This product distributed by
Takara Bio USA, Inc.

Ordering information
1.800.662.2566
ordersUS@takarabio.com

Technical support
1.800.662.2566
techUS@takarabio.com

TaKaRa
Clontech TaKaRa cellartis
takarabio.com

Genomic DNA from swabs

User manual

NucleoMag® DNA Swab

April 2019 / Rev. 01

MACHEREY-NAGEL
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
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
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
KingFisher® DUO Accessory Kit (Square-well Blocks, Deep-well tip combs, Plates for 8 x 12 NucleoMag® DNA Swab preps using KingFisher® DUO / DUO Prime platform.	744952	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.

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.

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 %.

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

The following components of the **NucleoMag® DNA Swab** kits contain hazardous contents.

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

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS-Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
MBL2	Ethanol 35–55 % + sodium perchlorate 15–40 % *Ethanol 35–55 % + Natriumperchlorat 15–40 %* CAS 64-17-5, 7601-89-0	 WARNING *ACHTUNG*	226, 302	210, 264W, 301+312, 330
MB3, MB4	Ethanol 20–35 % + sodium perchlorate 15–40 % *Ethanol 35–55 % + Natriumperchlorat 15–40 %* CAS 593-84-0	 WARNING *ACHTUNG*	226, 302	210, 264W, 301+312, 330

Hazard phrases

H226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.

H302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

Precaution phrases

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.

P264W Wash with water thoroughly after handling
Nach Gebrauch mit Wasser gründlich waschen

P301+312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt anrufen.

P330 Rinse mouth.
Mund ausspülen.

For further information please see Material Safety Data Sheets (***www.mn-net.com***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**www.mn-net.com**).*

 The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

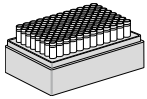
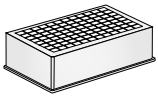
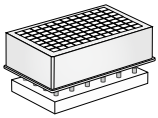
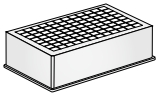
5 Protocols for the isolation of genomic DNA from swabs

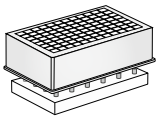
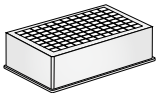
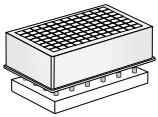
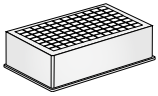
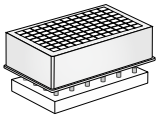
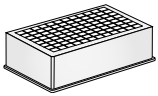
5.1 Protocol at a glance

- 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.

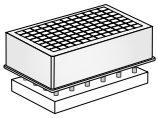
1	**Lyse sample** (e.g., buccal swabs)	**Add 30 µL Liquid Proteinase K and 270 µL Buffer FLB** **Mix** **56 °C, 45 min**	
2	**Separate and transfer lysate**	**Separate swab material to obtain 300 µL lysate. Transfer 300 µL of lysate to a Square-well Block**	
3	**Bind** DNA to NucleoMag® B-Beads	**300 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 at the Technical Support Bioanalysis (tech-bio@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
KingFisher® DUO Accessory Kit (set consists of Square-well Blocks, Deep-well tip combs, Plates for 8 x 12 NucleoMag® DNA Swab preps using KingFisher® DUO / DUO Prime platform.	744952	1 set

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

6.3 Product use restriction / warranty

NucleoMag® DNA Swab kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING,

WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

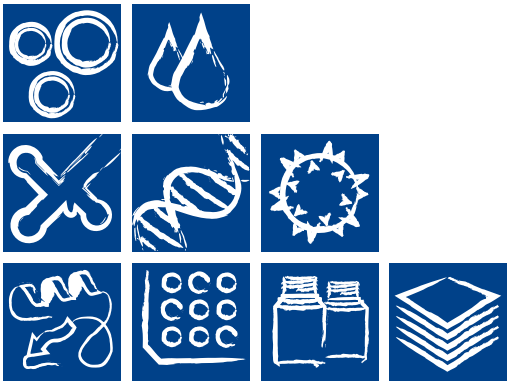
In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07 / 2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
tech-bio@mn-net.com



www.mn-net.com

MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

DE / International:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

A064321/0590.1