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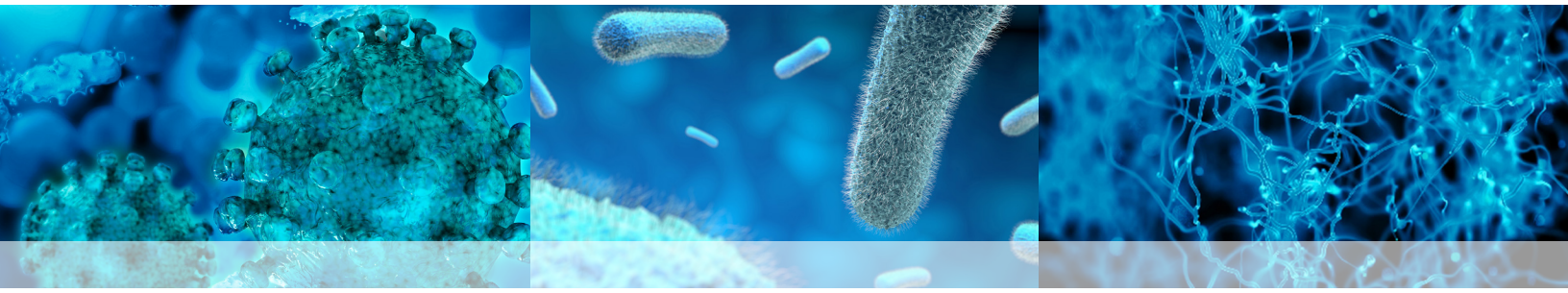
MACHEREY-NAGEL

NucleoMag® Pathogen

Automated purification of viral RNA / DNA and microbial DNA from clinical samples on the Freedom EVO® 150 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 viral or bacterial titers 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 DNA detection (1). Moreover, 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 processing of the NucleoMag® Pathogen kit from MACHEREY-NAGEL on the TECAN Freedom EVO® 150 liquid handling workstation using the Te-Shake™ in combination with the NucleoMag® SEP Magnetic Seperator for 96-well plate processing. We demonstrate this automated purification workflow for spiked viral RNA and DNA exemplarily. The tailored protocol allows processing of up to 96 samples per run (variable sample number).

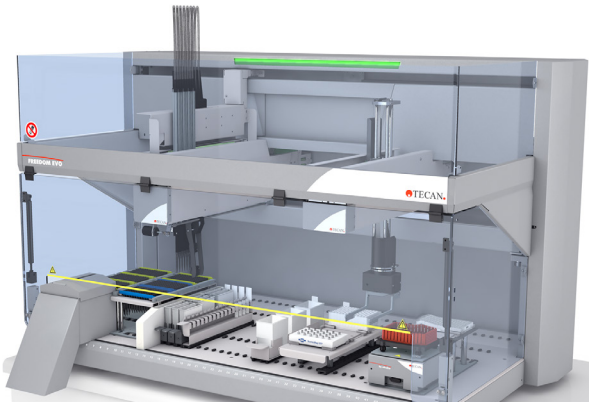
Product at a glance

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

Freedom EVO®	
Technology	8-channel LiHa Arm configured for disposable tips, 1000 µl syringes (alternative: AirLiha Arm), RoMa Arm for plate handling, Te-Shake™ for heating (RT-80°C) and shaking (100–1500 rpm)
Capacity	1–96 samples per batch
Special features	TouchTools™ software for intuitive touch screen guided operation, reduces training needs, optional integration of an Infinite® F or M NANO+ reader (Configurations of the Infinite 200 PRO family), in combination with the Freedom EVOware® Normalization Wizard, allowing for automated quantification and normalization

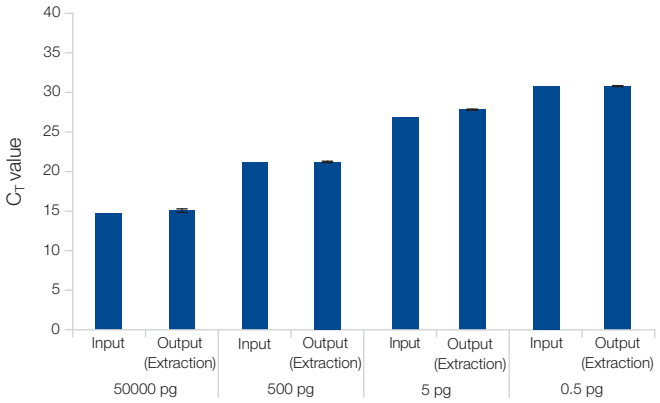
Material and methods

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 of liquid or homogenized sample material is mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer NPL1 prior to lysis incubation. The subsequent isolation is based on reversible adsoption of nucleic acids to paramagnetic beads (NucleoMag® B-Beads). Nucleic acid binding is enabled by addition of Binding buffer NPB2. After magnetic separation and removal of the supernatant, contaminants and salts are removed by three subsequent washing steps. The NucleoMag® B-Beads are air dried before highly pure nucleic acids are finally eluted under low ionic strength conditions in Elution Buffer NPE5.



