MACHEREY-NAGEL

NucleoSpin® 8 / 96 Plant II

Automated DNA purification in 96-well plate and 8-well strip format from plant samples using the NucleoSpin® 8 / 96 Plant II kits on the ep*Motion*® 5075 VAC plus TMX from Eppendorf®

Abstract

In this application note we describe the integration of the MACHEREY-NAGEL NucleoSpin® 8 / 96 Plant II kits into the ep*Motion*® 5075 VAC plus TMX automated pipetting system. The NucleoSpin® 8 / 96 Plant II kits are based on a well proven vacuum filtration based bind-wash-elute procedure. Protocols for the ep*Motion*® 5075 VAC plus TMX and ep*Motion*® 5075 VAC are available for medium throughput using the flexible 8-well strips or for high throughput the 96-well plates. With the NucleoSpin® 8 / 96 Plant II kit excellent DNA yields and outstanding quality of DNA from plant material is achieved. The extracted DNA is suitable for common downstream applications like restriction analysis, Southern Blotting, or PCR.

Introduction

The automated extraction of high molecular weight genomic DNA with reproducibility in yield and quality is the first and often limiting step in the processing of a broad range of different plant samples in the field of crop design, genotyping, and PCR based applications like SNP analysis. The automated pipetting system described here meets the requirements for a robust and user friendly procedure. Manual interactions are minimized due to automated liquid handling, robotic plate handling, integrated shaking, heating, and vacuum filtration.

With the NucleoSpin® 8 / 96 Plant II kit excellent DNA yields and outstanding quality of DNA from plant material is achieved. The extracted DNA is suitable for common downstream applications like restriction analysis, Southern Blotting, or PCR.

Product at a glance

NucleoSpin® 8 / 96 Plant II	
Technology	Silica membrane technology
Sample material	< 100 mg plant material can be processed
Preparation time	100 minutes for 96 samples (excluding lysis)
Typical yield	up to 30 µg DNA from plant tissue
Consistency	typical CVs for DNA yield are <15 %
DNA quality	fragment size up to 30–50 kbp, purity A_{260}/A_{280} 1.8–2.0
Elution volume	100–200 µL

Instrumentation

The instrument consists of an ep*Motion*® 5075 VAC plus TMX automated pipetting system equipped with a gripper tool, a thermo shaker and vacuum filtration manifold (Figure 1). The MACHEREY-NAGEL NucleoSpin® 8 / 96 Plant II kits were used throughout the study. Sample homogenization was performed using a bead based system (Retsch® Mixer Mill) with stainless steal beads. Plant leaves were frozen in liquid nitrogen and homogenized for 2 x 3 min. Following homogenization lysis buffer was added and samples were incubated at 65 °C for 30 min. Following a centrifugation step the cleared plant lysate was placed on the ep*Motion*® instrument and all further steps were performed by the instrument. Ready to run optimized method files are available for both the 8-well strip or 96-well plate kits for use with ep*Motion*® 5075 VAC plus TMX or ep*Motion*® 5075 VAC without orbital shaker / heater.

Material and methods

- Eppendorf ep*Motion*® 5075 VAC plus TMX
- Eppendorf ep*Motion*® 5075 VAC
- Vac Frame 2
- Vac Holder
- Reservoir 400 mL
- Collection Plate Adapter for MN Tube Strips
- Channeling Plate
- Reservoir Rack with Reagent Reservoirs
- NucleoSpin® 8 Plant II kit
- NucleoSpin® 96 Plant II kit
- Plant sample material, for example leaves, crushed seeds, flour

Product use limitations and safety information

Please read the MACHEREY-NAGEL NucleoSpin® 8 Plant II or NucleoSpin® 96 Plant II manual before performing the method for the first time.

Determination of yield and purity

Yield and purity of DNA were determined using a microplate reader (Biotek, Powerwave 200). DNA yield was calculated from A_{260} values. Purity was determined by calculating the A_{260}/A_{280} ratio.

Real-time PCR

Real-time PCR was performed with a NADH-dehydrogenase primer set using the Roche LightCycler® instrument with the Roche LightCycler® FastStart DNA Master SYBR Green I kit according to manufacturer's instructions.

Table 1: ep*Motion*® 5075 VAC plus TMX worktable details for NucleoSpin® 96 Plant II protocol.

Position	Labware	Comment
A2	MN Tube Strips	elution tubes*
A3	epT.I.P.S Motion 1000 µL	filtertips
B1	epT.I.P.S Motion 1000 µL	filtertips
B2	epT.I.P.S Motion 1000 µL	filtertips
B3	Reagent Reservoirs Position 1: – Position 2: Buffer PC Position 3: Buffer PW1 Position 4: Buffer PW2 Position 5: Buffer PW2 Position 6: Buffer PE Position 7: –	size 100 mL 100 mL 100 mL 100 mL 30 mL
Vacuum	NucleoSpin® Plant Binding Plate Vacuum Frame 2 Reservoir 400 mL with channelling plate	DNA binding plate (top) collar for vacuum manifold collects waste
C2	Sample Plate	samples
C3	MN Square-well Block	Mixing Plate
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting too

Table 2: ep*Motion*® 5075 VAC plus TMX worktable details for NucleoSpin® 8 Plant II protocol.

