



NucleoMag[®] RNA

Automated RNA purification from cells or tissue samples using the Opentrons OT-2

Application benefits

The combination of the proven NucleoMag[®] RNA technology and the OT-2 has several advantages that streamline your RNA purification workflows:

- Verified method for automated RNA isolation from cells and tissue samples
- Purification of RNA with reliable yield and purity
- Processing of up to 48 samples in parallel
- Protocols available in the Opentrons Protocol Library or via MACHEREY-NAGEL technical automation support: automation-bio@mn-net.com

Keywords

RNA, cells, animal and human tissue, magnetic beads, Opentrons OT-2

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 Opentrons OT-2 equipped with the Opentrons Magnetic Module, with the Single-Channel P1000 and with the 8-Channel P300 Pipettes. An optimized protocol using the Opentrons OT-2 with the NucleoMag[®] RNA extraction kit can be downloaded directly from the Opentrons Protocol Library.

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 OT-2	48 samples



Figure 1: The Opentrons OT-2 is equipped with the Opentrons Magnetic Module and Opentrons GEN2 Pipettes. The Magnetic Module uses high-strength magnetic bars that can be engaged to magnetize magnetic beads, and disengaged to allow magnetic beads to remain in solution.

Opentrons OT-2	
Technology	Automated liquid handling platform equipped with electronic pipettes and Magnetic Module (further modules are available for different applications).
Sample numbers	1 – 96 samples
Deck positions	Configurable platform with 11 deck slots
Pipetting volume	20 – 300 µL (P300 8-Channel Pipette) 100 – 1000 µL (P1000 Single-Channel Pipette) (Further Single-Channel and 8-Channel pipettes with different ranges are available for other applications)

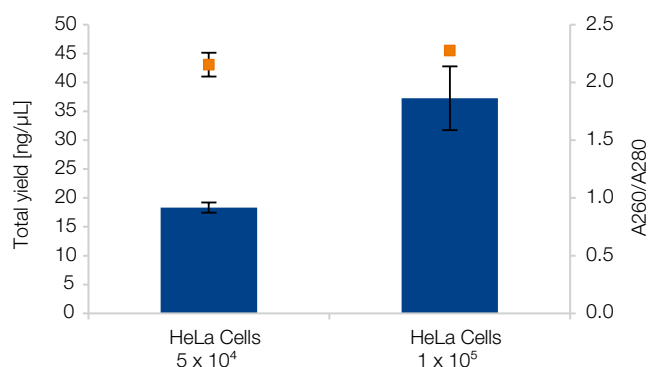
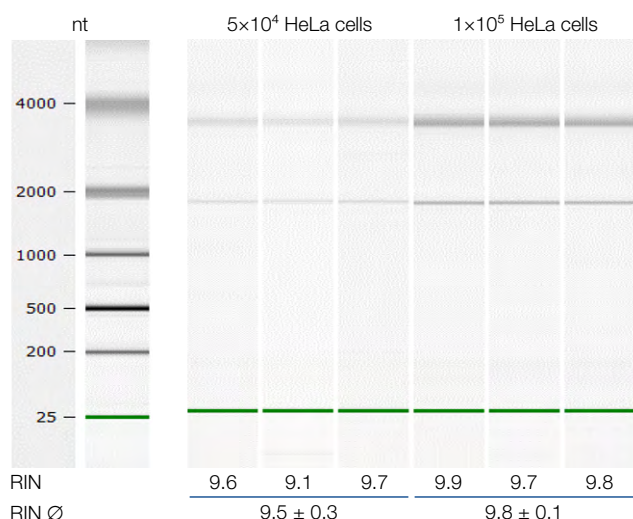
Material and Methods

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 and contaminants were removed via three subsequent washing steps. All pipetting steps and magnetic bead separations were carried out by the OT-2 and the Opentrons Magnetic Module.

Application data

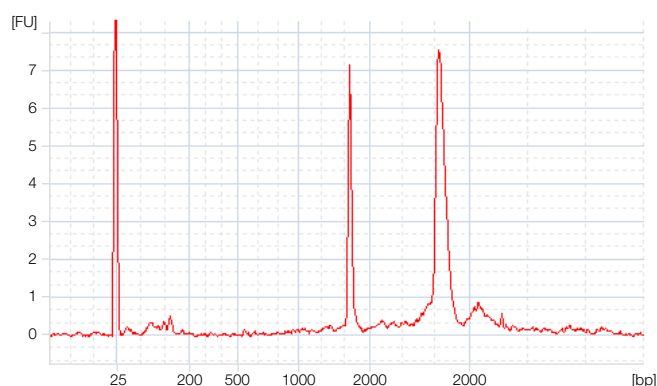
A



Reliable reproducibility in automated RNA purification

The figure shows total RNA yields and purities after the RNA extraction from three individual 5 × 10⁴ or 1 × 10⁵ HeLa cell samples. RNA was eluted in a final volume of 100 µL. Total RNA yield was determined by UV spectrometry averaging at 18.3 ± 0.9 ng/µL for 5 × 10⁴ HeLa cells and at 37.3 ± 5.5 ng/µL for 1 × 10⁵ HeLa cells (dark blue bars). Average purity of isolated RNA of all purified samples was measured at A260/A280 of 2.2 ± 0.1. The data show reliable reproducibility of yield and purity of total RNA.

B



Quality of isolated RNA from HeLa cells

(A) After total RNA isolated from three individual 5 × 10⁴ or three individual 1 × 10⁵ HeLa cell samples, the total RNA integrity was determined. RNA was isolated using the NucleoMag® RNA kit on the Opentrons OT-2. 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 9.1 with a mean of 9.76 (Standard deviation of ± 0.09). (B) The graph shows an exemplified Bioanalyzer result of extracted from 1 × 10⁵ HeLa cells (lane 4 above).

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	744350.1
		4 × 96 preps	744350.4
OT-2 pipetting robot	Automated liquid handling platform with Magnetic Module and electronic pipettes	OT-2 Pipetting Robot	999-00111 *
		Single-Channel P1000 Pipette	999-00004 *
		8-Channel P300 Pipette	999-00006 *
		Magnetic Module	999-00098 *

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 * For more detailed information, please visit www.opentrons.com. To contact Opentrons Sales or to schedule a demo, please email info@opentrons.com.