MACHEREY-NAGEL

NucleoMag® RNA

Automated purification of highly pure RNA from plant material on the platform KingFisher® Flex

TaKaRa
Available from and supported by Takara Bio USA, Inc.

Introduction

Advances in genome-wide transcriptome profiling technologies such as RNA-Seq, microarray analysis or other methods also fastens the identification of crucial genetic factors during plant development. Transcriptomic profiling combined with high throughput RNA sequencing helps to e.g., identify genes associated with broad-spectrum disease resistance against various phytopathogenic viruses, bacteria or fungi. Moreover deciphering of complex transcriptional networks by expression quantitative trait loci (eQTL) mapping analyses are a valuable approach to gain deeper knowledge of plant development and plant physiology. One common issue during RNA isolation from plant samples is the release of polyphenolic compounds and complex polysaccharides. By crosslinking with nucleic acids or disturbing DNA Polymerase activity, these compound based interferences have a strong impact on subsequent biomolecular applications. To circumvent these obstacles, MACHEREY-NAGEL developed a support protocol for the NucleoMag® RNA kit, allowing the rapid and reliable purification of highly pure RNA from plant material. The scalable kit was developed for high throughput processing such as a 96-well format using the detergent containing Lysis Buffer RL1, specifically designed for plant material. The optimized protocol allows the processing of 96 samples within 60 minutes including rDNase digestion performed on the KingFisher® Flex platform.

Product at a glance

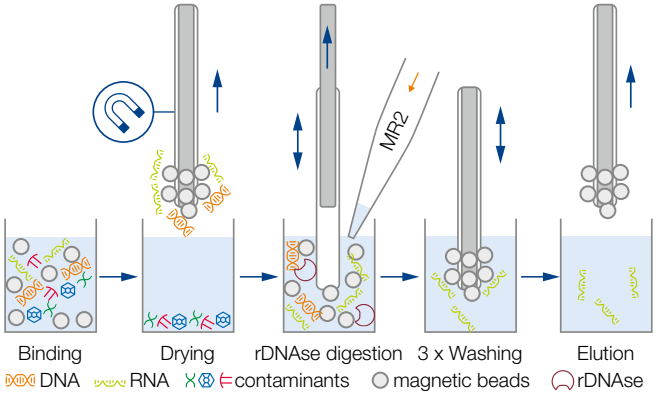
NucleoMag® RNA	
Technology	Magnetic bead technology
Sample material	20–50 mg plant material (wet weight)
Preparation time	Approx. 60 min on KingFisher® Flex for 96 samples including rDNase digestion
Typical yield	Depending on plant material and species
Elution volume	50–200 µL
Binding capacity	0.4 µg/µL beads

King Fisher® Flex	
Sample volume	20–5000 µL
Capacity	24 / 96 samples (8 plates per deck)
Heating / cooling	4–96 °C
Size / weight	60 x 38 x 68 cm / 28 kg

Material and methods

Homogenize 20–50 mg (wet weight) fresh plant material and lyse each sample with 400 µL RL1 and 7 µL TCEP. After centrifugation the cleared lysate is transferred to a Square-well Block for further processing.

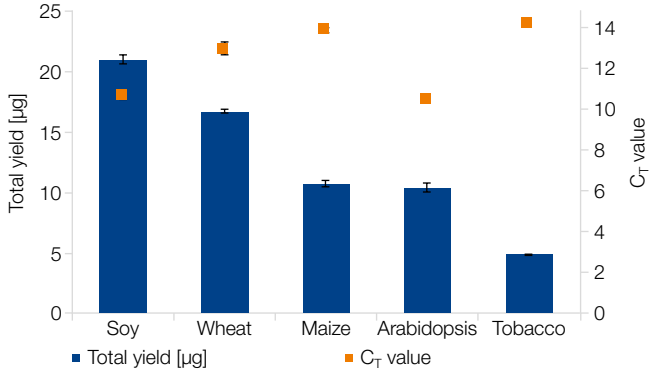
Subsequent DNA isolation is performed on the automation platform KingFisher® Flex. The isolation principle is based on reversible adsorption of nucleic acids to paramagnetic NucleoMag® B-Beads under appropriate buffer conditions.



