automated small-scale preparation of highly pure genomic DNA from buccal swab specimens. In addition, the kit is suited for isolation of DNA from other samples, such as dried blood spots or cigarette filters. The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). Manual preparation of 96 samples takes about 120 minutes. The purified DNA can be used directly as template for DNA amplifications (e.g., PCR), or any kind of enzymatic reactions.
- **NucleoMag® DNA Swab** allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on a common liquid handling platform or less than 30 minutes using a magnetic rod based system (excluding sample lysis). For more information about the automation process and the availability of ready to run scripts for certain platforms, please contact your local distributor or MN directly.
- 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), **NucleoMag® DNA Swab** can be processed completely at room temperature, however, elution at 56 °C will increase the yield by about 15–20 %.
- For research use only

2.3 Magnetic separation systems

For use of **NucleoMag® DNA Swab**, the use of the NucleoMag® SEP magnetic separator is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP or other common magnetic separators (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

- Load 600 µL dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100–200 µL dyed water to the wells of the collection plate and proceed as described above.

2.5 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage tube or bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads / binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Small elution volume possible	Number of tips needed
Magnetic mix	+	++	+	Low
Shaker	++	++	+++	Low
Pipetting	+++	+*	++	High

+: acceptable, ++: good, +++: excellent, * 8-channel pipetting device

2.6 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer MB6. Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, higher elution volumes might be necessary to cover the whole pellet.

Elution is possible at room temperature. Yield can be increased by 15–20 % if elution is performed at 56 °C.

3 Storage conditions and preparation of working solutions

Attention: *Buffers MBL2, MB3, and MB4 contain chaotropic salt! Wear gloves and goggles!*

Storage conditions:

- All components of the **NucleoMag® DNA Swab** kit should be stored at room temperature (18–25 °C) and are stable for at least one year.
- All buffers are delivered ready to use.

Before starting the **NucleoMag® DNA Swab** protocol, prepare the following:

- 80% ethanol for use in protocol step 6

4 Safety instructions

When working with the **NucleoMag® DNA Swab** 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 ***www.mn-net.com/msds***).

The waste generated with the **NucleoMag® DNA Swab** 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 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.

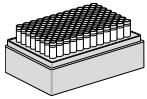
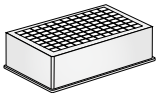
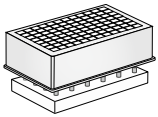
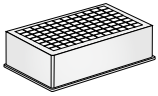
5 Protocols for the isolation of genomic DNA from swabs

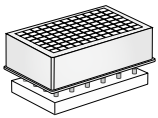
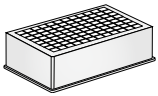
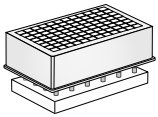
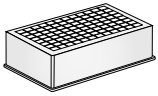
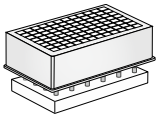
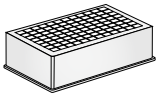
5.1 Protocol at a glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 15.

Before starting the preparation:

- Prepare an appropriate volume of ethanol 80% for use in step 6.

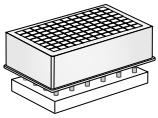
1	**Lyse sample** (e.g., buccal swabs)	**Add 30 µL Liquid Proteinase K and 270 µL Buffer FLB** **Mix** **56 °C, 45 min**	
2	**Separate and transfer lysate**	**Separate swab material to obtain 300 µL lysate. Transfer 300 µL of lysate to a Square-well Block**	
3	**Bind** DNA to NucleoMag® B-Beads	**300 µL lysate** **14 µL NucleoMag® B-Beads** **400 µL MBL2**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
4	**Wash** with MB3	Remove Square-well Block from NucleoMag® SEP **600 µL MB3**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	

