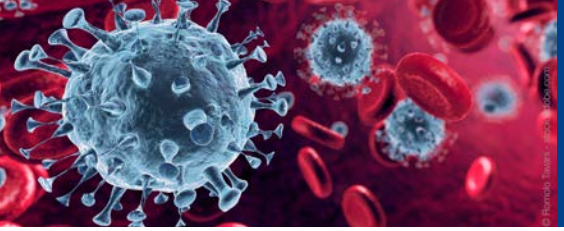


NucleoMag® Pathogen

Automated magnetic bead based isolation of viral nucleic acids and bacterial DNA from clinical samples on the MultiEX 032 workstation

Application benefits

Experience fast and secure nucleic acid purification with the NucleoMag® Pathogen kit and the MultiEX 032 system, featuring:

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

Keywords

Molecular diagnostics, nucleic acid extraction, automation, bacterial DNA, viral RNA, tissue, body fluids, swabs, stool, pathogen detection, disease prevention, magnetic beads, MultiEX, magnetic rod system, laboratory protocol optimization

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

Introduction

Isolation of nucleic acids from viral or bacterial pathogens present in clinical samples is the necessary first step for molecular tests that have become standard methodology in research and diagnostic laboratories. Due to the diversity of the sample material – swabs, blood, plasma, body fluids, tissue biopsies, etc. – the isolation procedure itself often poses challenges to laboratory staff and workflows. The purification needs to be able to handle this wide sample variation while providing robust and reliable results. 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. MACHEREY-NAGEL has established and tested the NucleoMag® Pathogen on the MultiEX 032. This way scientists and lab technicians can not only use the robust chemistry of the extraction kit but also benefit from the high reproducibility and data integrity of automation. To further lower the risk of manual handling the NucleoMag® Pathogen Prefilled Plates minimize handling and make automation easy to use.

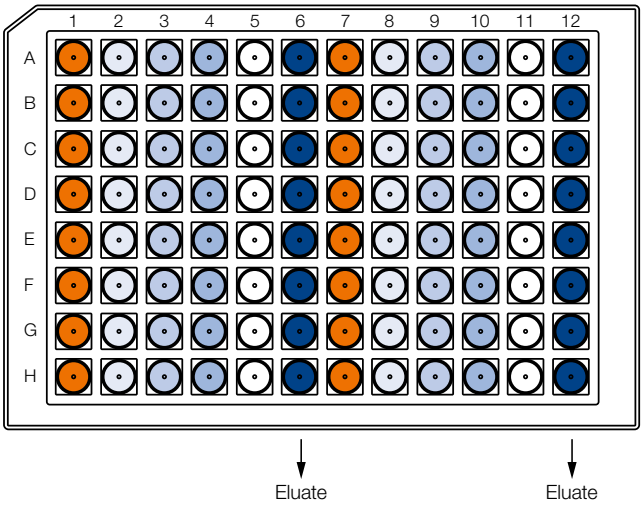
NucleoMag® Pathogen / NucleoMag® Pathogen Prefilled Plates	
Technology	Magnetic beads
Sample material	Human tissue, blood, cell-free biological fluid, stool, swabs
Target molecules	Viral RNA and DNA
Elution volume	50 – 100 µL (100 µL when using NucleoMag® Pathogen Prefilled Plates)
Fragment size	~300 bp – approx. 50 kbp
Sample numbers on MultiEX 032	32 samples with 2 plates and 4 tip combs
Processing time	Approx. 30 min (excluding lysis)

MultiEX 032	
Technology	Automated magnetic rod system
Sample number	1 – 32 samples
Deck positions	Two plate positions and 4 rows of magnetic rods
Capacity	Volume per well: 50 µL to 1000 µL
Size / footprint	349 x 325 x 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
Advantages	Fast automated processing for higher efficiency and reliability

