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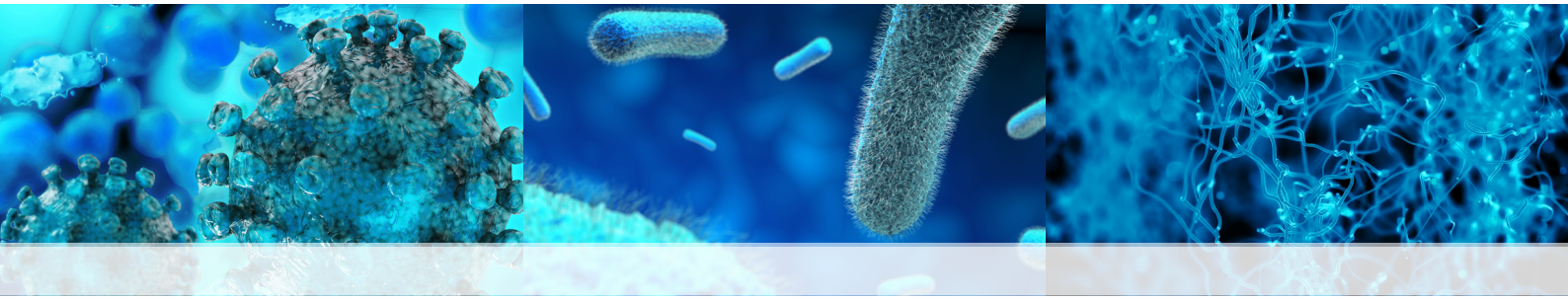
MACHEREY-NAGEL

NucleoMag® Pathogen

Automated purification of viral RNA / DNA and microbial DNA from clinical samples on the HAMILTON NIMBUS® Presto workstation



Introduction

Isolation of pathogen nucleic acids (e.g. viral RNA and DNA, bacterial DNA) from clinical samples is the basis for a large variety of molecular tests that have become standard methodology in research and diagnostic laboratories.

Due to the diversity of clinical sample material – swabs, blood, plasma, body fluids, tissue biopsies, etc. – the isolation procedure itself often poses challenges to laboratory staff and workflows. The purification process needs to be suitable for a wide variety of sample materials. In addition, the molecular diagnostic market demands extraction methods that are adaptable on automation platforms and reliable in terms of pathogen DNA detection.

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.

Together with Hamilton, MACHEREY-NAGEL has established its NucleoMag® technology on the NIMBUS Presto workstation. In this application note we demonstrate the utility and advantages of combining these technologies to fully automate your high throughput nucleic acid extractions for pathogen detection workflows.

Your advantages at a glance

- Proven NucleoMag® lysis and purification procedure suitable for diverse clinical samples
- Automated plate prefilling and plate handling by the Hamilton NIMBUS liquid handling system
- High speed nucleic acid purification by the integrated KingFisher™ Presto instrument
- Continue with downstream application without manual intervention

NIMBUS Presto Workstation	
Technology	Automated liquid handling platform (Hamilton NIMBUS) with integrated magnetic rod processing unit (KingFisher™ Presto)
Capacity	1–96 samples (≤ 200 µL sample volume)
Processable volume	50–5000 µL
Footprint	L 1359 mm | W 709 mm | H 889 mm

The NIMBUS Presto workstation combines liquid handling and magnetic rod processing for fully automated, high throughput nucleic acid extractions.

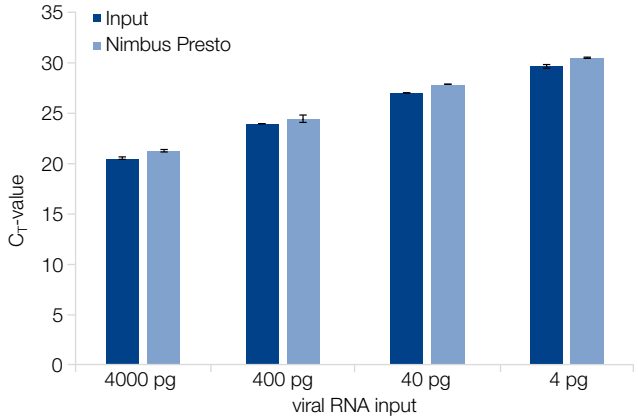
NucleoMag® Pathogen	
Technology	Magnetic beads
Sample material	≤ 200 µL whole blood, serum, plasma, ≤ 200 µL swab wash solution ≤ 25 mg tissue ≤ 200 µL feces
Elution volume	50–200 µL
Fragment size	300 bp–approx. 50 kbp
Preparation time	Approx. 70 min (excl. lysis) / 96 samples

Material and Methods

The NucleoMag® Pathogen kit is designed for common clinical sample material, such as whole blood, swabs, serum or plasma, feces, and tissue. Up to 200 µL of liquid or homogenized sample material (e.g. swab wash solution) is mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer NPL1 prior to lysis incubation. The subsequent isolation is based on reversible adsorption 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.