Position	Labware	Comment
A2	MN Tube Strips	elution tubes*
A3	epT.I.P.S Motion 1000 µLfiltertips	
B1		
B2	epT.I.P.S Motion 1000 µL	filtertips
B3	Reagent Reservoirs Position 1: – Position 2: Buffer PC Position 3: Buffer PW1 Position 4: Buffer PW2 Position 5: – Position 6: Buffer PE Position 7: –	size 100 mL 100 mL 100 mL 100 mL 30 mL
Vacuum	NucleoSpin® Plant Binding Plate Vacuum Frame 2 Reservoir 400 mL with channelling plate	DNA binding strips inserted into Column Holder A (top) collar for vacuum manifold collects waste
C2	Sample Plate	samples
C3	MN Square-well Block	Mixing Plate
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool

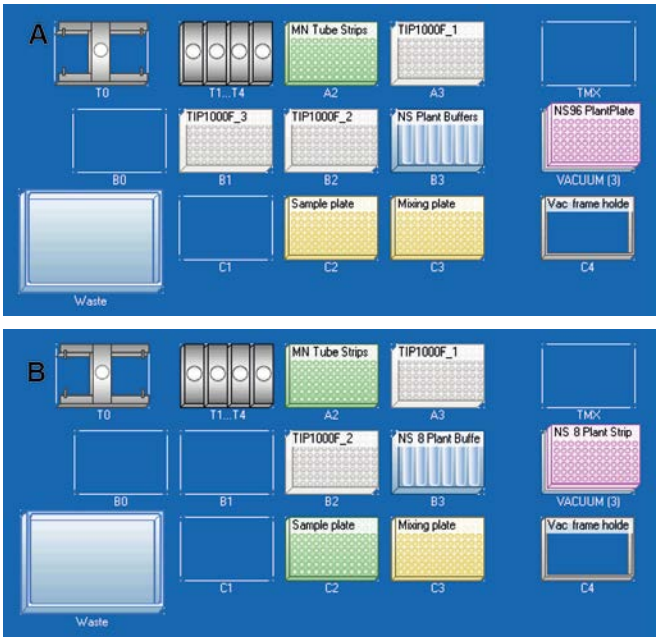

Figure 1: ep*Motion*® Editor

Screenshots from the ep*Motion*® Editor showing the setup of the ep*Motion*® 5075 VAC plus TMX worktable for use with the NucleoSpin® 96 Plant II kit (A) and NucleoSpin® 8 Plant II kit (B)

Results

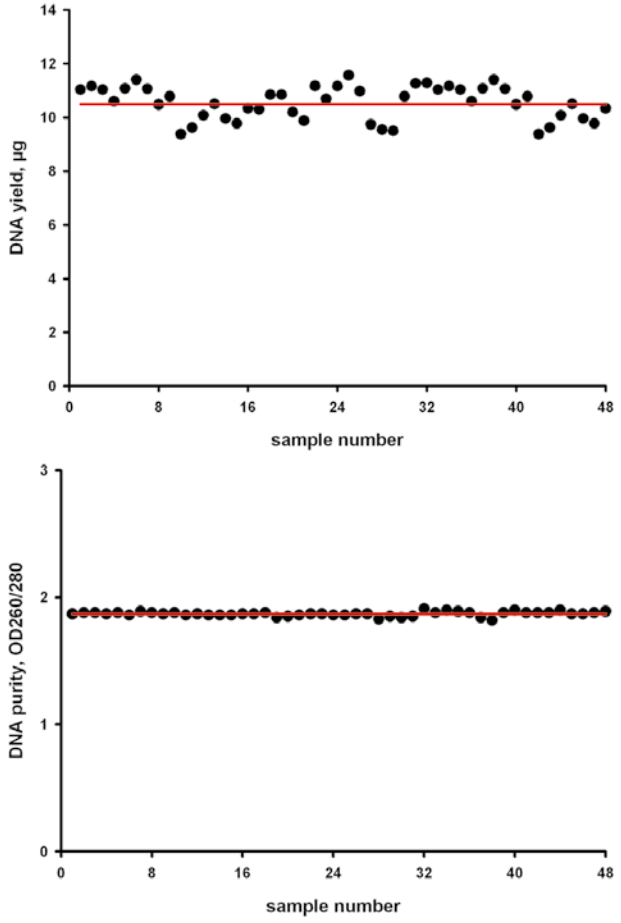

Figure 2: DNA isolation from wheat leaves using the NucleoSpin® 96 Plant II kit.

DNA yield (top) and purity (bottom). To investigate reproducibility of the DNA extraction method genomic DNA was isolated from a master lysate representing 50 mg of frozen wheat leaf tissue per extraction (n=48, identical sample lysate for all extractions) according to the standard procedure of NucleoSpin® 96 Plant II.

