

NucleoSpin® 96 RNA Plant and Fungi

Automated high throughput RNA purification from diverse plant and fungal samples using silica membrane-based kits with Eppendorf's epMotion® liquid handling workstations

Application benefits

Elevate your nucleic acid purification processes using MACHEREY-NAGEL's DNA/RNA purification kits in conjunction with Eppendorf's epMotion® liquid handling system:

- Flexible sample numbers: Process up to 96 samples in parallel
- Consistent recovery of RNA ensuring reliable reproducibility in both yield and purity
- Optimize speed while minimizing plastic consumption
- For questions about reagents or script support please contact: support@mn-net.com

Keywords

Plant RNA, fungal RNA, automated RNA purification, liquid handling system, high-purity, high-yield, transcriptome



Eppendorf epMotion® 5075vt

The Eppendorf epMotion® 5075vt offers versatility through the incorporation of various pipetting modules, a gripper, heater shaker, a vacuum chamber, and the easy to use epBlue software. This configuration allows for the processing of both silica membrane-based NucleoSpin® 8/96 and magnetic bead-based NucleoMag® DNA/RNA extraction kits.

Introduction

The extraction of RNA from plant species plays a pivotal role in research areas such as agricultural crop improvement, disease resistance, plant breeding, and plant developmental biology. Large-scale transcriptomic profiling and high-throughput RNA sequencing enable the identification of unique gene expression patterns and common expression signatures. For transcriptomic analyses, including qRT-PCR and RNA-seq, the extraction of high quality RNA is crucial. Therefore, these methods depend on reliable, high-throughput extraction workflows that ensure accurate and reproducible data.

RNA extraction from plant tissues is often hindered by the presence of plant metabolites such as polyphenolic compounds and complex polysaccharides, which can interfere with downstream applications. MACHEREY-NAGEL has developed a silica-membrane-based RNA purification kit designed for challenging plant and fungal sample material – NucleoSpin® 96 RNA Plant and Fungi – which delivers high RNA yields and purity across a wide range of plant and fungal species. The kit provides tailored protocols for diverse starting materials and combines fast convenient and time-efficient isolation of high-quality RNA through automated processing.

In this collaborative application note, we present the automated workflow on Eppendorf's epMotion® 5075vt liquid handler using the NucleoSpin® 96 RNA Plant and Fungi kit from MACHEREY-NAGEL. The newly optimized protocol supports the processing of 96-well plates, facilitating high-throughput sample preparation for gene expression and transcriptome analysis with reduced plastic consumption.

Products at a glance

NucleoSpin® 96 RNA Plant and Fungi	
Technology	Silica membrane
Sample material	50 – 500 mg of plant or fungal material
Target molecules	RNA
Typical yield	5 – 30 µg depending on sample material and quality
Fragment size	> 200 nt

epMotion® 5075vt	
Technology	Automated liquid handling platform equipped with interchangeable pipetting tools, gripper, heater-shaker, thermo module and integrated vacuum module (further modules are available for different applications).
Software	No-code epBlue 50.2
Sample numbers	1 – 96 samples
Deck positions	Configurable platform with 12 SLAS-/ANSI slots + liquid and solid waste
Pipetting volume	0.2 – 1000.0 µL

Material and methods

The extraction of plant and fungal RNA was performed using MACHEREY-NAGEL's NucleoSpin® 96 RNA Plant and Fungi kit on Eppendorf's epMotion® 5075vt. The verified protocol allows for flexibility in sample numbers up to 96 samples (variable sample number in multiples of 8). Sample disruption and lysis are recommended to be performed according to the NucleoSpin® 96 RNA Plant and Fungi kit user manual. In short, depending on the sample type 550 µL PFL and 10 – 50 µL PFR were used. Mechanical lysis of plant and fungal tissues was performed in MN Bead Tubes Type G on a Retsch Mill (30 Hz / 2 × 30 sec), followed by a heat incubation for 5 min at 56 °C. Afterwards, the steel beads were removed from samples using a magnet. To pre-clear the samples and pellet larger plant particles the lysates were centrifuged for 1 min at 14.000 x g and the supernatant was transferred to a 96-square-well block.

