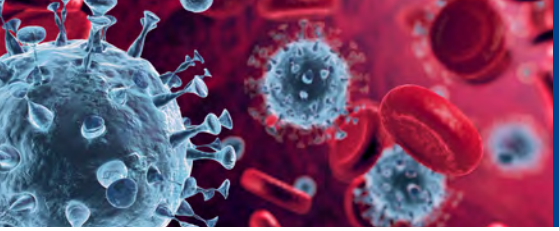

NucleoMag® Virus

Automated purification of viral RNA and DNA from biological fluids on the Opentrons OT-2

Application benefit

The combination of the NucleoMag® Virus kit kit and the OT-2 has several advantages that streamline your nucleic acid purification workflows:

- Verified method for fully automated nucleic acid purification workflow
- Reliable recovery of viral RNA/DNA with reliable reproducibility in 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

viral RNA, viral DNA, cell-free biological fluids, plasma, serum, pathogen detection OT-2

Introduction

As the ongoing covid-19 pandemic has shown us, the isolation of viral nucleic acids plays a pivotal role in research or diagnostic laboratories as well as in the field of epidemiology, immunology and virology. The ever more advancing and precise downstream analytical methods place high demands on the quality of the purified nucleic acids in terms of purity, yield and reproducibility. To meet these requirements, MACHEREY-NAGEL developed the NucleoMag® Virus kit allowing the automated isolation of nucleic acids from various cell-free body fluids such as plasma or serum. Magnetic bead technology is used to obtain viral RNA and DNA with highest purity and yield.

In this Application Note we demonstrate the automated purification of viral RNA and DNA from human saliva samples for subsequent qPCR analysis on the Opentrons OT-2 equipped with the Opentrons Magnetic Module, with the Single-Channel P1000 and with the 8-Channel P300.

An optimized protocol for using the Opentrons OT-2 with the NucleoMag® Virus kit can be downloaded directly from the Opentrons Protocol Library.

NucleoMag® Virus	
Technology	Magnetic beads
Sample material	Cell-free biological fluids Plasma Serum
Target molecules	Viral RNA and DNA
Elution volume	50-100 µL
Fragment size	~300 bp – approx. 50 kbp
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 for NGS clean-up. The Magnetic Modules uses high-strength magnetic bars that can be engaged to magnetize magnetic beads, and disengaged to allow magnetic beads to remain in solution.

OT-2 nucleic acid extraction workstation	
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® Virus kit is based on reversible adsorption of nucleic acids to paramagnetic NucleoMag® V-beads under appropriate binding conditions. Up to 200 µL saliva was mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer MVL. Reversible binding of nucleic acids to paramagnetic beads was enabled by adjustment with Binding Buffer MV2. Subsequent to the magnetic separation, the NucleoMag® V-Beads were washed to remove contaminants and salts using Wash Buffer MV3 and MV4. After air drying, highly pure nucleic acids were eluted in 100 µL elution buffer MV5. All pipetting steps and magnetic bead separation were carried out by the OT-2 and the Magnetic Module.

Application data

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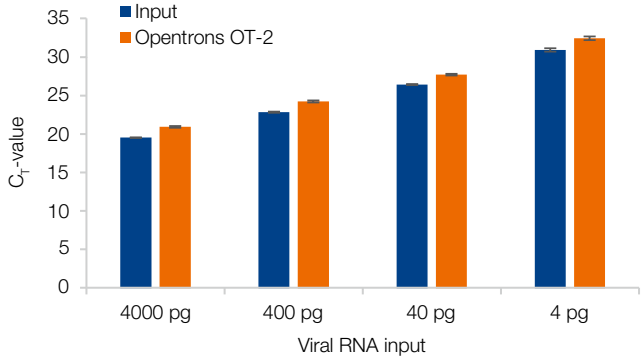
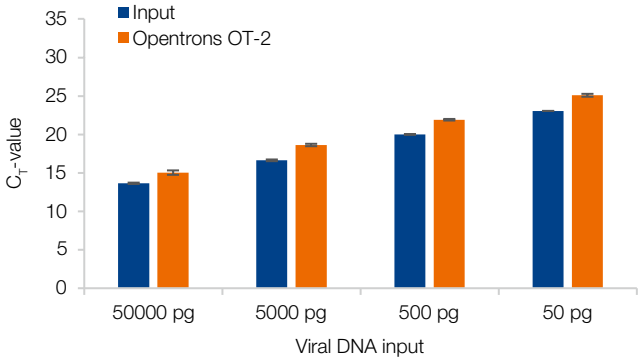


High sensitivity detection of viral DNA recovered from human saliva

DNA was isolated from human saliva samples (200 µL; n = 4 for each dilution) using the NucleoMag® Virus kit on the Opentrons OT-2 workstation. T7 bacteriophage DNA was spiked into human saliva in a dilution series. The recovery rate was determined by measuring the input value in comparison to the Ct value after DNA extraction (OT-2). The analysis was performed with a Taqman® PCR probe for T7-DNA using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.



High sensitivity detection of viral RNA recovered from human saliva

DNA was isolated from human saliva samples (200 µL; n = 4 for each dilution) using the NucleoMag® Virus kit on the Opentrons OT-2 workstation. Ms2 bacteriophage RNA was spiked into human saliva in a dilution series. The recovery rate was determined by measuring the input value in comparison to the Ct value after RNA extraction (OT-2). The analysis was performed with a Taqman® PCR probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Virus	Magnetic bead-based kit for the isolation of viral RNA / DNA, from cell-free biological fluids; including NucleoMag® V-Beads, buffers, Carrier RNA and Proteinase K	1 x 96 preps	744800.1
		4 x 96 preps	744800.4
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*
		Magnatic 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.