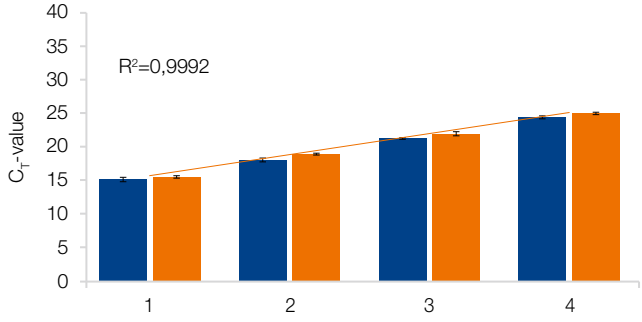
Material and Methods

The extraction of viral nucleic acids using the NucleoMag® Pathogen kit starts by lysing the clinical sample via the addition of Proteinase K and Lysis Buffer NPL1 followed by a 15 minute incubation. If solid particles remain within the lysate, it can be cleared via brief centrifugation before transferring cleared lysate to column 1 ● of the 96-well Deep-well Plate. In this application note prefilled plates were used and therefore 20 µL NucleoMag® B-Beads and 575 µL Binding Buffer NPB2 were already present in column 1. The other columns contain 600 µL of different Wash Buffer as shown in the layout to the right: NPW1 in column 2 ●, NPW2 in column 3 ● and 80% ethanol in column 4 ● as well as 50 – 100 µL Elution buffer NPE5 in column 6 ●. The kit is also available in bottles for manual Buffer distribution into the 96-deep-well plate to allow for maximum flexibility in number of samples. The prepared plate is placed in the instrument, the tip combs attached and the protocol selected and started. The MultiEX 032 fully automatically performs the mixing during the binding step, allowing for the reversible adsorption of nucleic acids to the paramagnetic NucleoMag® B-beads under optimal binding conditions. The beads are then transferred via magnetic separation to the other columns of the plate to perform wash steps, drying and elution. Once finished the obtained nucleic acids can directly be used for qRT-PCR and further downstream applications.

Plate Layout

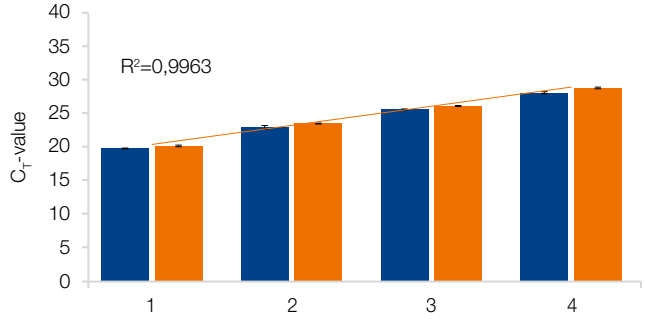


Application Data



Isolation of T7 DNA-Spike from human saliva

The NucleoMag® Pathogen kit was used on the MultiEX 032 system to isolate different concentrations of a T7 bacteriophage DNA target that was spiked into human saliva. The dilution series demonstrates a reliable recovery of nucleic acids for various viral titers (orange bars). The C_T-value of the inputs (blue bars) show a very small difference to the isolated DNA (orange bars), which demonstrates high recovery and sensitivity of the NucleoMag® Pathogen kit. Furthermore the very low error bars of biological replicates (n=4) show how automating on the MutliEX 032 can minimize the variation and increase the reliability and data integrity. This is even more clear when looking at the linear correlation (orange line) of the entire dilution series, which shows a near perfect coefficient of determination R^2 of 0.9992. qPCR was conducted by using the the BioLine SensiFast™ probe Lo-ROX kit on an Applied Biosystems® 7500 Real-Time PCR System.



Isolation of MS2 RNA-Spike from human saliva

Viral RNA extraction from a 1:10 dilution series of MS2 bacteriophage RNA-spikes within human saliva was performed with the NucleoMag® Pathogen kit on the MultiEX 032 system. The input concentrations of the dilution series are shown in the blue bars and the extracted RNA is indicated by the orange bars. The low standard deviation between the replicates (n=4) of each dilution showcase the high degree of standardization that can be achieved by automating the NucleoMag® Pathogen kit on the MultiEX 032 system. The qRT-PCR analysis was performed by using the BioLine SensiFast™ probe Lo-ROX One-step kit on an Applied Biosystems® 7500 Real-Time PCR System. The linear regression (orange line) of the extracted samples shows a very high R^2 coefficient of 0.9963 which indicates the dilution series is not altered allowing for very reliable RNA extraction across a range of viral titers without introducing a bias. The high degree of efficiency is also visualized due to the low increase of C_T-value of each individual extracted sample compared to the inputs.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Pathogen	Magnetic bead based kit for isolation of viral nucleic acids and bacterial DNA from Veterinary samples	96 preps 384 preps 2304 preps	744210.1 744210.4 744210.24
NucleoMag® Pathogen Prefilled Plates	The NucleoMag® Pathogen Prefilled Plates are ideal for the isolation of viral RNA/DNA and bacterial DNA from clinical samples. Suitable sample materials include blood, human tissue, stool, swabs and cell-free biological fluids. (The magnetic Rod tip combs are platform specific, for the MultiEX032 please don't use the included tip combs and order dedicated tip combs separately)	6 plates a 16 preps (96 preps total)	744211
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);
*For more detailed information, please reach out to your local distributor.