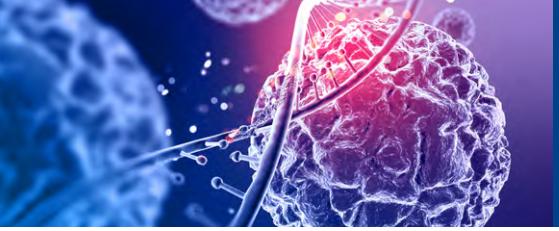


NucleoMag® Pathogen

Automated purification of viral RNA/DNA and bacterial DNA from human samples on the Opentrons OT-2

Application benefits

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

- Verified methods for fully automated nucleic acid purification workflow
- Reliable recovery of RNA/DNA with high 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, bacterial DNA, clinical specimens, pathogen detection, magnetic beads, OT-2

Introduction

For their demanding downstream analyses, clinical and translational research laboratories require fast and reliable DNA and RNA purification products for the recovery of high-quality nucleic acids from a diversity of human specimens, such as swabs, blood, plasma, serum, tissue biopsies or body fluids. Different starting materials pose special difficulties and challenges for nucleic acid extractions. To meet these requirements, MACHEREY-NAGEL developed the NucleoMag® Pathogen kit allowing the automated isolation of nucleic acids from various starting materials using magnetic bead technology. Viral nucleic acids and bacterial DNA are recovered with reliable purity and yield.

In this Application Note we demonstrate the automated purification of viral RNA and DNA from human serum 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 Pipettes.

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

NucleoMag® RNA	
Technology	Magnetic beads
Sample material	≤ 200 µL whole blood, serum, plasma, ≤ 200 µL swab wash solution ≤ 25 mg tissue ≤ 200 µL feces
Target molecules	Viral RNA/DNA and bacterial DNA from human samples
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 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)

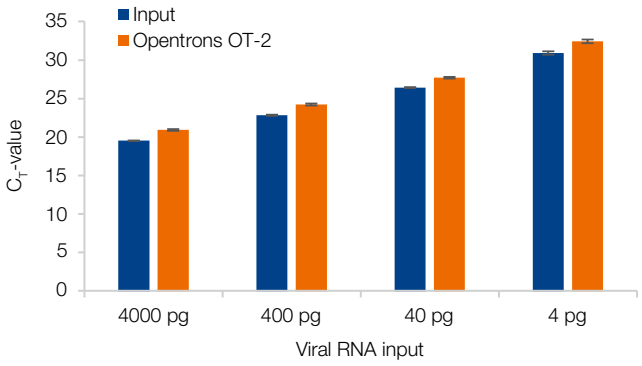
Material and Methods

The isolation procedure of the NucleoMag® Pathogen kit is based on reversible adsorption of nucleic acids to paramagnetic NucleoMag® B-beads under appropriate binding conditions. Up to 200 µL plasma material was mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer NPL1. Reversible binding of nucleic acids to paramagnetic beads was enabled by adjustment with Binding Buffer NPB2. Subsequent to the magnetic separation, the NucleoMag® B-Beads were washed to remove contaminants and salts using Wash Buffer NPW3, NPW4, and 80 % ethanol respectively. After air drying, highly pure nucleic acids were eluted in 100 µL elution buffer NPE5.

All liquid handling pipetting steps and magnetic bead separations were carried out by the OT-2 and Opentrons Magnetic Module.

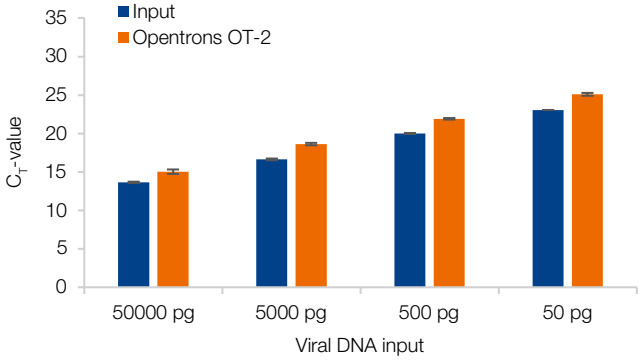
Application data

A



High sensitivity detection of viral RNA recovered from human plasma

RNA was isolated from human plasma samples (200 µL; n = 4 for each dilution) using the NucleoMag® Pathogen kit on the Opentrons OT-2 workstation. MS2 bacteriophage RNA was spiked into human plasma 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 MS2 using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.



High sensitivity detection of viral DNA recovered from human plasma

DNA was isolated from human plasma samples (200 µL; n = 4 for each dilution) using the NucleoMag® Pathogen kit on the Opentrons OT-2 workstation. T7 bacteriophage DNA was spiked into human plasma 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 T7 DNA using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Pathogen	Magnetic bead-based kit for the isolation of viral RNA / DNA, and bacteriall DNA from clinical samples; including NucleoMag® B-Beads, buffers, Carrier RNA and Proteinase K	1 × 96 preps 4 × 96 preps	744210.1 744210.4
OT-2 pipetting robot	Automated liquid handling platform with Magnetic Module and electronic pipettes	OT-2 Pipetting Robot Single-Channel P1000 Pipette 8-Channel P300 Pipette Magnetic Module	999 – 00111 * 999 – 00004 * 999 – 00006 * 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.

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