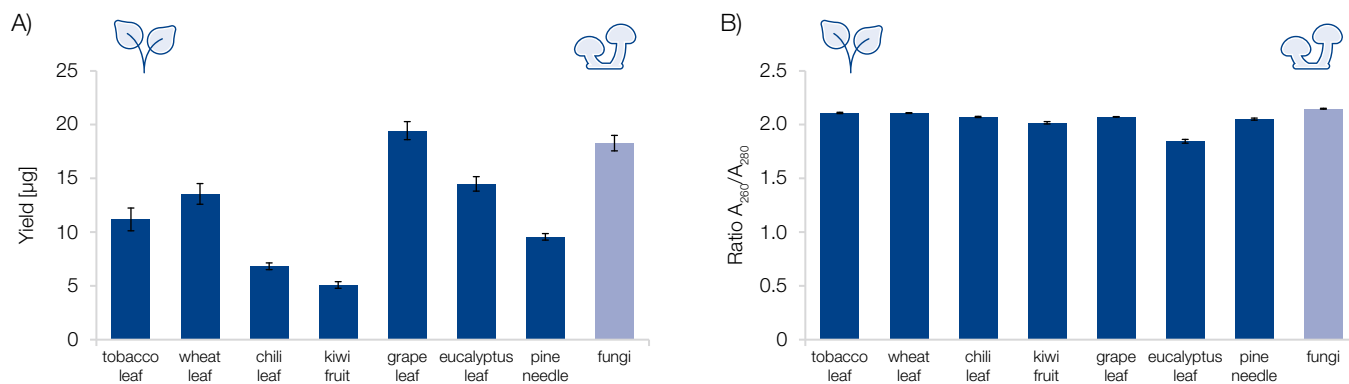
All subsequent steps are performed fully automated on the epMotion® 5075vt without manual intervention. The NucleoSpin® 96 RNA Plant and Fungi kit utilizes two different 96-well filter plates to achieve precise separation as well as high yield and quality of RNA. Crude lysates are cleared by the NucleoSpin® 96 Plant and Fungi filter plate to remove cellular debris. Subsequently binding conditions are adjusted by the addition of buffer PFB. Nucleic acids are then bound to the silica membrane of the NucleoSpin® 96 RNA Plant and Fungi binding plate during the binding step. Lysate clearance and RNA binding are performed using vacuum. The removal of contaminants, such as salts or proteins, from the silica membrane is performed by three washing steps. Finally, highly pure plant and fungal RNA is eluted in RNase-free H₂O. All pipetting and vacuum filtration steps were carried out by the Eppendorf epMotion® 5075vt.

Application data

Outstanding yields and purities across different plant and fungal samples

The NucleoSpin® 96 RNA Plant and Fungi and the epMotion® 5075vt combine the benefits of high-quality, high purity RNA extractions with a user friendly and automated system. The NucleoSpin® 96 RNA Plant and Fungi thereby delivers reliable yield and purities for various challenging plant and fungal species suitable for transcriptomic analysis. Plant leaf material from the model species tobacco (*Nicotiana tabacum*), monocot wheat (*Triticum aestivum*), dicot chili, as well as challenging

