NucleoMag® RNA

Automated RNA purification from cells or tissue samples using the NucleoMag® X32

Application benefits

The combination of the proven NucleoMag® RNA technology and the NucleoMag® X32 has several advantages that streamline your RNA purification workflows:

- Automated RNA isolation from cells and tissue samples
- Purification of RNA with reliable yield and purity
- Processing of up to 32 samples in parallel
- No programming required: Verified and pre-installed methods available

Keywords

RNA, cells, animal and human tissue, NucleoMag®, magnetic beads, magnetic rod system, NucleoMag® X32

Introduction

Purification of RNA from cells and tissue is the basis for genome-wide transcriptome studies, that can provide an in-depth understanding of gene expression networks and patterns, cross-cancer gene signatures or genetic biomarkers. RNA downstream analyses are placing high demands on the purified nucleic acids in terms of purity and integrity.

To meet these requirements, MACHEREY-NAGEL developed the NucleoMag® RNA kit. This magnetic bead-based extraction kit is scalable and was developed for high throughput processing. Purified RNA is of high purity and integrity and meets all the requirements imposed by sophisticated methods such as real-time PCR (RT-qPCR), cDNA synthesis, RNA-Seq or microarray analysis.

In this Application Note we demonstrate the automated RNA purification from cells and tissue using the NucleoMag® RNA kit on the NucleoMag® X32. The NucleoMag® X32 is a minimal footprint nucleic acid extraction system adopting magnetic bead technology. Capable of processing up to 32 samples at one time, the stand-alone instrument is easy to set up, program, and operate. Mixing, magnetic bead transfer, washing, and elution steps are performed automatically, saving valuable hands-on time.

The NucleoMag® X32 comes with verified scripts pre-installed on the device. For all scripts we also provide detailed protocol information with description of procedure steps, loading schemes, and required consumables. More information about the NucleoMag® X32 and further application notes can be found at *www.mn-net.com/de/NucleoMag-X32*.

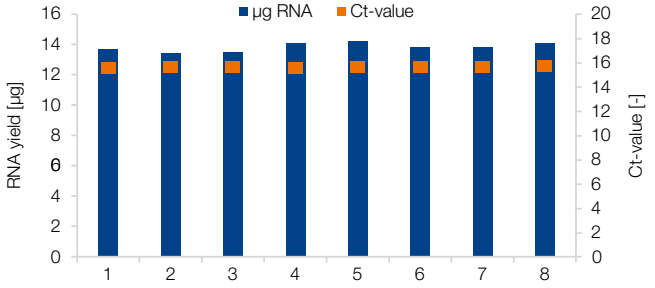
NucleoMag® RNA	
Technology	Magnetic beads
Sample material	Animal, human and plant tissue and cells
Lysate clarification	Centrifugation
Elution volume	50 – 200 µL
Fragment size	> 200 nt
Max. sample number on the NucleoMag® X32	32 samples

NucleoMag® X32	
Technology	Automated magnetic rod system
Display	7 inch-color touch screen
Capacity / volume per well	1 – 32 samples / 50 µL to 1000 µL
Dimensions	417 x 410 x 426 mm
Weight	30 kg
Contamination control	UV lamp, internal filter system
Website	*www.mn-net.com/de/NucleoMag-X32*

Material and Methods

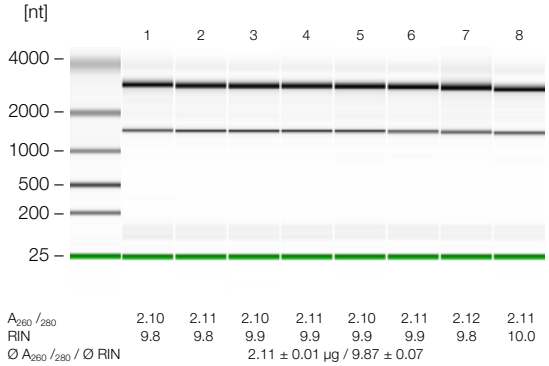
The isolation procedure of the NucleoMag® RNA kit is based on reversible adsorption of nucleic acids to paramagnetic NucleoMag® B-Beads under appropriate buffer conditions. Cells and tissue are lysed in presence of lysis buffer MR1 supplemented with TCEP. Following centrifugation and transfer of supernatant, binding of RNA to the NucleoMag® B-Beads was achieved by the addition of Binding Buffer MR2. DNA was digested in a rDNAse reaction mixture and rebinding of RNA to the NucleoMag® B-Beads was achieved by the manual provision of Binding Buffer MR2 followed by three subsequent washing steps using Wash Buffers MR3 and MR4 to remove further contaminants and salts. Pure RNA was finally eluted unter low ionic strength conditions in slightly alkaline Elution Buffer MR5. All binding, washing, and magnetic bead separation steps were carried out by the NucleoMag® X32 magnetic rod device.

Application data



Reliable reproducibillity in automated RNA purification

The figure shows total yields after RNA extraction from eight individual 5 × 10^5 HeLa cell samples. RNA was eluted in a final volume of 100 µL. Total RNA yield was determined by UV spectrometry averaging at 13.7 ± 0.2 µg. A subsequent qRT-PCR analysis (orange squares) was performed with a Taqman® Probe for a 130 bp Actin amplicon using the SensiFast™ Probe Lo-ROX One step kit from Meridian Bioscience® on an Applied Biosystems® 7500 Real-Time PCR System.



Quality of isolated RNA from HeLa cells

After total RNA isolated from eight individual 5 × 10^5 HeLa cell samples, the total RNA integrity was determined. RNA was isolated using the NucleoMag® RNA kit on the NucleoMag® X32. The quality of the RNA was determined by using the Bioanalyzer® 2100 and the total RNA 6000 Nano kit. The results demonstrate the reliable detection of clear bands for each sample and RIN values constantly above 9.8 with a mean of 9.87 (Standard deviation of ± 0.07). The purity of RNA was determined via UV spectrometry resulting in an average A260/280 of 2.11 ± 0.01.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® RNA	Magnetic bead-based kit for the isolation of RNA from cells and tissue; including NucleoMag® B-Beads, buffers, rDNase	1 × 96 preps 4 × 96 preps	744350.1 744350.4
NucleoMag® X32	Magnetic rod system for automated nucleic extraction using MACHEREY-NAGEL NucleoMag® kits, parallel processing of up to 32 samples	1	747020
96 Deep-well plates	96 deep-well plates for NucleoMag® X32	25	744955
Tip combs	8-place magnetic tip combs for NucleoMag® X32	50	744960

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