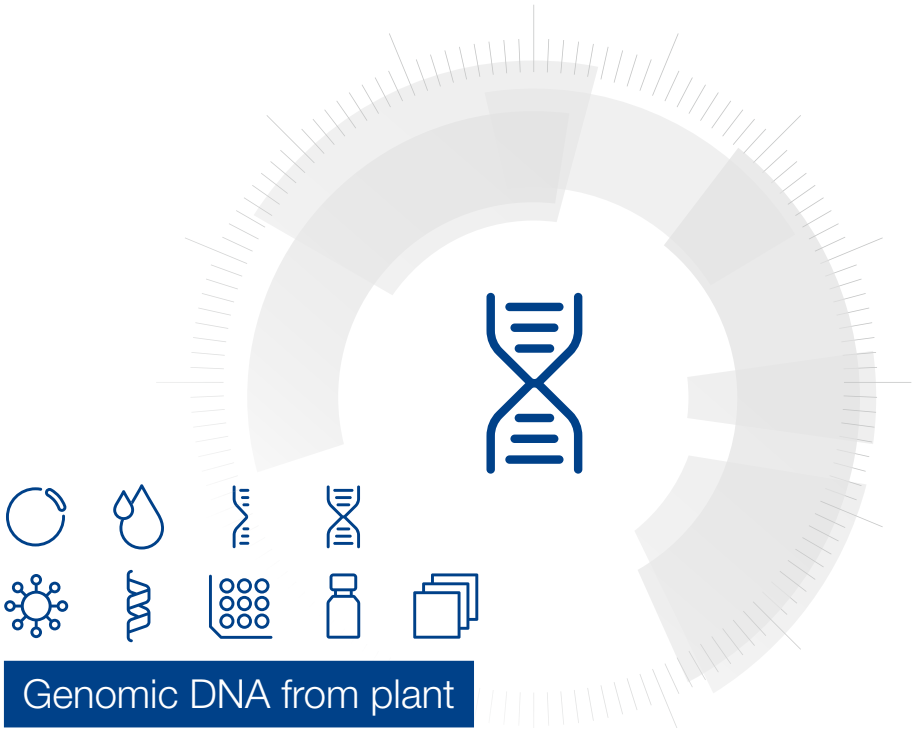
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from plant

■ NucleoMag® 384 Plant

November 2023 / Rev. 02

MACHEREY-NAGEL
www.mn-net.com

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

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1 Components

1.1 Kit contents

	NucleoMag® 384 Plant	
REF	**1 × 384 preps 744402.1**	**4 × 384 preps 744402.4**
NucleoMag® V-Beads	2 × 1.5 mL	5 × 1.5 mL
Lysis Buffer MC1	2 × 60 mL	500 mL
Binding Buffer MC2	50 mL	80 mL
Wash Buffer MC3	75 mL	300 mL
Wash Buffer MC4	75 mL	300 mL
Wash Buffer MC5	60 mL	250 mL
Elution Buffer MC6	30 mL	125 mL
RNase A (lyophilized)*	2 × 30 mg	7 × 30 mg
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* For preparation of working solutions and storage conditions see section 3

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

80 % ethanol

Equipment / Consumables

Product	REF	Pack of
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
Lysis plate for incubation of samples and lysis, e.g., Round well block (1 set consists of 1 Plate (96 × 1.2 mL round wells) each, and 12 Cap Strips)	740475 740475.24	4 sets 24 sets
Bead Tubes G for mechanical sample disruption, e.g., NucleoSpin® Bead Tubes G (50 tubes)	740817.50	50
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
e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells)	740673	20

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoMag® 384 Plant** kit before using this product. 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 online at ***www.mn-net.com***.

2 Product description

2.1 The basic principle

The **NucleoMag® 384 Plant** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Plant tissue is extracted with CTAB-Lysis Buffer MC1. Adjusting the binding conditions of nucleic acid with Binding Buffer MC2 and addition of paramagnetic beads can be carried out simultaneously. After magnetic separation and removal of supernatant, the paramagnetic beads are washed with Wash Buffers MC3, MC4, and 80 % ethanol to remove contaminants and salt. There is no need for a drying step as ethanol from previous wash steps is removed by Wash Buffer MC5. Finally, highly purified DNA is eluted with low-salt Elution Buffer MC6 and can directly be used for downstream applications. The **NucleoMag® 384 Plant** is designed for automated high-throughput processing on standard liquid handling instruments in a 384-well format.

