

NucleoMag[®] DNA Forensic

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MACHEREY-NAGEL

www.mn-net.com

Bioanalysis

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 0020 · 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

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1 Components

1.1 Kit contents

	NucleoMag [®] DNA Forensic		
REF	1 x 96 preps 744660.1	4 x 96 preps 744660.4	
NucleoMag [®] F-Beads	1.4 mL	2 x 2.6 mL	
Lysis Buffer FOL	50 mL	250 mL	
Binding Buffer FOB	100 mL	300 mL	
Wash Buffer FOW1	80 mL	200 mL	
Wash Buffer FOW2	25 mL	100 mL	
Elution Buffer FOE	13 mL	60 mL	
Liquid Proteinase K	2 x 1250 µL	9 mL	
Reducing Agent TCEP	14 mg	2 x 14 mg	
User manual	1	1	

 $^{^{\}star}$ For preparation of working solutions and storage, see section 3.

1.2 Consumables and equipment to be supplied by user

Product		REF	Pack of
•	Magnetic separator NucleoMag [®] SEP (see section 2.3)	744900	1
•	Separation plate for magnetic beads separation, Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
•	Lysis tubes for incubation of samples and lysis, 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 μ L u-bottom wells)	740486.24	24
•	For use of kit on KingFisher [®] 96 instrument: e.g., 96-well Accessory Kit A for KingFisher [®] (Square- well Blocks, Deep-well tip combs, Plates for 4 x 96 NucleoMag [®] Trace preps using KingFisher [®] 96 platform)	744950	1 set

1.3 About this user manual

It is strongly recommended that first time users of the **NucleoMag® 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 **NucleoMag[®] DNA Forensic** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Lysis is achieved by incubation of samples with Proteinase K at 56 °C (optional: at RT). For the adjustment of binding conditions under which nucleic acids bind to the paramagnetic beads or silica membrane, Buffer FOB and the NucleoMag[®] F-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are 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. The NucleoMag[®] DNA Forensic kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

- NucleoMag[®] DNA Forensic is designed for rapid manual and automated small-scale preparation of highly pure genomic DNA from buccal swabs or other forensic samples, for example, dried blood spots, 'trace and touch'-samples or cigarette filters. The kit is designed for use with NucleoMag[®] SEP magnetic separator plate (see ordering information) or any other magnetic separation system (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified DNA can be used directly as template for PCR, or any kind of enzymatic reaction.
- NucleoMag[®] DNA Forensic 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 the automation platform.
- 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 Forensic can be processed completely at room temperature, however, elution at 56 °C will increase the yield by about 15–20 %.
- NucleoMag[®] F-Beads are highly reactive, superparamagnetic beads. The binding capacity is 0.4 µg of gDNA per 1 µL of NucleoMag[®] F-Bead suspension; 1 µL of suspension contains 140 µg of beads
- The NucleoMag® DNA Forensic kit 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*.

• For research use only

2.3 Magnetic separation systems

For appropriate handling of the **NucleoMag® DNA Forensic** kit, the use of the magnetic separator NucleoMag® SEP 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 are suitable 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:

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

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 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

2.6 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer FOE. Elution can be carried out in a volume of $\ge 50 \ \mu$ L. It is essential to cover the NucleoMag[®] F-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 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 NucleoMag[®] DNA Forensic kit should be stored at room temperature (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 reversible development of salt precipitates

Before starting the NucleoMag[®] 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 H₂O 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.

	NucleoMag [®] DNA Forensic		
REF	1x 96 preps 744660.1	4 x 96 preps 744660.4	
Wash Buffer FOW2 (Concentrate)	25 mL Add 100 mL ethanol	100 mL Add 400 mL ethanol	
Reducing Agent TCEP	1 vial (14 mg) <i>Add 1 mL H₂O</i>	2 vials (14 mg/vial) Add 1 mL H ₂ O to each vial	

4 Safety instructions

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



Caution: Guanidine hydrochloride in buffer FOL, and 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 **NucleoMag® 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 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 14 (detailed protocol).

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	Bind DNA to NucleoMag [®] F-Beads	400 μL lysate	
		12 μ L NucleoMag [®] F-Beads	
		580 μL FOB	
		Mix by shaking for 10 min at RT	
		(Optional: Mix by pipetting up and down)	
		Remove supernatant after 2 min separation	
3	Wash with FOW1	Remove Square-well Block from NucleoMag [®] SEP	
		400 µL FOW1	
		Resuspend: Shake 1 min at RT	
		(Optional: Mix by pipetting up and down)	$ \longleftrightarrow $
		Remove supernatant after 2 min separation	

4	Wash with FOW2	1 st wash Remove Square-well Block from NucleoMag [®] SEP	
		400 µL FOW2	
		Resuspend: Shake 1 min at RT	
		(Optional: Mix by pipetting up and down)	
		Remove supernatant after 2 min separation	
5	Wash with FOW2	2 nd wash Remove Square-well Block on NucleoMag [®] SEP	
		400 µL FOW2	
		Resuspend: Shake 1 min at RT	
		(Optional: Mix by pipetting up and down)	
		Remove supernatant after 2 min separation	
6	Dry the magnetic beads	15 min at RT	
7	Elute DNA	Add 25–100 µL FOE	
		(Optional: Elute at 56 °C)	
		Shake 5 min at RT	
		(Optional: Mix by pipetting up and down)	\leftrightarrow
		Separate 2 min and transfer DNA into elution plate	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag[®] SEP) and suitable plate shakers (see section 2.2). 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.

