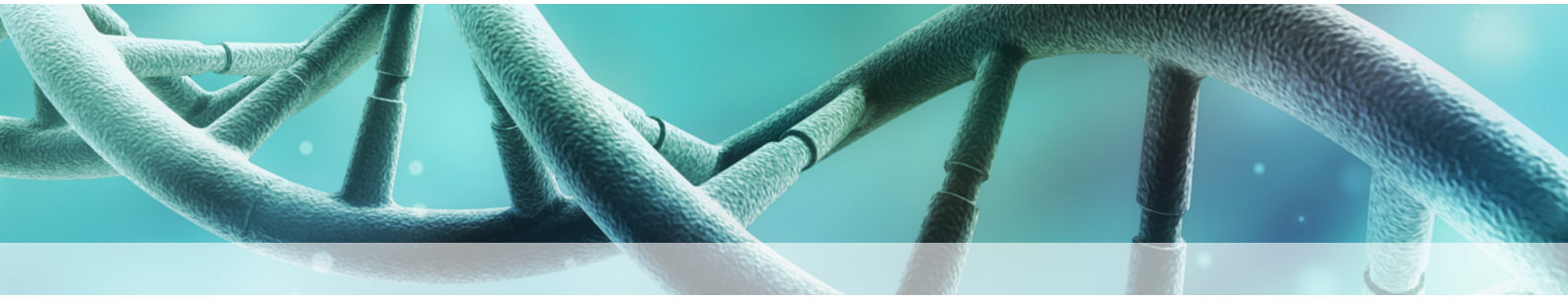
MACHEREY-NAGEL

NucleoMag® DNA FFPE

Automated purification of DNA from formalin-fixed, paraffin-embedded samples on the Hamilton NIMBUS® Presto workstation



Introduction

Clinical samples can be preserved in formaldehyde, embedded in paraffin wax, and stored at room temperature for decades without fear of decay or degradation. These pathologically interesting Formalin-Fixed, Paraffin-embedded (FFPE) samples have traditionally been examinied by histologists and immunohistochemists, but are moving further and further into the focus of molecular diagnostic researchers. However, the usage of FFPE material in genomic and NGS approaches involves several challenges associated with the limited quantity and quality of DNA and the removal of paraffin or formalin-mediated crosslinks.

To overcome these challenges, MACHEREY-NAGEL has developed the NucleoMag® DNA FFPE kit for the isolation of genomic DNA from FFPE tissue specimen. A patented odorless Paraffin Dissolver is used in this kit, replacing flammable and malodorous xylene or di-limonene commonly used for deparaffinization. The kit allows for the effective lysis in a convenient, two-phase system, followed by a decrosslinking step to ensure optimal performance in downstream applications. The purified DNA is ready for use in common downstream applications such as PCR, NGS, Blotting or various other enzymatic reactions.

Together with Hamilton, MACHEREY-NAGEL has established its NucleoMag® DNA FFPE kit on the NIMBUS Presto workstation. In this Application Note, we demonstrate the reliable genomic DNA purification from FFPE specimen in a fully automated manner for your high-throughput nucleic acid extraction workflow.

NucleoMag® DNA FFPE	
Technology	Magnetic beads
Sample material	FFPE, fixed samples, tissue sections
Sample amount	≤ 5 mg tissue, < 15 mg paraffin
Elution volume	50–200 µL
Typical DNA yield	depending on amount and quality of sample material
Average fragment size	300 bp-approx. 50 kbp
Preparation time	Approx. 70 min/96 preps (excl. lysis)

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

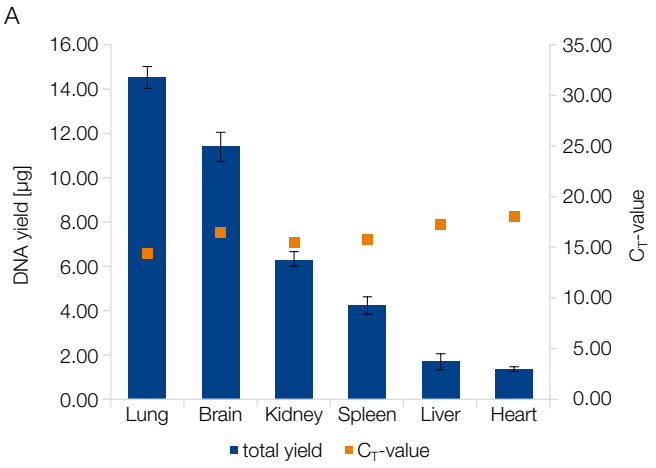
The NIMBUS Presto workstation combines liquid handling and magnetic rod processing for fully automated, high-throughput nucleic acid extractions.

