MACHEREY-NAGEL

NucleoMag® Pathogen

Automated purification of viral RNA and DNA and microbial DNA from clinical samples on the ep*Motion*® 5075t platform

Introduction

The isolation of viral RNA and DNA or microbial DNA from different starting material is often tedious for routine laboratories and requires different purification methods. Different starting materials pose special difficulties and challenges for nucleic acid extractions. Low virus or bacterial titers and hard to lyse pathogens often require troublesome procedures. Purification modifications for single pathogen targets and sample types are time consuming and inconvenient for routine assessments. The molecular diagnostic market demands extraction methods which are adaptable on automation platforms and reliable in terms of pathogen detection. The purification process needs to be suitable for a wide variety of sample materials.

To meet the requirements of the molecular diagnostic market MACHEREY-NAGEL developed the NucleoMag® Pathogen kit allowing the automated isolation of nucleic acids from various starting materials using magnetic bead technology.

This application note describes the automated process on the automated liquid handling workstation ep*Motion*® 5075t using the NucleoMag® Pathogen kit from MACHEREY-NAGEL. We show the automated purification workflow exemplarily for spiked viral RNA and DNA within human plasma sample material. The tailored protocol allows the processing of 96 samples in approx. 130 minutes.

Product at a glance

NucleoMag® Pathogen	
Technology	Magnetic beads
Sample material	< 200 µL whole blood, serum, plasma, < 25 mg tissue (e.g., ear notches), < 200 µL feces, < 200 µL swab wash solution
Target molecules	Viral RNA, viral DNA, and microbial DNA from clinical samples
Fragment size	300 bp–approx. 50 kbp
Elution volume	50–200 µL

Material and methods

The NucleoMag® Pathogen kit is designed for common clinical sample material, such as whole blood, serum or plasma, feces, tissue, or swabs. Up to 200 µL liquid or homogenized sample material is mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer NPL1 (please see the NucleoMag® Pathogen kit manual for more detailed information). The isolation principle is based on reversible adsorption of nucleic acids to paramagnetic beads (NucleoMag® B-Beads) under appropriate buffer conditions. The reversible binding of nucleic acids to paramagnetic beads the NucleoMag® B-Beads was enabled by adjustment with Binding Buffer NPB2. Subsequent to the magnetic separation, the NucleoMag® B-Beads are washed to remove contaminants and salts using Wash Buffer NPW3, NPW4, and 80 % ethanol respectively. After air drying the NucleoMag® B-Beads, highly pure nucleic acids are finally eluted under low ionic strength conditions in the slightly alkaline Elution Buffer NPE5.

