



Genomic DNA from plant

User manual

NucleoMag® 96 Plant

February 2012/Rev.02

Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	4
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Magnetic separation systems	6
2.4	Adjusting the shaker settings	7
2.5	Handling of beads	8
2.6	Storage and homogenization of samples	9
2.7	Elution procedures	9
3	Storage conditions and preparation of working solutions	10
4	Safety instructions	11
4.1	Risk and safety phrases	11
4.2	GHS classification	12
5	Protocol for the isolation of genomic DNA from plant samples	14
6	Appendix	19
6.1	Troubleshooting	19
6.2	Ordering information	21
6.3	Product use restriction/warranty	22

1 Components

1.1 Kit contents

REF	NucleoMag® 96 Plant		
	1x 96 preps 744400.1	4 x 96 preps 744400.4	24 x 96 preps 744400.24
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 (lyophilized)*	15 mg	2 x 30 mg	12 x 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

* For preparation of working solutions and storage conditions see section 3.

Equipment/Consumables

Product	REF	Pack of
<ul style="list-style-type: none"> • Magnetic separation system e.g., NucleoMag[®] SEP (see section 2.3) 	744900	1
<ul style="list-style-type: none"> • 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
<ul style="list-style-type: none"> • 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
<ul style="list-style-type: none"> • 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) e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells) 	740486.24 740673	24 20
For use of kit on KingFisher[®] 96 instrument:		
<ul style="list-style-type: none"> e.g., KingFisher[®] 96 Accessory Kit B (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag[®] 96 Plant preps using KingFisher[®] 96 platform) 	744951	1 set

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 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® 96 Plant** kit can be used either manually or automated on standard liquid handling instruments.

2.2 Kit specifications

NucleoMag® 96 Plant is designed for rapid manual and automated small-scale preparation of DNA from plant samples.

The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). Manual 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® 96 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.

2.3 Magnetic separation systems

For use of **NucleoMag® 96 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

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

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

Adjusting shaker speed for binding and wash steps:

- Load 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.

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: 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 $\geq 50 \mu\text{L}$. 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® 96 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® 96 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® 96 Plant			
REF	1 x 96 preps 744400.1	4 x 96 preps 744400.4	24 x 96 preps 744400.24
RNase A (lyophilized)	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

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.

4.1 Risk and safety phrases

Component	Hazard contents	Hazard symbol	Risk phrases	Safety phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>Gefahrstoff-symbol</i>	<i>R-Sätze</i>	<i>S-Sätze</i>
MC2	Isopropanol 50–100 % <i>Isopropanol 50–100 %</i>	 F**  Xi**	R 11-36-67	S 7-16-24-26-39
MC3, MC4	Sodium perchlorate 5–20 % + ethanol 20–35 % <i>Natriumperchlorat 5–20 % + Ethanol 20–35 %</i>	*	R 10	S 16
RNase A	RNase A, lyophilized <i>RNase A, lyophilisiert</i>	 Xn	R 42/43	S 22-24

Risk phrases

R 10	Flammable. <i>Entzündlich</i>
R 11	Highly Flammable. <i>Leichtentzündlich</i>
R 36	Irritating to eyes. <i>Reizt die Augen.</i>
R 42/43	May cause sensitization by inhalation and contact with skin. <i>Sensibilisierung durch Einatmen und Hautkontaktmöglich.</i>
R 67	Vapours may cause drowsiness and dizziness. <i>Dämpfe können Schläfrigkeit und Benommenheit verursachen.</i>

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.




Safety phrases

- S 7 Keep container tightly closed.
Behälter dicht geschlossen halten.
- S 16 Keep away from sources of ignition – No smoking!
Von Zündquellen fernhalten – Nicht rauchen!
- S 22 Do not breathe dust.
Staub nicht einatmen.
- S 24 Avoid contact with the skin.
Berührung mit der Haut vermeiden.
- S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Bei Berührung mit den Augen sofort gründlich mit Wasser abspülen und Arzt konsultieren.
- S 39 Wear eye / face protection.
Schutzbrille / Gesichtsschutz tragen.

4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
MC2	Isopropanol 50–100 % <i>Isopropanol 50–100 %</i>	 Danger <i>Gefahr</i>	225, 319, 336	210, 233, 280, 305+351+338, 337+313, 403+235
MC3, MC4	Sodium perchlorate 5–20 % + ethanol 20–35 % <i>Natriumperchlorat 5–20 % + Ethanol 20–35 %</i>	 Warning <i>Achtung</i>	226	210, 233, 403+235
RNase A	RNase A, lyophilized <i>RNase A, lyophilisiert</i>	 Danger <i>Gefahr</i>	317, 334	261, 280, 302+352, 304+341, 333+313, 342+311, 363

Hazard phrases

- H 225 Highly flammable liquid and vapour.
Flüssigkeit und Dampf leicht entzündbar.
- H 226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.
- H 317 May cause an allergic skin reaction.
Kann allergische Hautreaktionen verursachen.
- H 319 Causes serious eye irritation.
Verursacht schwere Augenreizung.
- H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.
- H 336 May cause drowsiness or dizziness.
Kann Schläfrigkeit und Benommenheit verursachen.