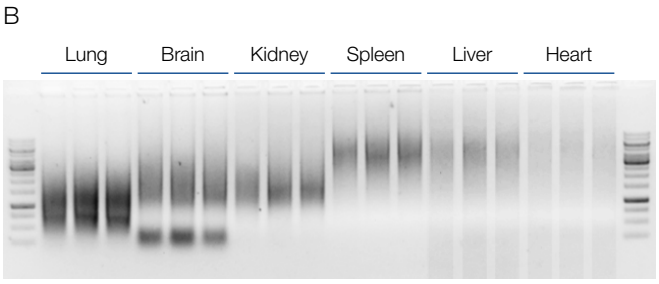
Material and Methods

In this Application Note, we demonstrate the automated purification workflow using the MACHEREY-NAGEL NucleoMag® DNA FFPE kit and the Hamilton NIMBUS Presto system. Automated genomic DNA extraction is shown exemplarily for six different mouse FFPE samples.

The paraffin of FFPE sections is dissolved using the patented Paraffin Dissolver followed by a proteinase K mediated lysis step in a two-phase system to solubilize the fixed tissue and release DNA into solution. To effectively eliminate the crosslinks originating from formalin-treatment, the released DNA is further treated with a special Decrosslinking Buffer. The lower aqueous phase containing the genomic DNA is then purified via reversible adsorption using a magnetic bead-based purification procedure on the Hamilton NIMBUS Presto system.

Application data





Automated isolation of genomic DNA from various mouse FFPE samples

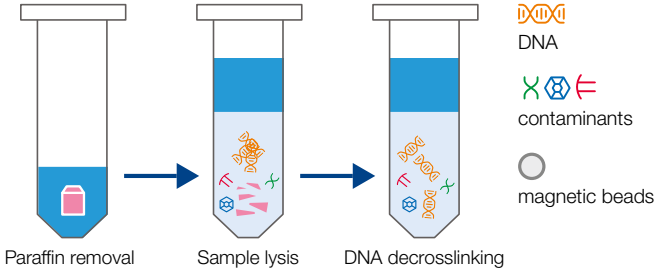
(A) DNA was isolated from various mouse FFPE samples (n = 3; < 15 mg paraffin) using the NucleoMag® DNA FFPE kit on the Hamilton NIMBUS Presto system. The total yield was determined by UV spectrometry (dark blue bars). A subsequent qPCR analysis was performed with a Taqman® Probe for a GAPDH amplicon using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System (orange squares). The results demonstrate a reliable qPCR-performance for all tested FFPE samples. (B) Purifed genomic DNA was visualized via agarose gel electrophoresis (7.5 µL per lane; 1 % TAE gel; M: GeneRuler™ 1 Kb DNA Ladder - Thermo Scientific). Yield and fragmentation of DNA largely depend on sample quality (formalin fixing protocol used) and sample-to-paraffin ratio. The size of purified DNA fragments are in range of most sequence-oriented applications.

A rapid, fully automated solution for nucleic acid isolation from FFPE samples

MACHEREY-NAGEL and Hamilton deliver a tailored solution for your high-throughput genomic DNA purification from formalin fixed, paraffin embedded samples. We have adapted the NucleoMag® DNA FFPE kit procedure on the NIMBUS Presto workstation to meet the expectations of FFPE analysis.

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

- Fully automated sample extraction after external lysis
- 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
- Patented Paraffin Dissolver
- Superior DNA decrosslinking for improved performance in typical downstream applications



The paraffin is removed from the tissue with the Paraffin Dissolver. Tissue digestion is performed in a convenient two-phase system with an effective lysis buffer. The released DNA in the lower aqueous phase is still highly crosslinked. Heat incubation in a special decrosslinking buffer is carried out to separate the DNA.

Subsequent DNA isolation is performed on the Hamilton NIMBUS Presto platform combining the advantages of the automated plate-prefilling and -handling performed by the NIMBUS workstation and the high-speed extraction procedure of the integrated KingFisher™ Presto unit.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® DNA FFPE	Magnetic bead-based kit for the isolation of genomic DNA from formalin-fixed paraffin-embedded samples	1 x 96 preps	744320.1
		4 x 96 preps	744320.1
NIMBUS® Presto	Automated liquid handling platform with 4 pipetting channels, a CO-RE Gripper, barcode scanner, and many additional features		Hamilton*

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