2.2 Kit specifications

NucleoMag® 384 Plant is designed for an automated preparation of DNA from plant samples in a 384-well format. The kit is designed for use with static magnetic separator plates. The purified DNA can be used directly as template for qPCR, next generation sequencing, or any kind of enzymatic reactions.

NucleoMag® 384 Plant allows easy automation on common liquid handling instruments. The actual processing time depends on the configuration of the instrument and the magnetic separation system used.

For research use only

2.3 Magnetic separation systems

For the **NucleoMag® 384 Plant** kit, the use of a static magnetic separator plate in combination with a 96- or 384-multichannel device is recommended. Separation is carried out in a 384-well plate (not provided in the kit). The kit is also suitable for common 384-well plate magnetic separators

Magnetic separator	Separation plate or tube
Magnetic separator VP 771G-4A (V&P Scientific, Inc.) Alpaqua 384 Post Magnet Plate (Alpaqua Engineering, LLC)	384-well plates (deep well), (Greiner BioOne)

Static magnetic pins

Separators with static magnetic pins are 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.

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 105 µL (for checking the binding step settings) or 80 µL (for checking the washing steps settings) 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 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.

* Contact MN Technical Service for optimized program files and support protocols.

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	Number of tips needed
Shaker	++	++	Low
Pipetting	+++	+*	High

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

2.6 Storage and homogenization of samples

We recommend the use of young plant samples and to keep plants for about 12 h in the dark before collecting samples in order to reduce polysaccharide content.

Plant samples can be stored frozen, under ethanol or lyophilized. In many cases lyophilized, dried material can be easier processed and gives higher yield. If using dried samples, reduce the amount of starting material by the factor 5 (e.g., use 10 mg dried plant leaves instead of 50 mg fresh weight).

As plant tissue is very robust, the lysis procedure is most effective with well homogenized, powdered samples. Suitable methods include grinding with pestle and mortar in the presence of liquid nitrogen or using steel beads. We also recommend the use of other commercial homogenizers, bead mills, for example, Crush Express for 96-well homogenization (contact Saaten-Union Resistenzlabor GmbH, D-33818 Leopoldshohe), Tissue Striker (www.KisanBiotech.com) or Geno/Grinder 2000 (www.spexcsp.com or for Germany www.c3-analysentechnik.de).

Methods to homogenize samples

Homogenize samples in NucleoSpin® Bead Tubes G, Rack of Tube Strips or a Round well block with Cap Strips (see ordering Informations 6.2) filled with e.g. steel beads.

Alternatively homogenizing of larger samples can be performed by VA steel beads (diameter: 7 mm): Put 4 – 5 beads and plant material together into a 15 mL plastic tube (Falcon), chill the tube in liquid nitrogen and vortex for about 30 seconds (e.g., with a Multi Pulse Vortexer, contact Schütt Labortechnik GmbH, Postfach 3454, D-37024 Göttingen, Germany). Repeat this chilling and vortexing procedure until the entire plant material is ground to a powder. Chill the tube once more and remove the beads by rolling them out gently or with a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube! This leads to sticking and loss of plant material attached to the beads.

2.7 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer MC6. 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 55 °C.

3 Storage conditions and preparation of working solutions

Attention: *Buffers MC3 and MC4 contain chaotropic salt! Buffer MC2 is highly flammable and irritant. Wear gloves and goggles!*

Storage conditions:

- All components of the **NucleoMag® 384 Plant** kit should be stored at room temperature (18 – 25 °C) and are stable for up to one year.
- All buffers are delivered ready-to-use.

Before starting any **NucleoMag® 384 Plant** protocol, prepare the following:

- **RNase A:** Before first use, add the indicated volume of water to each vial of the lyophilized RNase A. **Store RNase A at 4 °C**.
- **80 % ethanol:** Use molecular biology grade ethanol, dilute with appropriate water to 80 %.