		Remove supernatant after 2 min separation	
5	**Wash** with MB4	Remove Square-well Block from NucleoMag® SEP **600 µL MB4** **Resuspend: Shake 5 min at RT** *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
6	**Wash** with ethanol 80 %	Remove Square-well Block from NucleoMag® SEP **700 µL ethanol 80 %**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
7	**Dry beads**	**10 min at RT**	
8	**Elute** DNA	Remove Square-well Block from NucleoMag® SEP **50–100 µL MB6** *(Optional: Elute at 56 °C)*	

Shake 5 min at RT

(Optional: Mix by pipetting up and down)

Separate 2 min and transfer DNA into elution plate



5.2 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Sample collection

Collect the samples with cotton, Dacron, or C.E.P., or flocked 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.

Note: Swabs may be collected from alternative epithelial surfaces. Other types of small-scale sample matrices containing cellular material (e.g., blood spots, cigarette buds, surface swabs) may be used with this kit.

Before starting the preparation:

- Prepare an appropriate volume of ethanol 80% for use in step 6

1 Lyse samples

Calculate the amount of lysis stock required: for each sample, **30 µL Liquid Proteinase K solution** + **270 µL Buffer FLB** are required. Prepare lysis stock solution accordingly and vortex.

Note: Never prepare the lysis stock solution more than 15 min before addition to the samples. Proteinase K tends to self-digestion when incubated in Buffer FLB without substrate.

Place the swab head in a suitable lysis tube.

Transfer 300 µL of the resulting solution to each lysis tube containing the swab head. Close the individual tubes. **Mix** by vigorous shaking for 10–15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tube.

Note: The swab heads should be submerged in the lysis solution. Therefore, depending on type or size of buccal swab used, the FLB buffer volume may have to be increased to up to 400 µL. Increasing volume of Proteinase K is not required.

Alternatively, perform lysis with Buffer FLB / Proteinase K in a NucleoSpin® Forensic Filter or a NucleoSpin® Trace Filter Plate (see ordering information). These accessories allow convenient separation of lysate from swab material by centrifugation and reduce loss of lysate.

Incubate the tubes containing the samples at **56 °C** for **45 min** or overnight at room temperature. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes are securely closed.

2 Separate and transfer lysate

Separate swab material from lysed sample. Remove buccal swab and squeeze out to obtain **300 µL lysate**.

Note: When using increased volumes (> 270 µL) of Buffer FLB in step 1 of the procedure, transfer the respective amount of lysed sample to a new Square-well Block for further processing.

Note: When using NucleoSpin® Forensic Filters, spin the filter tube 1 min at > 10,000 x g *to separate lysate and swab. Discard the filtering cartridge including the swab material and proceed with the lysate in the collection tube. When using the NucleoSpin® 96 Trace Filter Plate, centrifuge the NucleoSpin® Trace Filter Plate stacked onto a 96 well Square-well Block for 5 min at 4,000 x* g *to separate lysate and swab. The centrifuge must be able to accommodate the NucleoSpin® 96 Trace Filter Plate stacked on a Square-well Block (bucket height: min. 75 mm).*

3 Bind DNA to NucleoMag® B-Beads

To each **lysate of 300 µL** from the previous step, add **14 µL of NucleoMag® B-Beads** and **400 µL of Binding Buffer MBL2**. Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Note: NucleoMag® B-Beads and Buffer MBL2 can be premixed. Per well to be processed, mix 14 µL of NucleoMag® B-Beads with 400 µL Buffer MBL2. Vortex briefly. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are required.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might be not visible in this step. Remove supernatant from the opposite side of the well.

4 Wash with MB3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MB3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by pipetting up and down repeatedly (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

5 Wash with MB4

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MB4** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by pipetting up and down repeatedly (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

6 Wash with ethanol 80 %

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **700 µL ethanol 80 %** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by pipetting up and down repeatedly (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

7 Dry beads

Dry the magnetic beads by incubating the Square-well Block for **10 min** at **room temperature** in order to remove traces of ethanol.

