NucleoMag® RNA

Flexible magnetic bead based isolation of RNA from tissue and cell samples on the MultiEX 032 workstation

Application benefits

Experience fast and flexible RNA purification with the NucleoMag® RNA kit and the MultiEX 032 system, featuring:

- Verified methods ensuring a reliable automated purification process
- Consistent recovery of RNA with high reproducibility in yield and quality
- Increasing throughput capability, processing up to 32 samples in parallel
- No programming or complex setup required: Verified and pre-installed methods available

Keywords

Total RNA, nucleic acid extraction, automated RNA purification, isolation, tissue, cell culture, transcriptomics, gene expression, magnetic beads, MultiEX 032, magnetic rod system, laboratory protocol optimization

MultiEX 032
Scripts are pre installed and biologically verified on the MultiEX 032 workstation.

Introduction

Purification of RNA from cells and tissue is fundamental for genome-wide transcriptome studies, which explore gene expression networks and patterns, cross-cancer gene signatures or genetic biomarkers. Downstream analysis in this type of research demand high standards of RNA purity and integrity. With the development of the NucleoMag® RNA kit MACHEREY-NAGEL has committed itself to satisfy these requirements. The high purity and integrity of the purified RNA meets the specifications 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 MultiEX 032 automatic magnetic separating instrument. The established and verified protocol can be pre-installed on the device for easy automation with the click of a button.

NucleoMag® RNA	
Technology	Magnetic beads
Sample material	Animal or human tissue and cells
Target molecules	RNA
Elution volume	50 – 200 µL
Fragment size	> 200 nt
Sample numbers on MultiEX 032	32 samples with 2 plates and 4 tip combs
Processing time	Approx. 60 min (excluding lysis)

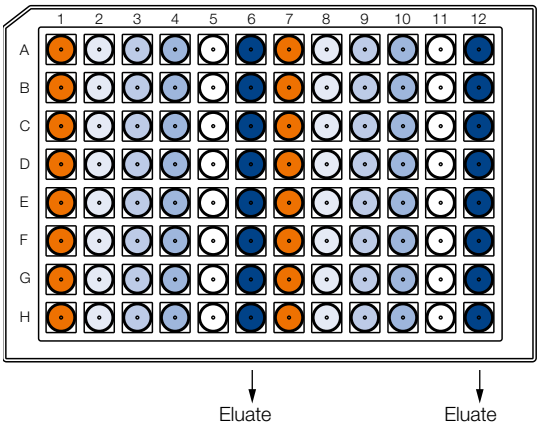
MultiEX 032	
Technology	Automated magnetic rod system
Sample number	1 – 32 samples
Deck positions	Two plate positions with four rows of magnetic rods
Capacity	Volume per well: 50 µL to 1000 µL
Size / footprint	349 × 325 × 390 mm (width x depth x height)
Usage	Ready to use verified scripts
Ease of Use	10-inch color touch screen
Contamination control	Built-in UV lamp for disinfection

Material and Methods

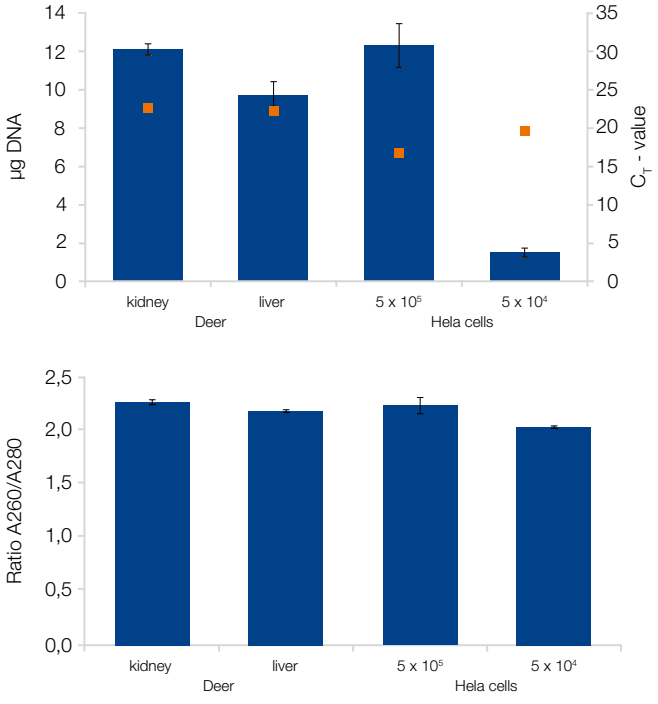
The isolation procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Cells and tissue are lysed in presence of lysis buffer MR1 supplemented with TCEP. Following centrifugation and transfer of clear lysate to column 1 ● of the 96-deep-well plate is performed. All further components of the NucleoMag® RNA kit are distributed to the extraction plate as shown in the plate layout below. The automated extraction on the MultiEX 032 is started by placing the plate into the instrument, attaching the tip covers and selecting the protocol. Binding of RNA to the NucleoMag® B-Beads takes place in protocol Part A and is achieved by adjusting the chemical conditions with Binding Buffer MR2. DNA is eliminated via an enzymatic rDNase digest. The protocol part A stops after this step and 350 µL Binding Buffer MR2 are added to column 2 / 8 ● by the user before continuing with part B. Contaminants are removed via three subsequent washing steps, followed by drying and elution. All mixing, magnetic separation and bead transfer steps are performed automatically by the MutliEX032 instrument.

