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Genomic DNA from Plant

User manual

NucleoMag 96 Plant

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MACHERY-NAGEL



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1 Kit contents

Cat. No.	NucleoMag 96 Plant		
	1 x 96 preps 744 400.1	4 x 96 preps 744 400.4	24 x 96 preps 744 400.24
NucleoMag C-Beads	3 ml	12 ml	72 ml
Lysis Buffer MC1	60 ml	240 ml	3 x 480 ml
Binding Buffer MC2	2 x 25 ml	8 x 25 ml	3 x 400 ml
Wash Buffer MC3	75 ml	2 x 150 ml	2 x 900 ml
Wash Buffer MC4	75 ml	2 x 150 ml	2 x 900 ml
Wash Buffer MC5	75 ml	2 x 150 ml	2 x 900 ml
Elution Buffer MC6	25 ml	100 ml	600 ml
RNase A	15 mg	2 x 30 mg	12 x 30 mg
Elution plate, U-bottom (including one Self- adhering PE foil)	1	4	24
Protocol	1	1	1

Material to be supplied by user:

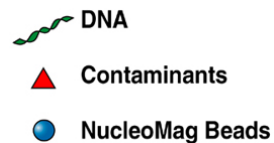
- 80% Ethanol
- Separation plate, e.g. Square-well Block
- Homogenization tubes, e.g. MN Tube Strips and Cap Strips

(see ordering information, section 6.2).

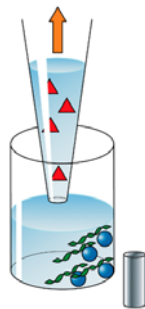
2 Product description

2.1 The basic principle

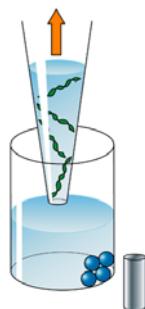
The NucleoMag 96 Plant procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Plant tissue is extracted with CTAB-Lysis Buffer MC1. Adjusting the binding conditions of nucleic acid with Binding Buffer MC2 and addition of paramagnetic beads can be carried out simultaneously. After magnetic separation and removal of supernatant the paramagnetic beads are washed 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 96 Plant kit can be used either manually or automated on standard liquid handling instruments.



Plant tissue is extracted with Lysis Buffer MC1. Binding conditions are adjusted and the NucleoMag Plant Beads are added to the sample.



DNA is bound to the NucleoMag Plant Beads. Beads are held back in the well while contaminants are washed away.



DNA is eluted from the beads and recovered, while beads are held back in the well by the magnet. DNA is ready-to-use in downstream applications.

2.2 Magnetic separation systems

For use of NucleoMag 96 Plant the use of the magnetic separator NucleoMag SEP is recommended. Separation is carried out in an MN Square-well Block (see ordering information). The kit can also be used with other common separators. See suppliers ordering information for suitable separation plates.

Magnetic separator	Separation plate or tube
NucleoMag SEP (Cat. No. 744 900)	Square-well Block (MN, Cat. No. 740 670)
Promega MagnaBot	Square-well Block (MN, Cat. No. 740 670)
Tecan Te-MagS	1.5 ml tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, e.g. NucleoMag SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a plate shaker, e.g. H+P Variomag[®] Teleshake (H+P Labortechnik AG, Bruckmannring 28, D-85764 Oberschleißheim, Germany, www.hp-lab.de), 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, e.g. Te-MagS (for automated use only): 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.

2.3 Adjusting the shaker settings

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

- Apply 600 µl dyed water (select desired elution buffer volume) to the wells of the separation plate. Position the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check 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 and elution step.

2.4 Handling of beads

Distribution of beads

A homogenous 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 vortex 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 on each system. It is recommended to use 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 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

* 8-channel pipetting device

2.5 Elution procedures

Purified genomic DNA can be eluted directly with the supplied Elution Buffer MC6. Elution can be carried out in a volume of $\geq 50 \mu\text{l}$. It is essential to cover the NucleoMag Plant Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (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 high 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.