8 Elute DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **Buffer MB6 (50–100 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5–10 min** at **room temperature** or **56 °C**. Alternatively, resuspend beads completely by pipetting up and down repeatedly and incubate for **5–10 min** at **room temperature** or **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified genomic DNA to the Elution Plate.

Note: Yield can be increased by 15–20 % by using preheated elution buffer (56 °C) or by incubating the bead / elution buffer suspension at 56 °C for 10 min.

5.3 Detailed protocol for KingFisher® Flex

Note: The required method files "NucleoMag® DNA Swab Flex" as well as for other instruments are available through the Technical Support Bioanalysis (support@mn-net.com).

Important: Always prepare deep-well block with samples first and add reagents exactly in the order as given below.

Sample collection

Collect the samples with cotton, Dacron, or C.E.P., or flocked 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.

Note: Swabs may be collected from alternative epithelial surfaces. Other types of small-scale sample matrices containing cellular material (e.g. blood spots, cigarette buds, surface swabs) may be used with this kit.

1 Lyse samples

Calculate the amount of lysis stock required: for each sample, **30 µL Liquid Proteinase K** solution + **270 µL Buffer FLB** are required. Prepare lysis stock solution accordingly and vortex.

Note: Never prepare the lysis stock solution more than 15 min before addition to the samples. Proteinase K tends to self-digestion when incubated in Buffer FLB without substrate.

Place the swab head in an appropriate lysis tube.

Transfer 300 µL of the resulting solution to each lysis tube containing the swab head. Close the individual tubes. **Mix** by vigorous shaking for 10–15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tube.

Note: The buccal swab heads should be submerged into the lysis solution. Therefore, depending on type or size of buccal swab used the FLB buffer volume has to be increased to up to 400 µL. Increasing volume of Proteinase K is not required.

Alternatively, perform lysis with Buffer FLB / Proteinase K in NucleoSpin® Forensic Filters or a NucleoSpin® Trace Filter Plate (see ordering information). These accessories allow convenient separation of lysate from swab material by centrifugation and reduce loss of lysate.

Incubate the tubes containing the samples at **56 °C** for **45 min** or overnight at room temperature. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes are securely closed.

2 Separate and transfer lysate

Separate swab material from lysed sample. Remove buccal swab and squeeze out to obtain **300 µL lysate**.

When using increased volumes (> 270 µL) of Buffer FLB in step 1 of the procedure, transfer the respective amount (< 500 µL) of lysed sample to a KingFisher® Deep-well Block.

Note: When using NucleoSpin® Forensic Filters, spin the filter tube 1 min at > 10,000 x g *to separate lysate and swab. Discard the filtering cartridge including the swab material and proceed with the lysate from the collection tube. When using the NucleoSpin® 96 Trace Filter Plate, centrifuge the NucleoSpin® Trace Filter Plate stacked on a Square-well Block for 5 min at 4,000 x* g *to draw the lysate out of the swab material. The centrifuge must be able to accommodate the NucleoSpin® 96 Trace Filter Plate stacked on a Square-well Block (bucket height: min. 75 mm).*

Transfer 300 µL of cleared lysate to each well of the KingFisher® Deep-well Block.

3 Prepare sample plate

Add **14 µL of NucleoMag® B-Beads** and **400 µL of Binding Buffer MBL2** to each well of the sample plate.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Note: NucleoMag® B-Beads and Buffer MBL2 can be premixed. Per well to be processed, mix 14 µL of NucleoMag® B-Beads with 400 µL Buffer MBL2. Vortex briefly. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are required.

4 Prepare wash and elution plates

Wash plates:

Fill **600 µL Buffer MB3** to each well of an empty KingFisher® Deep-well Block.

Fill **600 µL Buffer MB4** to each well of an empty KingFisher® Deep-well Block.

Fill **700 µL Buffer 80 % ethanol** to each well of an empty KingFisher® Deep-well Block.

Elution plate:

Fill **50–100 µL Buffer MB6** to each well of an empty KingFisher® Elution Plate.