Before starting the preparation:

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

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 μ L of Liquid Proteinase K, 5 μ L TCEP (14 mg/mL) and 450 μ L Lysis Buffer FOL to a reaction tube containing the sample. Mix well by vigorous shaking or by repeated pipetting up and down for 10–15 s. Spin briefly (15 s; 1,500 x g) to collect any sample at the bottom of the tube.

<u>Note:</u> 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.

Alternatively, perform lysis with Buffer FOL / Proteinase K/TCEP in a NucleoSpin[®] Trace Filter Plate (see ordering information). This plate allows convenient separation of lysate from swab material by centrifugation and reduces loss of lysate.

Incubate at **56** °C for **60 min** with shaking. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes are securely closed. Alternatively, lysis step can be performed in Tube Strips (see ordering information).

Following the lysis incubation, spin down to collect any sample from the lysis

tube lids and transfer each lysate to the wells of a Square-well Block. When using buccal swabs, remove buccal swab and squeeze out to obtain **400 µL lysate**.

When using the NucleoSpin[®] Trace Filter Plate, centrifuge the NucleoSpin[®] Trace Filter Plate stacked onto a 96 well Square-well Block for 5 min at 5,600 x g to draw the lysate out of the swab material.

2 Bind DNA to NucleoMag® F-Beads

To each lysate of 400 μ L from the previous step, add **12** μ L of **NucleoMag® F-Beads** and **580** μ L of **Binding Buffer FOB**. 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.

<u>Note:</u> NucleoMag[®] F-Beads and Buffer FOB can be premixed. Per well to be processed, mix 12 µL of NucleoMag[®] F-Beads with 580 µL Buffer MB2. Vortex briefly. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are required.

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

Separate the magnetic beads against the side of the wells by placing the Squarewell 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.

<u>Note:</u> 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.

3 Wash with FOW1

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

Add 400 μ L Buffer FOW1 to each well and resuspend the beads by shaking until the beads are resuspended completely (1-3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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.

4 Wash with FOW2

1st wash

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

Add $400 \mu L$ Buffer FOW2 to each well and resuspend the beads by shaking until the beads are resuspended completely $(1 - 3 \min)$. Alternatively, resuspend beads completely by repeated pipetting up and down.

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 FOW2

2nd wash

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

Add 400 μ L Buffer FOW2 to each well and resuspend the beads by shaking until the beads are resuspended completely (1-3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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 Dry NucleoMag® F-Beads

Air dry the beads for 10-15 min at room temperature.

7 Elute DNA

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

Add desired volume of **Buffer FOE** (25–100 μ L) to each well of the Square-well Block and resuspend the beads by shaking 5–10 min at room temperature. Alternatively, resuspend beads completely by repeated pipetting up and down 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 either elution plates or tube stripes (see ordering informations)

<u>Note:</u> 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.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions		
	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).		
	Insufficient Elution buffer volume		
	Beads pellet must be covered completely with elution buffer.		
	Insufficient performance of elution buffer during elution step		
Poor DNA yield	 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.		
	Aspiration and loss of beads		
	• Time for magnetic separation was too short or aspiration speed was too high.		
	Insufficient washing procedure		
Low purity/ Low sensitivity	 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 repeated pipetting up and down. 		

Problem	Possible cause and suggestions			
	Carry-over of ethanol from wash buffers			
Suboptimal performance	• Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications.			
of DNA in	Ethanol evaporation from wash buffers			
applications	• Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.			
	Time for magnetic separation too short			
Carry-over of beads	 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.			
	Contamination of the rims			
Cross contamination	• 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 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 pieces 250 pieces 1000 pieces
NucleoSpin [®] Trace Filter Plate	740677	20
NucleoMag [®] SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Square-well Blocks, ethylene oxide treated	740481EO	4
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
96-well Accessory Kit A for KingFisher®	744950	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.

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

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MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany DE +49 24 21 969-0 info@mn-net.com FR +33 388 68 22 68 sales-fr@mn-net.com

CH +41 62 388 55 00 sales-ch@mn-net.com

US +1 888 321 62 24 sales-us@mn-net.com

A056901/xxxx