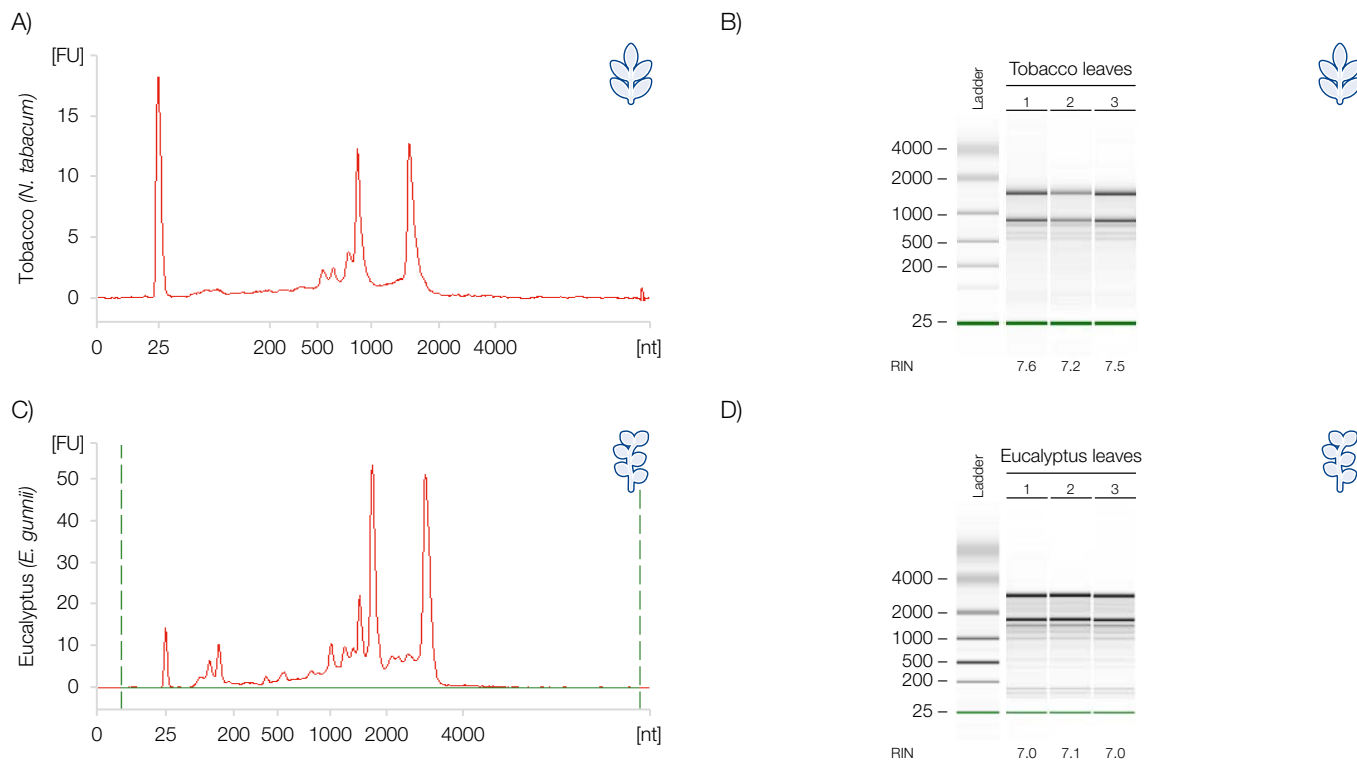
samples such as sour kiwi fruits or samples rich in secondary metabolites and other compounds including grape leaf (*Vitis vinifera*), eucalyptus leaf (*Eucalyptus gunnii*) and pine needle (*Pinus* spp.) deliver high yields and purities. As a representative for the Basidiomycota, we extracted RNA from the meadow mushroom (*Agaricus campestris*) also resulting in high yield and purity.



High RNA yields and purities of plant and fungal species

RNA was extracted from seven different plant species from different tissues and one fungal species. 100 mg tobacco leaf tissue, 85 mg wheat leaf, 85 mg chili leaf, 250 mg kiwi fruit, 95 mg grape leaf, 50 mg pine needles and 85 mg fungal tissue were used as sample input material (n = 8). Automated RNA extraction was performed on the epMotion® 5075vt using the NucleoSpin® 96 RNA Plant and Fungi kit. RNA quantity (A: plant tissue, dark blue bars; fungal tissue, light blue bar) and purity (B: plant tissue, dark blue bars; D: fungal tissue, light blue bar; Ratio A₂₆₀/A₂₈₀) were determined photometrically. Results show high RNA yields and purities for various plant species and tissues.