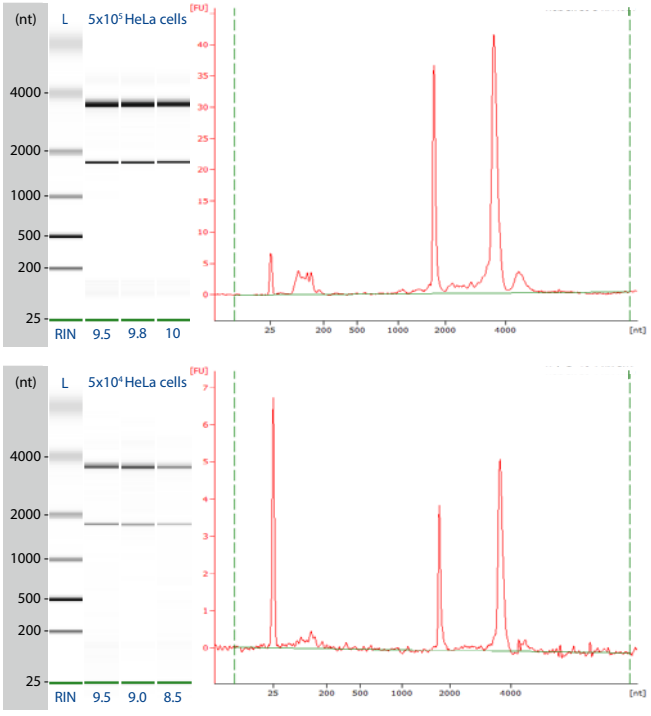
Plate Layout

Column	Reagents
● 1 | 7	Lysate (350 µL), NucleoMag® B-Beads (28 µL), Binding Buffer MR2 (350 µL)
○ 2 | 8	rDNAse reaction mixture (300 µL)
● 3 | 9	Wash Buffer MR3 (600 µL)
● 4 | 10	Wash Buffer MR4 (900 µL)
○ 5 | 11	Wash Buffer MR4 (900 µL)
● 6 | 12	RNase-free H2O (100 µL)



Application Data





RNA extraction from 20 mg tissue and cell culture

RNA extraction was conducted from 20 mg tissue (deer kidney, liver) and two cell culture concentrations (5 × 10^5 and 5 × 10^4 HeLa cells) using the NucleoMag® RNA kit on MultiEX 032 system. The yield (blue bars, top) was determined photometrically alongside the purity (blue bards, bottom) and does show high RNA recovery and A260/A280 ratios. qRT-PCR was conducted on an Applied Biosystems® 7500 Real-Time PCR System using the BioLine SensiFast™ probe Lo-ROX One-Step kit. The resulting C_T-values (orange squares, top) show very low standard deviation between biological replicates (n= 4), underlining the superior reproducibility achieved with this automated NucleoMag® RNA workflow.

Analysis of RNA integrity and quality via capillary gel electrophoresis

RNA isolated from 5 × 10^5 (top) and 5 × 10^4 (bottom) HeLa cells using the NucleoMag® RNA kit on the MultiEX 032 was analysed on the 2100 Bioanalyzer instrument using the Bioanalyzer RNA 6000 Nano kit from Agilent. The results show highly pure RNA with distinct peaks of 18S and 28S ribosomal subunits.

The high RNA integrity is reflected by the near perfect RIN values of 9.5 to 10 for the higher concentrated sample, while the 10^4 cells show the same band pattern with still high but slightly lower RIN's between 8.5 to 9.5 due to the lower overall centration of the initial sample amount. This shows the high RNA quality that can be achieved with the automated NucleoMag® RNA kit on the MultiEX 032.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® RNA	Flexible magnetic bead based isolation of RNA from tissue and cell samples; including buffers and magnetic beads. (Plastics are robot specific and need to be purchased separately)	96 preps 384 preps 2304 preps	744350.1 744350.4 744350.24
96 Deep-well plates	96-Deep-Well plates for processing of NucleoMag® kits with magnetic rod systems	25 pieces	744955
MultiEX 032	Magnetic rod system for bead based automated nucleic acid purification	1 – 32 samples	*

NucleoMag® is a registered trademark of MACHEREY-NAGEL (contact: automation-bio@mn-net.com); Applied Biosystems® is a registered trademark of Applied Biosystems.

*For more detailed information, please reach out to your local distributor