5 Run purification protocol on instrument

Start the isolation of nucleic acids on the KingFisher® Flex instrument.

Start the method file 'NucleoMag® DNA Swab Flex'

Insert plates as indicated on the KingFisher® instrument display.

Method starts with a mixing step (binding step) after setting up the last plate to the instrument.

6 Remove eluted nucleic acids

The instrument stops after the final elution step. Follow the instructions on the instrument's display and unload the plates from the instrument.

Purified DNA can be used in any suitable downstream analysis.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Elution buffer volume insufficient* • Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *Incubation after dispensing beads to lysate* • Mix immediately after dispensing NucleoMag® B-Beads / Buffer MBL2 to the lysate. *Aspiration and loss of beads* • Time for magnetic separation was too short or aspiration speed was too high.
Low purity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely, mix by pipetting up and down repeatedly.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Low purity* • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely, mix by pipetting up and down repeatedly.

Problem	Possible cause and suggestions
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the Square-well Block when transferring the lysate. If the rim of the wells is contaminated, seal the Square-well Block with Self-adhering PE Foil (see ordering information) before starting the shaker.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® DNA Swab	744601.1 744601.4 744601.24	1 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® Forensic Filters	740988.10 740988.50 740988.250	10 50 250
NucleoSpin® Forensic Filters (Bulk)	740988.50B 740988.250B 740988.1000B	50 250 1000
NucleoSpin® Trace Filter Plate	740677	20
NucleoMag® SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Self-adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Cap Strips	740638	30 strips
KingFisher® 96 Accessory Kit A (set consists of Square-well Blocks, Deep-well tip combs, Elution Plates; for 4 x 96 NucleoMag® DNA Swab preps using KingFisher® 96 platform)	744950	1 set
96 Deep-well plates for magnetic rod systems	744955	25
8-well Tip Combs for magnetic rod system	744960	50
8-well Accessory Kit magnetic rod systems	744961	1 set

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

6.3 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.

Last updated: 08/2022, Rev. 04

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-333
support@mn-net.com

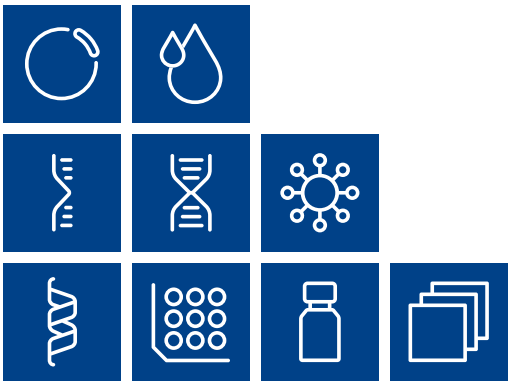
Trademarks:

KingFisher® is a registered trademark of Thermo Fisher Scientific

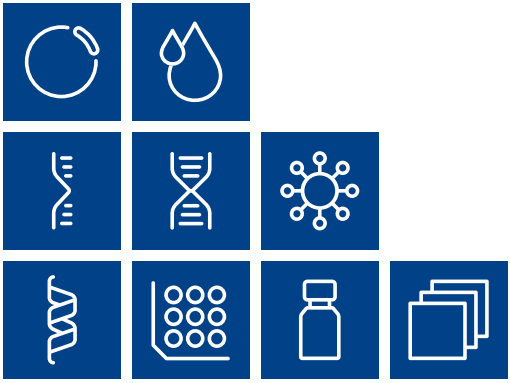
NucleoMag® is a registered trademark of MACHEREY-NAGEL GmbH & Co KG

Te-MagS™ is a trademark of Tecan Group Ltd., Switzerland

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.



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



MACHEREY-NAGEL

www.mn-net.com

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany

DE +49 24 21 969-0 info@mn-net.com
CH +41 62 388 55 00 sales-ch@mn-net.com
FR +33 388 68 22 68 sales-fr@mn-net.com
US +1 888 321 62 24 sales-us@mn-net.com