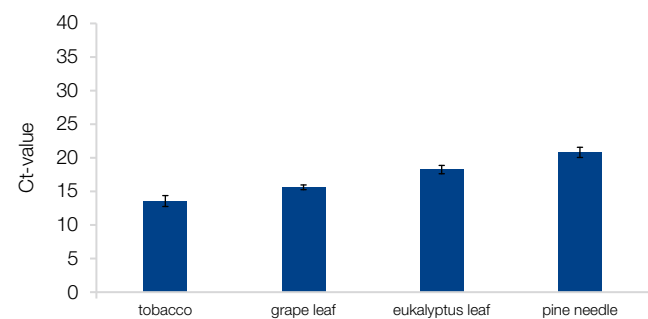
High quality of isolated RNA from plant tissue



Isolation of high-quality RNA from challenging sample material

RNA was isolated from various plant species. 1 μ L of purified RNA from tobacco leaves (A, B) and eucalyptus (C, D) were analyzed on the Agilent 2100 Bioanalyzer using the RNA Nano 6000 assay. Images show the electropherograms (A, C) and agarose gel analysis (B, D) of RNA isolated with the NucleoSpin® 96 RNA Plant and Fungi kit. The results show that the extraction of RNA from both model species and challenging samples results in high quality RNA indicated by high RIN values.

High sensitivity of isolated RNA in qRT-PCR assays



Isolation of high-quality RNA

RNA extraction was conducted from leaf material of tobacco, grape, eucalyptus and pine using the NucleoSpin® 96 Plant and Fungi kit on the epMotion® 5075vt. Subsequent qRT-PCR analysis, targeting a highly conserved photosynthetic relevant part of the chloroplast genome, was conducted using the SensiFast™ probe Lo-ROX one-step kit from BioLine on an Applied Biosystems® 7500 Real-Time PCR System. Results show a high amplification of RNA across all sample materials, indicating the efficient removal of inhibitors and bias free lysis of plant samples (n = 8 each).

Speed up and automate your RNA extraction

MACHEREY-NAGEL and Eppendorf deliver a fully automated solution for your high throughput RNA extraction. We adapted the NucleoSpin® 96 RNA Plant and Fungi kit on the epMotion® 5075vt to speed up your nucleic acid purification workflow completely hands off, and reduce plastic consumption.

- Flexible sample numbers (multiple of 8) and fast processing of 96 samples completely hands off (excluding mechanical lysis).
- Reliable performance and excellent yields using NucleoSpin® 96 RNA Plant and Fungi kit on the epMotion® 5075vt.
- Effortless automation and walk-away protocols to save time during your routine tasks and complex workflows.

Application Note NucleoSpin® 96 RNA Plant and Fungi

Eppendorf and MACHERY-NAGEL as strong collaboration partners



Download Application Notes

Scan the QR code or click the link below to read more about the collaboration between MACHERY-NAGEL and Eppendorf and read more about our scripts and methodologies of our DNA, RNA and protein purification solutions on Eppendorf's epMotion® systems.



www.mn-net.com/eppendorf

Evolve your liquid handling journey with the epMotion®

For more information on automation solutions with liquid handling systems, scan the QR code or click the link below.



www.eppendorf.com/own-your-solution/automated/

Ordering information

Product	Specifications	Pack of	REF
NucleoSpin® 96 RNA Plant and Fungi	Silica membrane-based kit for the isolation of RNA from challenging plant and fungal samples, including NucleoSpin® RNA Plant and Fungi filter plate, NucleoSpin® RNA Plant and Fungi binding plate, MN wash plates with paper sheets, Square-well Block, Rack of Tube Strips and buffers	1 x 96 / 4 x 96 preps	740128.1 / .4
MN Bead Tubes Type G	5 mm steel beads in 2 mL screw cap tubes for the disruption of plant and fungal tissue, compatible with common bead beating devices	50 tubes	740817.50
MN Bead Plates Type G	96-well plates with pack of prefilled tube stripes (8 x 12), prefilled with 5 mm steel beads for the disruption of plant and fungal, compatible with common plate bead beating devices	1 x set / 4 x sets	740855.1 / .4
MN Beads Type G (bulk)	5 mm steel beads for the disruption of plant and fungal tissue, DNA and nuclease free	500 g	740817.B.250
Eppendorf epMotion® 5075vt*	Automated liquid handling platform including MultiCon PC, a completely self-contained housing, vacuum system with accessories, gripper, ThermoMixer®, epBlue software, keyboard, mouse, system for solid and liquid waste, 100–240 V/50–60 Hz		5075 000 044*

*For more detailed information, please visit www.eppendorf.com. To contact Eppendorf SE, please email eppendorf@eppendorf.com or visit www.eppendorf.com/contact.

Want to schedule an e-demo?
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