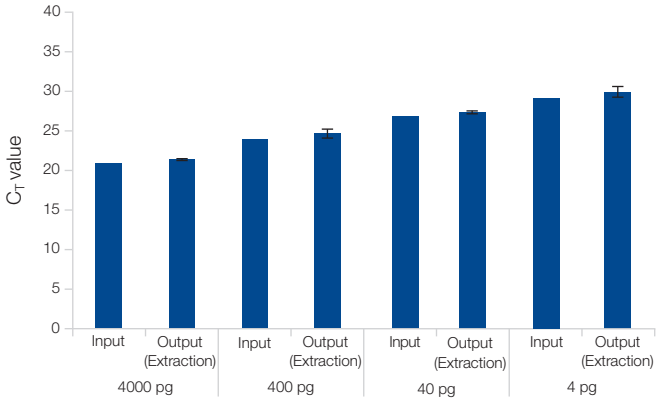
Example configuration on a Freedom EVO® 150

Application data



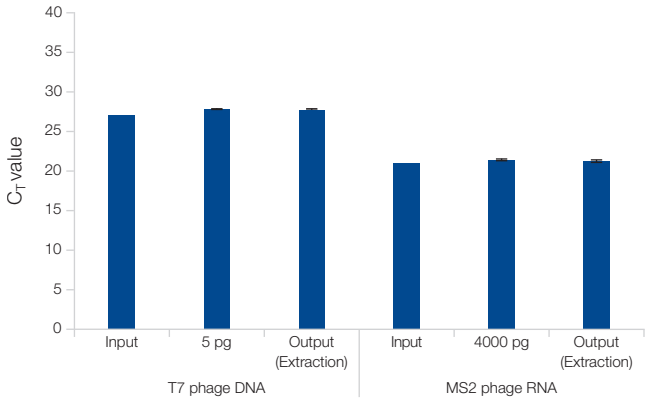
qPCR analysis of T7 DNA recovered from PBS / saline

DNA was isolated from PBS / saline sample material (n = 3 for each dilution; 200 µL each sample) using the NucleoMag® Pathogen kit on the Freedom EVO® 150 platform. T7 bacteriophage DNA was spiked in a serial dilution and the recovery rate was determined by measuring the Input value in comparison to the C_T value after DNA extraction (Output). The analysis was performed with a Taqman® Probe for T7 DNA using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. The determined quantity was extrapolated using a corresponded standard curve. During the whole dilution series the recovery rate was between 97.1 % and 99.8 %.



qRT-PCR analysis of MS2 RNA recovered from PBS / saline

RNA was isolated from PBS / saline sample material (n = 3 for each dilution; 200 µL each sample) using the NucleoMag® Pathogen kit on the Freedom EVO® 150 platform. MS2 bacteriophage RNA was spiked in a dilution series and the recovery rate was determined by measuring the input value in comparison to the C_T value after DNA extraction (output). The analysis was performed with a Taqman® Probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. The determined quantity was extrapolated using a corresponded standard curve. During the whole dilution series the recovery rate was between 97.4 % and 98.2 %.



Co isolation of T7 DNA and MS2 RNA recovered from PBS / saline

Viral DNA and RNA was isolated simultaneously from PBS / saline sample material (n = 3 for each dilution; 200 µL each sample) using the NucleoMag® Pathogen kit on the Freedom EVO® 150 platform. C_T values of extracted T7 bacteriophage DNA and MS2 bacteriophage RNA were analyzed individually by qPCR or qRT-PCR in comparison to the original input and each individual dilution (5 pg or 4000 pg) of the respective DNA or RNA spike. The analysis was performed with a Taqman® Probe for T7 DNA using the SensiFast™ Probe Lo-ROX kit or with a Taqman® Probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. The determined quantity was extrapolated using a corresponded standard curve. During the whole dilution series the recovery rate of both, RNA and DNA was between 97.1 % and 98.8 %.

Automate your viral and microbial nucleic acid extraction from clinical samples

MACHEREY-NAGEL and TECAN deliver a tailored solution for your high throughput viral RNA, viral DNA, and microbial DNA extraction from clinical sample material. We adapted the NucleoMag® Pathogen procedure on the Freedom EVO® 150 system to automate your nucleic acid purification workflow.

- Excellent recovery of nucleic acids from diverse clinical sample materials
- All required reagents provided, including carrier RNA, Proteinase K, and ready to use buffers
- Tailored protocol for processing variable sample numbers

References

(1) Tsalik et al., 2018 “New Molecular Diagnostic Approaches to Bacterial Infections and Antibacterial Resistance” Annual Review of Medicine

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
NucleoMag® SEP	Static magnetic separator	1	744900
Square-well Block (separation plate)	96-well deep-well block with 2.5 mL square-wells, u-bottom for magnetic separation	4 / 24	740481 / .24
Elution plate U-bottom (elution plate)	96-well microplate with 300 µL u-bottom, including self adhering foil	24	740486.24