2.6 Storage and homogenization of samples

We recommend to use young plant samples and if possible 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: 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.

3 Storage conditions and preparation of working solutions

Attention:

Buffers MC3 and MC4 contain chaotropic salt! Wear gloves and goggles!

Buffer MC2 is highly flammable and irritant.

All components of the **NucleoMag 96 Plant** kit should be stored at room temperature (20-25°C) and are stable for up to one year.

All buffers are delivered ready-to-use.

Before starting any **NucleoMag 96 Plant** protocol prepare the following:






- **RNase A:** Add the indicated volume of water to RNase A. Store at 4°C.
- **80% Ethanol:** use molecular biology grade ethanol, dilute with appropriate water to 80%.

	NucleoMag 96 Plant		
Cat. No.	1 x 96 preps 744400.1	4 x 96 preps 744400.4	24 x 96 preps 744400.24
RNase A (lyophilized)	1 x 15 mg add 1.25 ml water	2 x 30 mg add 2.5 ml water to each vial	12 x 30 mg add 2.5 ml water to each vial

4 Safety instructions – risk and safety phrases

The following components of the NucleoMag 96 Plant kits contain hazardous contents.

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

Component	Hazard Contents	Hazard Symbol	Risk Phrases	Safety Phrases	
MC2	isopropanol	 F*	Highly flammable. Irritating to eyes. Vapours may cause drowsiness and dizziness.	R 11-36-67	S 7-16-24/25-26
		 Xi*			
MC3	sodium perchlorate in ethanol	 Xn**	Flammable. Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16
MC4	sodium perchlorate in ethanol	 Xn**	Harmful if swallowed. Flammable	R 22-10	S 13-22-27
RNase A	RNase A, lyophilized	 Xi*	May cause sensitization by inhalation and skin contact	R 42/43	S 7-16-22

Risk Phrases

R 10	Flammable
R 11	Highly flammable
R 22	Harmful if swallowed
R 36	Irritating to eyes
R 36/38	Irritating to eyes and skin
R 42/43	May cause sensitization by inhalation and skin contact
R 67	Vapours may cause drowsiness and dizziness

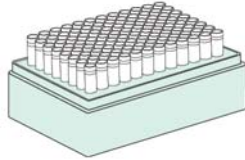
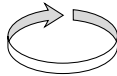
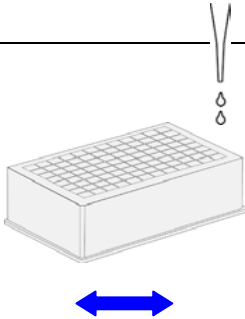
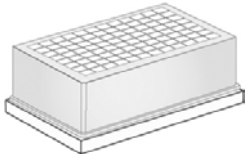
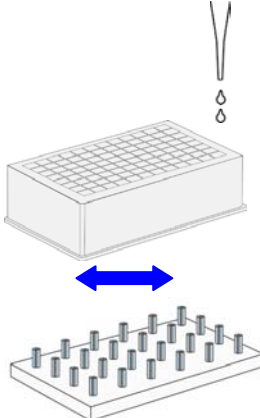
Safety Phrases

S 7	Keep container tightly closed
S 13	Keep away from food, drink and animal feedingstuffs
S 16	Keep away from sources of ignition - No smoking!
S 22	Do not breathe dust
S 24	Avoid contact with skin
S 24/25	Avoid contact with skin and eyes
S 27	Take off immediately all contaminated clothing

* Label not necessary, if quantity below 25 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

** Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 General procedure

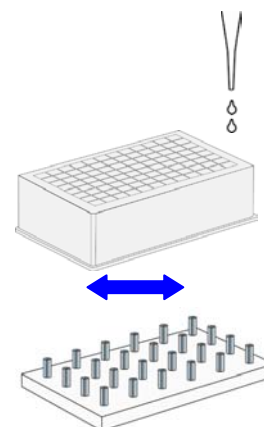
<p>1 Homogenize and lyse plant samples (20-50 mg).</p>	<p>Homogenization, add 500 µl MC1 buffer, mix and incubate at 56°C, 30 min</p>	
<p>2 Clear lysate by centrifugation, transfer 400 µl of cleared lysate to a Square-well Block for further processing.</p>	<p>5,600 x g</p>	
<p>3 Bind DNA to NucleoMag Plant Beads.</p>	<p>400 µl cleared lysate 30 µl C-beads 400 µl MC2 shake 5 min at RT</p>	
<p>4 Remove supernatant.</p>	<p>2 min separation</p>	
<p>5 MC3 wash step.</p>	<p>600 µl MC3 shake 5 min, RT</p> <p>optional: mix by pipetting up and down</p> <p>2 min separation</p>	

6 MC4 wash step.

600 μ l MC4
shake 5 min, RT

optional: mix by
pipetting up and
down

2 min separation

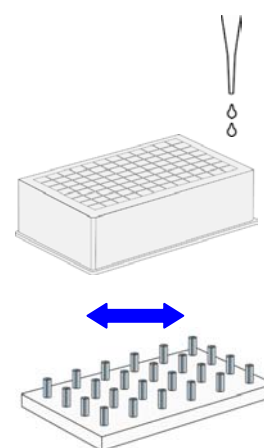


7 80% Ethanol wash step.

600 μ l 80% Ethanol,
shake 5 min, RT

optional: mix by
pipetting up and
down

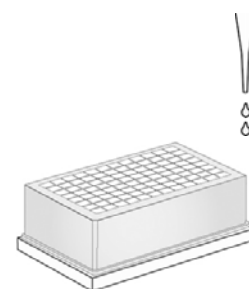
2 min separation



8 MC5 wash step.

600 μ l MC5
60 sec incubation
aspirate and discard
supernatant

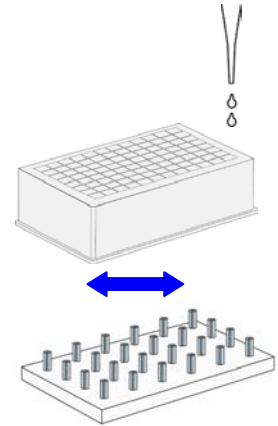
*Note: Do not
resuspend beads in
MC5 buffer*



9 Elute genomic DNA and transfer to Elution Plate.

50 – 200 μ l MC6
shake 5 min, RT
2 min separation
transfer

Optional:
Elution at 55°C



5.1 Standard protocol for the purification of genomic DNA

This protocol is designed for magnetic separators with static pins (e.g. NucleoMag SEP) and suitable plate shakers (e.g. H+P Variomag[®] Teleshake). This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments. For the availability of ready-to-run scripts please contact your local distributor or MN directly.

1 Homogenize and lyse sample material

Homogenize about 20-50 mg (lyophilized) plant tissue, e.g. using microtube strips in a mixer mill, and add 500 µl MC1 lysis buffer. Do not moisten the rim. Close the individual wells with cap strips. Mix by vigorous shaking for 15-30 sec. Spin briefly for 30 sec at 1,500 x g to collect any sample from the cap strips. Incubate the closed strips at 56°C for 30 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 lysate

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

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

Note: See recommendations for suitable plates or tubes and compatible magnetic separators section 2.3.

4 Add 400 µl Binding Buffer MC2 and 30 µl of NucleoMag Plant Beads to each well of the Square-well Block. Mix immediately by shaking (5 min) at room temperature.

Alternatively, pipette up and down 10 times and incubate 5 min at room temperature.