Workflow on automation platform

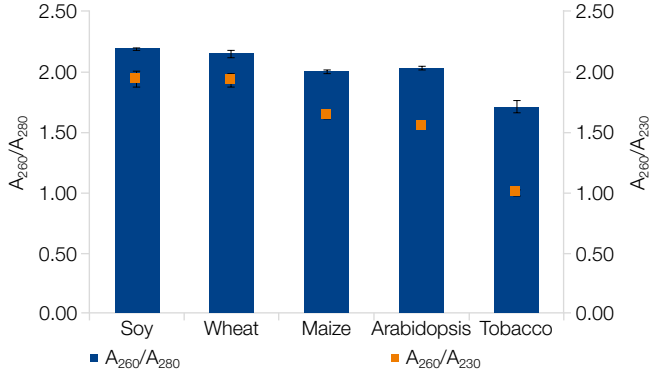
After binding of nucleic acids to the NucleoMag® B-Beads, DNA is digested in a rDNAse reaction mixture and rebinding of RNA to the NucleoMag® B-Beads is achieved by the manual provision of Binding Buffer MR2. After magnetic separation, the paramagnetic beads are washed three times to remove further contaminants and salts using two different Wash Buffers MR3 and MR4. Pure genomic DNA is finally eluted under low ionic strength conditions in the slightly alkaline Elution Buffer MR5.

Application data



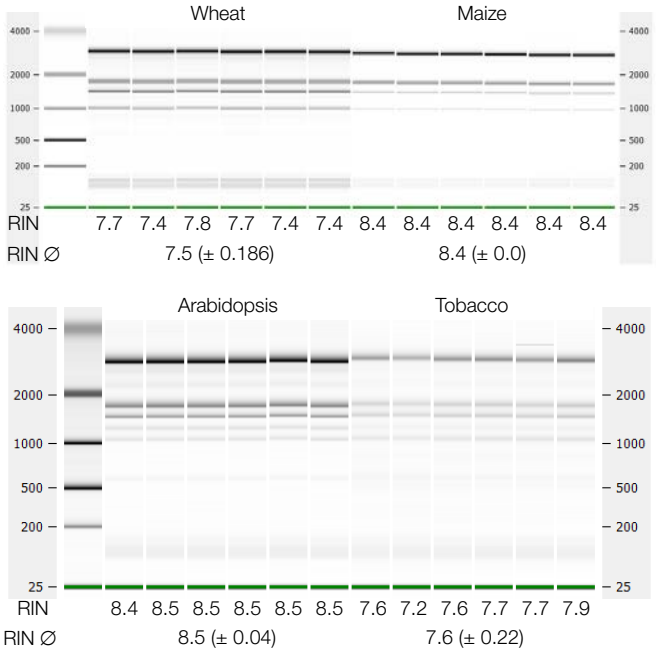
Isolation of total RNA from young leaves of different plant species

Total RNA was isolated from 25 mg fresh leaves of different plant species using the NucleoMag® RNA kit in combination with the RL1 Lysis Buffer and TCEP on a KingFisher® Flex platform. The total yield was determined by UV spectrometry (dark blue bars). A subsequent qRT-PCR analysis (orange squares) was performed for a 152 bp amplicon using the SYBR® Green quantitative RT-qPCR Kit from Sigma on a Roche LightCycler® System.



Purity of isolated RNA from leaves of different plant species

Total RNA was isolated from 25 mg fresh leaves of different plant species using the NucleoMag® RNA kit in combination with the RL1 Lysis Buffer and TCEP on a KingFisher® Flex platform. Purity of isolated RNA was assessed by UV-spectrometry resulting into an average A_{260}/A_{280} value between 1.99 to 2.18 (dark blue bars). The average A_{260}/A_{230} value (orange squares) for most plant species varies between 1.63 to 1.93.



Quality of isolated total RNA from different plant species

After total RNA isolated from fresh wheat, maize, arabidopsis and tobacco leaves (6 x 25 mg each), the total RNA integrity was determined. RNA was isolated using the NucleoMag® RNA kit in combination with the RL1 Lysis Buffer and TCEP on a KingFisher® Flex platform. The representative RNA integrity was measured by capillary gel electrophoresis using the Agilent Bioanalyzer® 2100 system and the Agilent RNA 6000 Nano kit. The results demonstrate the reliable detection of clear bands for the relevant RNA species. All RNA samples show RIN values above 7.2 and 8.4 respectively with constantly consistent results.

Automate your RNA extraction from plant material

MACHEREY-NAGEL delivers a ready to go solution for your high throughput RNA extraction from various plants. We adapted the NucleoMag® RNA procedure to isolate RNA from plant material on instruments of the KingFisher® series to speed up your nucleic acid purification workflow.

- Reliable performance and excellent yields from various plant sample material
- Speed up your RNA extraction by processing of 96 samples in less than 60 minutes (excluding lysis)

Ordering information

Product	Specifications	Preps	REF
NucleoMag® RNA	NucleoMag® B-Beads, buffers, TCEP, RNase-free rDNase	1 x 96 4 x 96	744350.1 744350.4
Lysis Buffer RL1	Plant lysis buffer with a high detergent concentration	2 x 96	740385.125
KingFisher® Accessory Kit B*	Deep-well Plates, Deep-well Tip Combs, and Elution Plates for 4 x 96 NucleoMag® RNA preps using the KingFisher® Flex platform	4 x 96	744951

*For use on KingFisher® Flex