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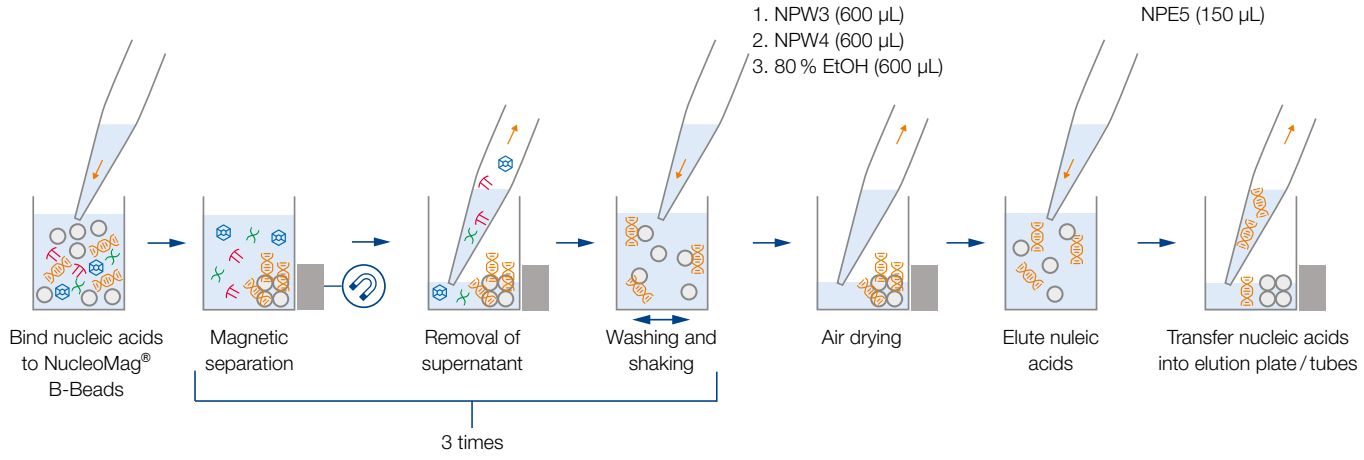
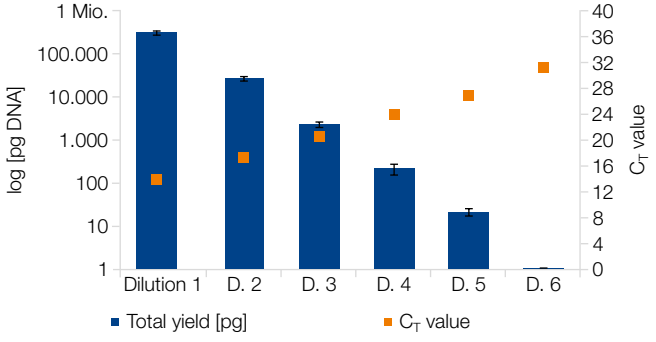
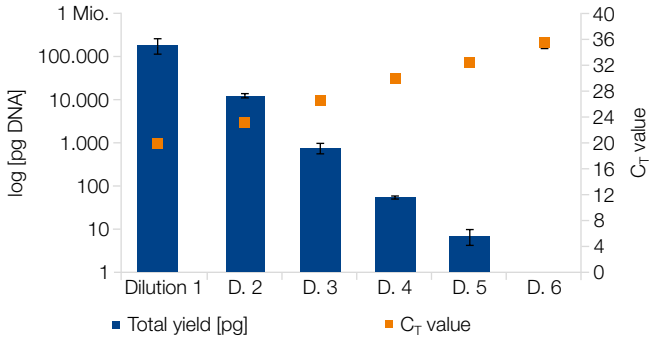
Application data





qPCR analysis of T7 DNA recovered from human plasma

DNA was isolated from human plasma sample material (n = 3; 200 µL each sample) using the NucleoMag® Pathogen kit on the ep*Motion*® 5075t. T7 bacteriophage DNA was spiked in a serial dilution of 1–1:100.000 (Dilution 1-D.6; blue bars). Sensitivity was determined by a subsequent Taqman® Probe for T7 DNA using the SensiFast™ Probe Lo-ROX kit (Bioline) on an Applied Biosystems® 7500 Real-Time PCR System (orange squares). The determined quantity was extrapolated using a corresponded standard curve.



qRT-PCR analysis of MS2 RNA recovered from human plasma

RNA was isolated from human plasma sample material (n = 3; 200 µL each sample) using the NucleoMag® Pathogen kit on the ep*Motion*® 5075t. MS2 bacteriophage RNA was spiked in a serial dilution of 1:1–1:100.000 (Dilution 1-D.6; blue bars). Sensitivity was determined by a subsequent Taqman® Probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit (Bioline) on an Applied Biosystems® 7500 Real-Time PCR System. (orange squares). The determined quantity was extrapolated using a corresponded standard curve.

Automate your viral and microbial nucleic acid extraction from clinical samples

MACHEREY-NAGEL and Eppendorf® deliver a tailored solution for your high throughput viral RNA, viral DNA, and microbial DNA extraction. We adapted the NucleoMag® Pathogen procedure on the ep*Motion*® 5075t system to automate your purification workflow.

- Excellent recovery of nucleic acids from diverse clinical sample material
- Start right away with included carrier RNA, Proteinase K and ready to use buffers
- Optimized purification workflow on the ep*Motion*® 5075t to process 96 samples within 130 minutes

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Pathogen	Kit based on magnetic bead technology for the isolation of viral RNA, viral DNA, and microbial DNA from clinical samples including NucleoMag® B-Beads, buffers, Carrier RNA and Proteinase K	1 x 96 / 4 x 96	744210.1 / .4
ep*Motion*® 5075t	Basic device incl. Eppendorf ThermoMixer®,epBlue™ software, mouse, waste box, 100–240 V ±10 % / 50–60 Hz ±5 %, 0.2 µL–1 mL		5075000302