We demonstrate this automated purification workflow for spiked viral RNA and DNA exemplarily. The tailored protocol allows flexible processing of up to 96 samples per run.

Application data



High sensitivity detection of viral RNA recovered from liquid samples

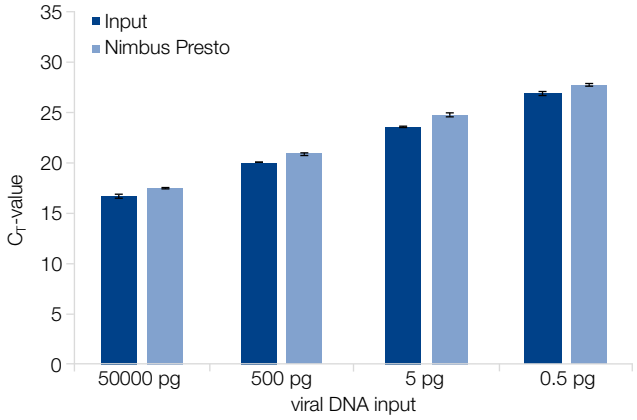
RNA was isolated from liquid samples (200 µL; n = 3 for each dilution) using the NucleoMag® Pathogen kit on the NIMBUS Presto workstation. MS2 bacteriophage RNA was spiked into a sample solution in a dilution series. The recovery rate was determined by measuring the input value in comparison to the C_T value after RNA extraction (NIMBUS Presto). 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.

	1	2	3	4	5	6	7	8	9	10	11	12
A	20.46	*	20.47	*	20.51	*	20.45	*	21.1	*	20.7	*
B	*	20.67	*	20.38	*	20.31	*	20.45	*	20.45	*	20.42
C	20.4	*	20.43	*	20.61	*	20.55	*	20.47	*	20.46	*
D	*	20.55	*	20.32	*	20.48	*	20.42	*	20.34	*	20.33
E	20.54	*	20.29	*	20.46	*	20.58	*	20.55	*	20.34	*
F	*	20.45	*	20.32	*	20.44	*	20.36	*	20.33	*	20.38
G	20.43	*	20.38	*	20.3	*	20.59	*	20.32	*	20.12	*
H	*	20.45	*	20.2	*	20.51	*	20.45	*	20.13	*	20.03

■ No sample (*C_T undetermined; negative control)
■ Sample (C_T; positive control)

Absence of cross contamination

Positive (T7 bacteriophage DNA) and negative (no DNA) control samples (200 µL each) were arranged in a checkerboard pattern on a 96-well deepwell plate and subjected to the NucleoMag® Pathogen kit procedure on the NIMBUS Presto workstation. Presence of DNA in the eluates was examined by qPCR 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. Absence of qPCR signal (C_T undetermined) in the negative control samples indicates a cross contamination free workflow.



High sensitivity detection of viral DNA recovered from liquid samples

DNA was isolated from liquid samples (200 µL; n = 3 for each dilution) using the NucleoMag® Pathogen kit on the NIMBUS Presto workstation. T7 bacteriophage DNA was spiked into a sample solution in a dilution series. The recovery rate was determined by measuring the input value in comparison to the CT value after DNA extraction (NIMBUS Presto). 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.

A rapid, fully automated solution for pathogen nucleic acid extraction from clinical samples

MACHEREY-NAGEL and Hamilton deliver a tailored solution for your high throughput viral RNA, viral DNA, and microbial DNA extraction needs from various clinical sample materials. We adapted the NucleoMag® Pathogen procedure on the NIMBUS Presto workstation to meet the expectations of the molecular diagnostic market.

Here, we demonstrate the successful use of the NucleoMag® Pathogen kit for isolation of viral RNA and DNA from liquid sample material and downstream qPCR assays.

The powerful combination of the NucleoMag® technology and the NIMBUS Presto workstation has several advantages over standard nucleic acid purification procedures:

- Save hands-on time by using automated plate-prefilling and plate-handling performed by the NIMBUS workstation
- Benefit from the high-speed extraction procedure of the integrated KingFisher™ Presto unit
- Reliable recovery and performance in downstream assays

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Pathogen	Magnetic bead-based kit for the isolation of viral RNA / DNA, and microbial DNA from clinical samples; including NucleoMag® B-Beads, buffers, Carrier RNA and Proteinase K	1 x 96 preps 4 x 96 preps	744210.1 744210.4
NIMBUS Presto	Automated liquid handling platform with 4 pipetting channels, a CO-RE gripper, barcode scanner, and many additional features		Hamilton*

NucleoMag® is a registered trademark of MACHEREY-NAGEL; Hamilton® and NIMBUS® are a registered trademarks of HAMILTON; KingFisher™ is a trademark of Thermo Fisher Scientific; SensiFast™ is a trademark of Bioline Reagents; Taqman® is a registered trademark of Roche Diagnostics

* For more detailed information, please visit www.hamiltoncompany.com/robotics. To find a Hamilton subsidiary or distributor in your area, please visit www.hamiltoncompany.com/contacts.