

NucleoMag[®] Plant

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Bioanalysis

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1 Components

1.1 Kit contents

		NucleoMag® Plan	t
REF	1 × 96 preps 744400.1	4 × 96 preps 744400.4	24 × 96 preps 744400.24
NucleoMag [®] C-Beads	3 mL	12 mL	75 mL
Lysis Buffer MC1	60 mL	250 mL	3 × 500 mL
Binding Buffer MC2	50 mL	200 mL	3 × 400 mL
Wash Buffer MC3	75 mL	300 mL	2 × 900 mL
Wash Buffer MC4	75 mL	300 mL	2 × 900 mL
Wash Buffer MC5	125 mL	500 mL	2 × 1000 mL
Elution Buffer MC6	30 mL	125 mL	1000 mL
RNase A (lyophilized)*	15 mg	2 × 30 mg	12 × 30 mg
Elution Plate U-bottom (including Self adhering Foil)	1	4	24
User manual	1	1	1

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

Reagents

• 80 % ethanol

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also 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 or updated revisions.

^{*} For preparation of working solutions and storage conditions see 3

Equipment/Consumables

Product	REF	Pack of
Magnetic separation system e.g., NucleoMag [®] SEP (see section 2.3)	744900	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
• 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	740486	24
microtiterplate with 300 μL u-bottom wells) e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 μL flat-bottom wells)	740673.24	20
For use of kit on KingFisher [®] Flex instrument: 96-well Accessory Kit B for KingFisher [®] (Deep-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag [®] Plant preps using KingFisher [®] Flex platform)	744951	1 set

2 Product description

2.1 The basic principle

The **NucleoMag**[®] **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**[®] **Plant** kit can be used either manually or automated on standard liquid handling instruments.

2.2 Kit specifications

NucleoMag[®] Plant is designed for rapid manual and automated small-scale preparation of DNA from up to 20–50 mg wet weight plant sample.

The kit is designed for use with NucleoMag[®] SEP magnetic separator plate (see ordering information, section 6.2) or other magnetic separation systems (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 qPCR, next generation sequencing, or any kind of enzymatic reactions.

NucleoMag[®] Plant 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.

For research use only.

2.3 Magnetic separation systems

For use of **NucleoMag[®] Plant**, 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 (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 (e.g., King Fisher® instruments)

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 crosscontamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

- Load 1000 µL (for checking the settings for the binding step) or 600 µL (for checking the settings for the washing steps) 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.

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

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	Number of tips needed
Magnetic mix	+	++	Low
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, etc.

Methods to homogenize samples

Commercial homogenizers, for example, Crush Express for 96-well homogenization (contact Saaten-Union Resistenzlabor GmbH, D-33818 Leopoldshöhe), Tissue Striker (www.KisanBiotech.com) or Geno/Grinder 2000 (www.spexcsp.com or for Germany www.c3-analysentechnik.de).

Homogenizing samples by VA steel beads (diameter: 3 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, 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 remove them 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. Alternativeley, prefilled and ready to use MN Bead Tubes Type G (5 mm steel beads) allow an easy and convenient sample homogenization procedure.

High throughput homogenization: Add the plant tissue to the individual tubes of the Tube Strips. Add one 3 mm stainless steel bead to each tube and close the individual tubes with Cap Strips. Freeze the sample in liquid nitrogen and insert the Rack of Tube Strips in a suitable homogenization tool (e.g., mixer mill). For disruption, shake the samples for 60–90 s at 30 Hz or until a homogenous plant powder has been formed. If necessary, repeat shaking once. Fresh plant material can also be homogenized with lysis buffer, however, homogenization of fresh plant material with lysis buffer may cause shearing of DNA. For frozen plant material thawing should be avoided during the homogenization. Samples should be frozen in liquid nitrogen before homogenization. Lyophilized or silicagel dried material can be homogenized with or without lysis buffer. Homogenization of lyophilized tissue with lysis buffer may result in higher yield but also may cause shearing of DNA. Alternativeley, prefilled and ready to use MN Bead Plate Type D allows an easy and convenient sample homogenization procedure.

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 \geq 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[®] Plant kit should be stored at 15–25 °C and are stable until: see package label.
- All buffers are delivered ready to use.

Before starting any NucleoMag® 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 [®] Plant		
REF	1 × 96 preps 744400.1	4 × 96 preps 744400.4	24 × 96 preps 744400.24
RNase A (lyophilized)	15 mg Add 1.25 mL water	2 × 30 mg Add 2.5 mL water to each vial	12 × 30 mg Add 2.5 mL water to each vial

4 Safety instructions

When working with the **NucleoMag® 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*).



Caution: Sodium perchlorate in buffer MC3 and MC4 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[®] 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 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

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:

Check if RNase A was prepared according to section 3.