With the NucleoSpin® 96 Plant II kit yields of 10–12 µg DNA from 50 mg wheat leaf tissue with a purity (A_{260}/A_{280} ratio) of 1.87 (Figure 2) were obtained. Mean values (red bold plots in Figure 2) and standard deviations (red dotted plots in Figure 2) are summarized in Table 3, further supporting the excellent reproducibility of the method. The purified gDNA is of high molecular weight as shown by agarose gel analysis in Figure 3 and can readily be used in common downstream applications.

Table 3: DNA yield and purity of isolated from 50 mg wheat leaf using the NucleoSpin® 96 Plant II kit.

	DNA yield (µg)	DNA purity (A260/A280)
Average yield	10.5	1.87
Standard deviation	0.5	0.02
Min.	9.4	1.82
Max.	11.6	1.91

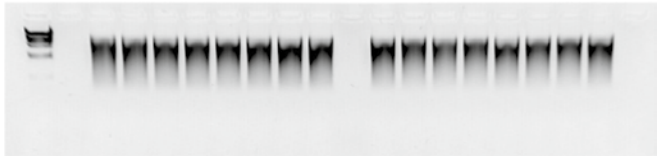

Figure 3: Agarose gel analysis.

From selected purified samples 10 µL of the eluates were loaded on a 0.8 % agarose gel. Size standard: λHindIII. High molecular weight DNA was obtained.

The NucleoSpin® Plant II kit is also available in a 8-well strip format. In another experiment yield and purity of the 96-well plate and the 8-well strip kit were compared. The results are shown in Table 4.

Table 4: DNA yield and purity of isolated from 50 mg wheat leaf using the NucleoSpin® 96 Plant II or the NucleoSpin®96 Plant II kit, respectively.

	NucleoSpin®96 Plant II	NucleoSpin®8 Plant II
Average DNA yield (µg)	10.5	10.5
Average purity	1.84	1.86

Purified DNA was also tested for real-time PCR applications. PCR reactions targeting the NADH dehydrogenase gene were set up. Results are shown in Figure 4. All samples could be amplified and the expected PCR fragments were obtained.

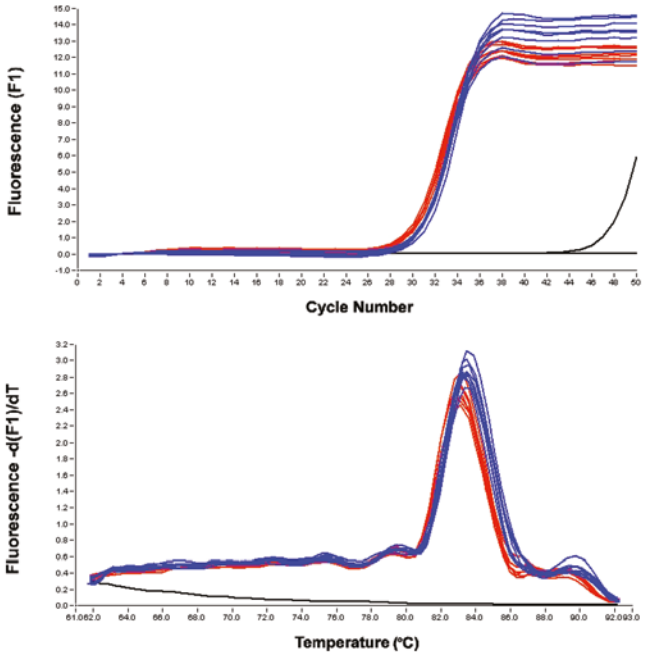

Figure 4: Real-time PCR targeting the NADH dehydrogenase gene.

To investigate run to run reproducibility, eight purified DNA samples from two independent ep*Motion*® runs using the same sample lysate (first run: red plots, second run: blue plots) were amplified in a real-time PCR experiment. Consistent and reproducible threshold cycles (top) and the expected fragment (850 bp) is obtained as verified by melting point analysis (bottom) and gel electrophoresis. Mean threshold cycles are: first run 30.4, second run 29.7.

Conclusion

Combining MACHEREY-NAGEL NucleoSpin® 8 / 96 Plant II chemistry with the ep*Motion*® 5075 VAC plus TMX Workstation, the ep*Motion*® 5075 VAC respectively, provides a reliable and flexible method for the automated DNA extraction from plant leaf tissue. The solution eliminates human errors and minimizes the risk of cross-contamination. The automation of the process replaces labor intensive manual protocols, and saves the user's time. NucleoSpin® 8 Plant II kit as well as NucleoSpin® 96 Plant II kit can be used with the same hardware allowing the user to switch between the two methods according to the requirements in sample throughput.

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® 8 Plant II	Kit based on silica membrane technology for the isolation of DNA from plant samples in 8-well strip format.	12 x 8 / 60 x 8	740669 / .5
NucleoSpin® 96 Plant II	Kit based on silica membrane technology for the isolation of DNA from plant samples in 96-well plate format.	2 x 96 / 4 x 96 / 24 x 96	740663.2 / .4 / .24
Starter Set A (for NucleoSpin® 8 Plant II only)	For use of NucleoSpin® 8-well strips on vacuum manifolds (e.g., Vac Frame 2, or NucleoVac 96 Vacuum Manifold).		740682

For more information regarding the automated use of MN products, please contact your local representative or visit MN directly under *www.mn-net.com*