Precaution phrases

- P 210 Keep away from heat/sparks/open flames/hot surfaces – No smoking.
Von Hitze/Funken/offener Flamme/heißen Oberflächen fernhalten. Nicht rauchen.
- P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.
- P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.
- P 280 Wear protective gloves / eye protection.
Schutzhandschuhe/Augenschutz tragen.
- P 302+352 IF ON SKIN: Wash with plenty of soap and water.
Bei Kontakt mit der Haut: Mit viel Wasser und Seife waschen.
- P 304+341 IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing.
Bei Einatmen: Bei Atembeschwerden an die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.
- P 305+351+313 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing
BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.
- P 333+313 IF skin irritation or a rash occurs: Get medical advice / attention.
Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.
Bei Symptomen der Atemwege: Giftinformationszentrum oder Arzt anrufen.
- P 337+313 Get medical advice / attention.
Bei anhaltender Augenreizung: Ärztliche Rat einholen / ärztliche Hilfe hinzuziehen.
- P 403+235 Store in a well ventilated place. Keep cool.
Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.
- P 363 Wash contaminated clothing before reuse.
Kontaminierte Kleidung vor erneutem Tragen waschen.

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

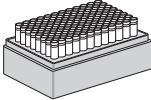
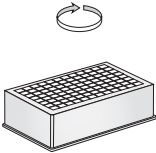


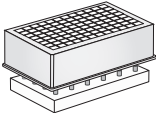
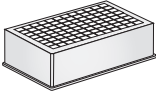
5 Protocol for the isolation of genomic DNA from plant samples

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 17.

Before starting the preparation:

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

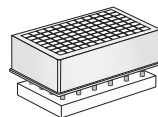
1 Homogenize and lyse plant sample material (20–50 mg)	500 μ L MC1 Mix 56 °C, 30 min	
2 Clear lysates by centrifugation, transfer 400 μ L of cleared lysate to a Square-well Block for further processing	5,600 x g, 20 min 400 μ L cleared lysate	
3 Bind DNA to NucleoMag® C-Beads	30 μ L NucleoMag® C-Beads 400 μ L MC2	
	Mix by shaking for 5 min at RT <i>(Optional: Mix by pipetting up and down)</i>	
	Remove supernatant after 2 min separation	
4 Wash with MC3	Remove Square-well Block from NucleoMag® SEP 600 μ L MC3	

Resuspend: Shake 5 min at RT

*(Optional: Mix by pipetting
up and down)*



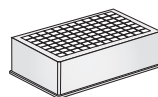
**Remove supernatant
after 2 min separation**



5 Wash with MC4

Remove Square-well Block
from NucleoMag® SEP

600 µL MC4

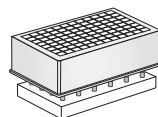


Resuspend: Shake 5 min at RT

*(Optional: Mix by pipetting
up and down)*



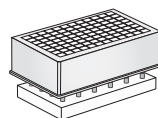
**Remove supernatant
after 2 min separation**



**6 Wash with
80 % ethanol**

Remove Square-well Block
from NucleoMag® SEP

600 µL 80 % ethanol

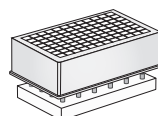


Resuspend: Shake 5 min at RT

*(Optional: Mix by pipetting
up and down)*



Remove supernatant



7 Wash with MC5

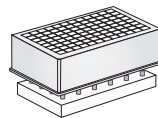
Leave Square-well Block
on NucleoMag® SEP



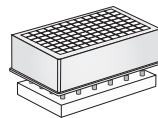
600 µL MC5

Incubate for 45–60 s

*Note: Do not resuspend
the beads in Buffer MC5!*



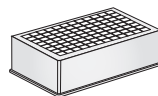
Remove supernatant



8 Elute DNA

Remove Square-well Block
from NucleoMag® SEP

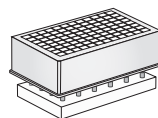
50–200 µL MC6
(Optional: Elute at 55 °C)



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)** or **< 10 mg (lyophilized) plant tissue**, for example, using microtube strips in a mixer mill, and add **500 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 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 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.

Note: 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.

Note: NucleoMag® C-Beads and Buffer MC2 can be premixed. For 96 samples, mix at least 2880 µL of NucleoMag® C-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® 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 Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet 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.

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 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.

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	<p><i>Elution buffer volume insufficient</i></p> <ul style="list-style-type: none"> • Beads pellet must be covered completely with elution buffer
	<p><i>Insufficient performance of elution buffer during elution step</i></p> <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.
	<p><i>Beads dried out</i></p> <ul style="list-style-type: none"> • Do not let the beads dry as this might result in lower elution efficiencies.
	<p><i>Partial elution in Wash Buffer MC5 already</i></p> <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.
Low purity	<p><i>Aspiration of attracted bead pellet</i></p> <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.
	<p><i>Incubation after dispensing beads to lysate</i></p> <ul style="list-style-type: none"> • Mix immediately after dispensing NucleoMag[®] C-Beads / Buffer MC2 to the lysate.
	<p><i>Insufficient washing procedure</i></p> <ul style="list-style-type: none"> • Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag[®] SEP.

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
<i>Carry-over of beads</i>	<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	REF	Pack of
NucleoMag [®] 96 Plant	744400.1	1 x 96 preps
	744400.4	4 x 96 preps
	744400.24	24 x 96 preps
NucleoMag [®] SEP	744900	1
Square-well Blocks	740481	4
	740481.24	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	4 sets
	740477.24	24 sets
Cap Strips	740638	30 strips
KingFisher [®] 96 Accessory Kit B (set consists of Square-well Blocks, Deep-well tip combs, Elution Plates; for 4 x 96 NucleoMag [®] 96 Plant preps using KingFisher [®] 96 platform)	744951	1 set

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

6.3 Product use restriction/warranty

NucleoMag® 96 Plant kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

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

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

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

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

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

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

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

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

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

components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

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

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

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

Last updated: 07/2010, Rev. 03

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

Trademarks:

KingFisher is a registered trademark of Thermo Fisher Scientific
NucleoMag is a registered trademark of MACHEREY-NAGEL GmbH & Co KG
Te-MagS is a trademark of Tecan Group Ltd., Switzerland

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.