Homogenize and lyse		
plant sample material (20–50 mg)	Homogenize plant sample material (20 – 50 mg)	
	500 µL MC1	
	Mix	
	56 °C, 30 min	
Clear lysates by centrifugation, transfer	5,600 x <i>g</i> , 20 min	Ċ
400 µL of cleared lysate to a Square-well Block for further processing	400 µL cleared lysate	
Bind DNA to NucleoMag®	30 µL NucleoMag [®] C-Beads	
C-Beads	400 µL MC2	
	Mix by shaking	\leftrightarrow
	for 5 min at RT	
	(Optional: Mix by pipetting up and down)	
	Remove supernatant after 2 min separation	
	(20 – 50 mg) Clear lysates by centrifugation, transfer 400 µL of cleared lysate to a Square-well Block for further processing	(20–50 mg) (20–50 mg) (20–50 mg) 500 μL MC1 Mix 56°C, 30 min Clear lysates by centrifugation, transfer 400 μL of cleared lysate to a Square-well Block for further processing Bind DNA to NucleoMag [®] C-Beads 30 μL NucleoMag [®] C-Beads 400 μL MC2 Mix by shaking for 5 min at RT (Optional: Mix by pipetting up and down) Remove supernatant

4	Wash with MC3	Remove Square-well Block from NucleoMag [®] SEP	
		600 µL MC3	
		Resuspend: Shake 5 min at RT (Optional: Mix by pipetting up and down)	\Leftrightarrow
		Remove supernatant after 2 min	
5	Wash with MC4	Remove Square-well Block from NucleoMag [®] SEP	
		600 µL MC4	
		Resuspend: Shake 5 min at RT (Optional: Mix by pipetting up and down)	\Leftrightarrow
		Remove supernatant after 2 min separation	
6	Wash with 80 % ethanol	Remove Square-well Block from NucleoMag [®] SEP	
		600 μL 80 % ethanol	
		Resuspend: Shake 5 min at RT (Optional: Mix by pipetting up and down)	↔
		Remove supernatant	

- 7 Wash with MC5
- Leave Square-well Block on NucleoMag[®]

600 µL MC5



Incubate for 45-60 s

Note: Do not resuspend the beads in Buffer MC5!

Remove supernatan



8 Elute DNA

Remove Square-well Block from NucleoMag[®] SEP

50-200 μL MC6 (Optional: Elute at 55 °C)

Shake 5 min at RT (Optional: Mix by pipetting up and down)

Separate 2 min and transfer DNA into elution plate / tubes



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.

Before starting the preparation:

• Check if RNase A was prepared according to section 3.

1 Homogenize and lyse sample material

Homogenize about 20–50 mg (fresh plant tissue), for example, using mictrotube strips in a mixer mill. Add 500 μ L Buffer MC1 to the homogenized plant tissue (20–50 mg fresh or < 10 mg lyophilized tissue). 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 30 min.

<u>Optional:</u> If samples contain large amounts of RNA, we recommend the addition of 10 μ L RNase A solution (stock solution 12 mg/mL) to the MC1 Lysis mixture.

2 Clear lysates

Centrifuge the samples for **20 min** at a full speed (**5,600 – 6,000 x** *g*). Remove cap strips.

Transfer **400 µL of the cleared lysate** (equilibrated to room temperature) to a Square-well Block. Do not moisten the rims of the well.

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

3 Bind DNA to NucleoMag® C-Beads

Add **30 µL of NucleoMag[®] C-Beads** and **400 µL Buffer MC2** to each well of the Square-well Block. 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[®] C-Beads and Buffer MC2 can be premixed. For 96 samples, mix at least 2880 μ L of NucleoMag[®] C-Beads with 38,4 mL of Buffer MC2, mix by vortexing. Use 430 μ L of the suspension per well. Be sure to resuspend the NucleoMag[®] C-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 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 is not visible in this step. Remove supernatant from the opposite side of the well.

4 Wash with MC3

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

Add **600 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 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 MC4

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

Add **600 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 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 80 % ethanol

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

Add **600 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 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 Wash with MC5 ! Leave the Square-well Block on the NucleoMag[®] SEP magnetic separator.

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

Gently add **600 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.

<u>Note:</u> 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 Square-well Block from the NucleoMag[®] SEP magnetic separator.

Add desired volume of **Buffer MC6 (50-200 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5-10 min** at **56 °C**. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5-10 min** at **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.

<u>Note:</u> 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			
	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. 			
Poor DNA yield	Partial elution in Wash Buffer MC5 already			
POOI DINA Yiela	 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[®] C-Beads / Buffer MC2 to the lysate. 			
	Insufficient washing procedure			
Low purity	 Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag[®] SEP. 			
	Carry-over of ethanol from 80 % ethanol wash solution			
Suboptimal performance of DNA in	 Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. 			
downstream applications	Low purity			
	See above			

Problem	Possible cause and suggestions			
	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. 			
Deads	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 plant 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
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Product	REF	Pack of
NucleoMag [®] Plant	744400.1 744400.4 744400.24	1 × 96 preps 4 × 96 preps 24 × 96 preps
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
MN 96 Bead Plate Type D (Rack of prefilled tube strips (8 × 12) containing 3 mm steel beads; suitable in conjunction with mixer mill)	740853.4 740853.24	4 24
Cap Strips	740638	30 strips
96-well Accessory Kit B for KingFisher [™] (set consists of Square-well Blocks, Deep-well tip combs, Elution Plates; for 4 × 96 NucleoMag [®] Plant preps using KingFisher [™] Flex platform)	744951	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.

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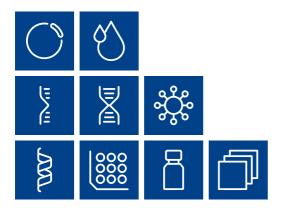
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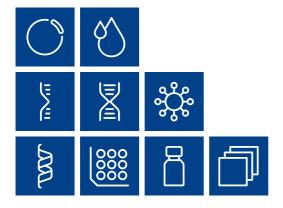
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Plasmid DNA Clean up RNA DNA Viral RNA and DNA Protein High throughput Accessories Auxiliary tools



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