	NucleoMag® 384 Plant	
REF	**1 × 384 preps 744402.1**	**4 × 384 preps 744402.4**
RNase A (lyophilized)	2 × 30 mg Add 2.5 mL water to each vial	7 × 30 mg Add 2.5 mL water to each vial

4 Safety instructions

When working with the **NucleoMag® 384 Plant** 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® 384 Plant** 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 plant samples in a 384-well format

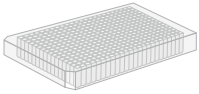
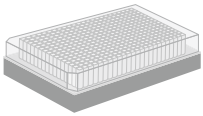
5.1 Protocol at a glance

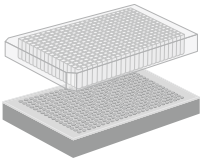
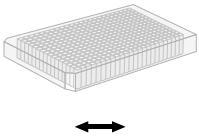
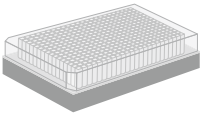
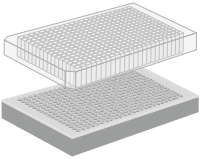
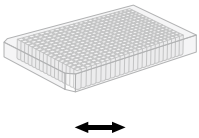
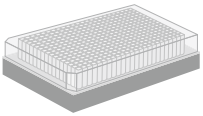
For additional equipment and hardware requirements, refer to section 1.2 and 2.3 respectively.

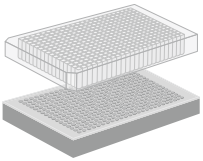
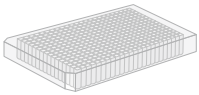
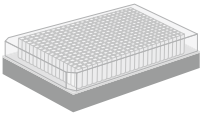
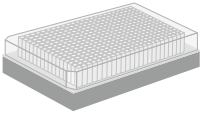
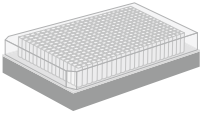
For detailed information on each step, see page 14.

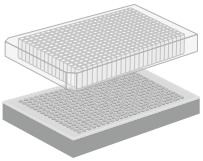
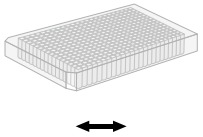
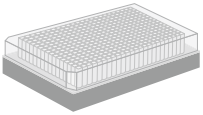
Before starting the preparation:

Check if RNase A was prepared according to section 3.

1	**Homogenize and lyse** plant sample material (~30 mg)	**Add 200 µL MC1 and 10 µL RNase A** **Mix** **56 °C, 60 min**	
2	**Clear lysates** by centrifugation, transfer 50 µL of cleared lysate for further processing	**Centrifuge at 5,600 x *g*, 3 min** **Transfer 50 µL cleared lysate**	
3	**Bind** DNA to NucleoMag® V-Beads	**Add 4 µL NucleoMag® V-Beads and 50 µL MC2** *(Note: Resuspend the NucleoMag® V-Beads before removing them from the storage bottle)*	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 3 min separation	

4	**Wash** with MC3	Remove separation plate from magnetic separator **Add 80 µL MC3**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
5	**Wash** with MC4	Remove separation plate from magnetic separator **Add 80 µL MC4**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	

6	**Wash** with 80 % ethanol	Remove separation plate from magnetic separator **Add 80 µL 80 % ethanol**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
7	**Wash** with MC5	**Leave** Square-well Block on the magnetic separator! **Add 80 µL MC5** **Incubate for 45 – 60 s** ***Note:*** *Do not resuspend the beads in Buffer MC5!*	
		Remove supernatant	

8	**Elute** DNA	Remove separation plate from magnetic separator **Add 60 µL MC6** *(Elution volume possible from 50 – 200 µL; Optional: Elute at 55 °C)*	
		Shake 10 min at RT *(Optional: Mix by pipetting up and down)*	
		Separate 2 min and transfer DNA (supernatant) into elution plate / tubes	

5.2 Detailed protocol

This protocol is designed for magnetic separators with static pins and suitable plate shakers (see section 2.3). It is recommended to use a suitable 384-well separation plate (see section 2.3). Alternatively, isolation of DNA can be performed in other formats (see section 6.2). This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Before starting the preparation:

Check if RNase A was prepared according to section 3.

1 Homogenize and lyse sample material

Homogenize about **30 mg (fresh)** or **< 10 mg (lyophilized) plant tissue**, for example, using mictrotube strips in a mixer mill, and add **200 µL Buffer MC1**. Do not moisten the rim. Close the individual wells with cap strips. Mix by vigorous shaking for 15 – 30 s. Spin briefly for 30 s at 1,500 x *g* to collect any sample from the cap strips. Incubate the closed strips at **56 °C** for **60 min**.

Optional: If samples contain large amounts of RNA, we recommend the addition of 10 µL RNase A solution (stock solution 12 mg/mL) to the MC1 lysis mixture.

2 Clear lysates

Centrifuge the samples for **3 min** at a full speed (**5,600 – 6,000 x *g***). Remove cap strips.

Transfer **50 µL of the cleared lysate** (equilibrated to room temperature) to a suitable separation plate. Do not moisten the rims of the well.