Note: NucleoMag Plant Beads and Binding Buffer MC2 can be premixed. For 96 samples mix at least 2880 µl of NucleoMag Plant Beads with 38,4 ml of buffer MC2, mix by vortexing. Use 430 µl of the suspension per well.

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

- 5** Separate the magnetic beads against the side of the wells by placing the Square-well Block 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.

Remove the Square-well Block from the magnetic separator.

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.

- 6** Add 600 µl Wash Buffer MC3 to each well and wash the bead/DNA complex by shaking (5 min) at room temperature.

Alternatively, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the Square-well Block on the magnetic separator as described above. Aspirate and discard the supernatant.

Remove the Square-well Block from the magnetic separator.

- 7** Add 600 µl Wash Buffer MC4 to each well and wash the bead/DNA complex by shaking (5 min) at room temperature.

Alternatively, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the Square-well Block on the magnetic separator as described above. Aspirate and discard the supernatant.

- 8** Add 600 µl 80% ethanol to each well and wash the bead/DNA complex by shaking (5 min) at room temperature.

Alternatively, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the Square-well Block on the magnetic separator as described above. Aspirate and discard the supernatant.

Do not remove the Square-well Block from the magnetic separator.

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

- 9** Add 600 µl Wash Buffer MC5 to each well and incubate for 60 s while the beads are still separated on the magnet. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in Wash Buffer MC 5. This step is to remove traces of ethanol and eliminates a drying step.

- 10** Add desired volume of Elution Buffer MC6 (50 – 200 µl) to each well and resuspend the bead/DNA complex by shaking (5 – 10 min).

Alternatively, pipette up and down 10 times and incubate 5 – 10 min.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the magnetic separator. Wait 2 min until all the beads have been attracted to the magnet. Transfer the supernatant containing the purified genomic DNA to the Elution Plate.

Note: The yield can be increased by 15 – 20% by using prewarmed 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	<i>Elution buffer volume insufficient</i> <ul style="list-style-type: none"> • Beads pellet must be covered completely with elution buffer
	<i>Insufficient performance of elution buffer during elution step</i> <ul style="list-style-type: none"> • Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step.
	<i>Beads dried out</i> <ul style="list-style-type: none"> • Do not let the beads dry as this might result in lower elution efficiencies.
	<i>Partial elution in Wash Buffer MC5 already</i> <ul style="list-style-type: none"> • 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.
	<i>Aspiration of attracted bead pellet</i> <ul style="list-style-type: none"> • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate.
	<i>Incubation after dispensing beads to lysate</i> <ul style="list-style-type: none"> • Mix immediately after dispensing NucleoMag Plant Beads/Binding Buffer MC2 to the lysate.
	Low purity

Problem	Possible cause and suggestions
Suboptimal performance of DNA in downstream applications	<i>Carry-over of ethanol from 80% ethanol wash solution</i> <ul style="list-style-type: none">• Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications.
	<i>Low purity</i> <ul style="list-style-type: none">• see above
Carry-over of beads	<i>Time for magnetic separation too short</i> <ul style="list-style-type: none">• Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.
	<i>Aspiration speed too high (elution step)</i> <ul style="list-style-type: none">• High aspiration speed during the elution step may cause bead carry over. Reduce aspiration speed for elution step.
Cross contamination	<i>Contamination of the rims</i> <ul style="list-style-type: none">• 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

Product	Cat. No.	Pack of
NucleoMag 96 Plant	744 400.1	1 x 96
NucleoMag 96 Plant	744 400.4	4 x 96
NucleoMag 96 Plant	744 400.24	24 x 96
NucleoMag SEP	744 900	1
Square-well Blocks	740 670	20
Self-adhering PE foil	740 676	50 sheets
MN Tube Strips	740 637	5 sets
Cap Strips	740 638	30 strips

6.3 Product use restriction / warranty

NucleoMag 96 Plant kit components were developed, designed and sold **for research purposes only**. They are suitable **for *in vitro* uses only**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoMag 96 Plant** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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