Note: See recommendations for suitable plates or tubes and compatible magnetic separators section 1.2.

3 Bind DNA to NucleoMag® V-Beads

Add **4 µL of NucleoMag® V-Beads** and **50 µL Buffer MC2** to each well of the separation plate. 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.

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

Separate the magnetic beads against the side of the wells by placing the separation plate on a suitable magnetic separator. Wait at least **3 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 is not visible in this step. Remove supernatant from the opposite side of the well.

4 Wash with MC3

Remove the separation plate from the magnetic separator.

Add **80 µL Buffer MC3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the separation plate on the 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 MC4

Remove the separation plate from the magnetic separator.

Add **80 µL Buffer MC4** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the separation plate on the 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 80 % ethanol

Remove the separation plate from the magnetic separator.

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

Separate the magnetic beads by placing the separation plate on the magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting

7 Wash with MC5

Leave the separation plate on the magnetic separator.

Note: Supernatant is colorless, magnetic bead pellet is clearly visible.

Gently add **80 µL Buffer MC5** to each well and incubate for **45 – 60 s** while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in Wash Buffer MC5. This step is to remove traces of ethanol and eliminates a drying step!

8 **Elution**

Remove the separation plate from the magnetic separator.

Add desired volume of **Buffer MC6 (50 – 200 µL)** to each well of the separation plate 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.

Separate the magnetic beads by placing the separation plate on the 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 pre-warmed elution buffer (55 °C) or by incubating the bead / elution buffer suspension at 55 °C for 10 min.

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. *Partial elution in Wash Buffer MC5 already* Keep the beads on the magnet while dispensing Wash Buffer MC5. Do not resuspend beads in this buffer, and do not incubate beads in this buffer for more than 2 min, as this buffer is water-based and might elute the DNA already. *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® V-Beads / Buffer MC2 to the lysate.
Low purity	*Insufficient washing procedure* Use only the appropriate combinations of separator and plate, for example.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol from 80 % ethanol wash solution* Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Low purity* See above

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 processing plate when transferring the plant lysate. If the rim of the wells is contaminated, seal the processing plate with Self-adhering PE Foil (see ordering information) before starting the shaker.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® 384 Plant	744402.1 744402.4	1 × 384 preps 4 × 384 preps
NucleoMag® SEP Just for use with 96-well plates, such as 740481 (Deep-well plate). Not recommended for the use of 384-well blocks.	744900	1
Square-well Blocks (96-well blocks with square wells for use with NucleoMag® SEP 744900)	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
Round well block with Cap Strips (set consists of 1 Plate (96 × 1.2 mL round wells) each, and 12 Cap Strips	740475 740475.24	4 sets 24 sets
NucleoSpin® Bead Tubes G (2 mL tubes with steel beads for sample homogenization)	740817.50	50
Elution Plate U-bottom	740486.24	24
Elution Plate Flat-bottom	740673	20
Cap Strips	740638	30 strips

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.

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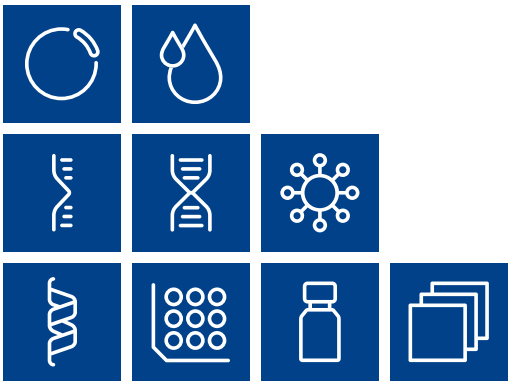
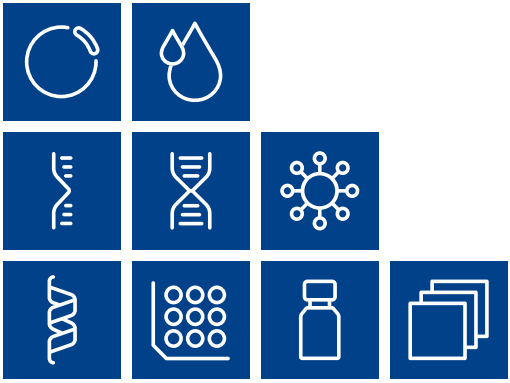
Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-333
support@mn-net.com



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



www.mn-net.com

MACHEREY-NAGEL

Management System
EN ISO 13485:2016
ISO 9001:2015
www.tuv.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
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