MACHEREY-NAGEL

NucleoMag® DNA FFPE

Automated purification of DNA from formalin-fixed, paraffin-embedded samples on ep*Motion*® 5075t

Introduction

The collection and storage of formalin-fixed, paraffin-embedded (FFPE) clinical samples is a standard procedure in hospitals worldwide and represent an invaluable resource for retrospective analyses. To breakdown and identify molecular correlations between, e.g., carcinogenesis, patient treatment, and disease outcomes, the purification of substantial amounts of high quality DNA is essential. However, there are several issues in using FFPE material for subsequent downstream molecular applications. The fixation process leads to cross-linked nucleic acids resulting in severe fragmentations. Moreover, FFPE extraction protocols often involve tedious manual steps and hazardous chemicals such as xylene.

MACHEREY-NAGEL now provides the automation ready, magnetic bead based NucleoMag® DNA FFPE extraction kit to speed up the purification process. MACHEREY-NAGEL developed an odorless Paraffin Dissolver (patented) allowing the effective lysis in a convenient two phase system, followed by a decrosslinking step to ensure optimal performance in downstream applications. The extracted DNA can be directly used as a template for PCR, NGS, blotting, or various other enzymatic reactions.

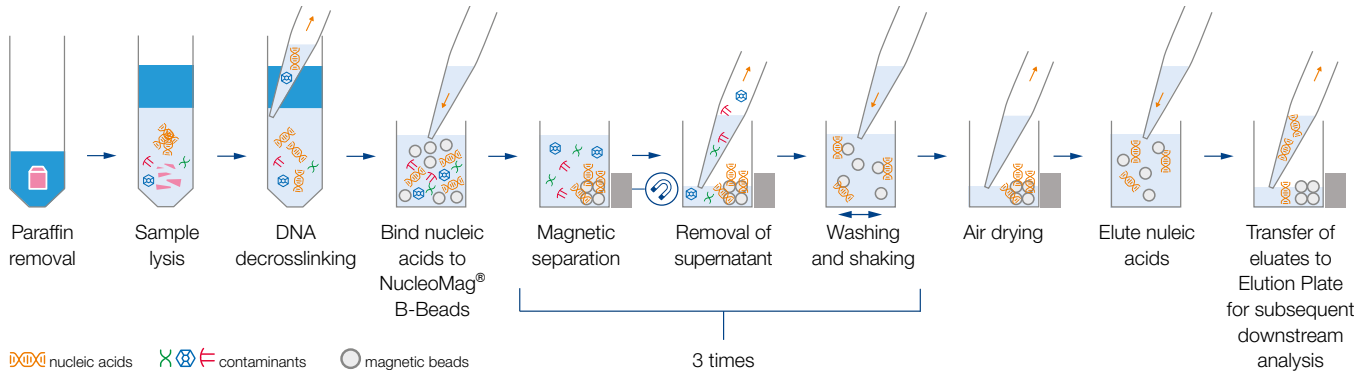
MACHEREY-NAGEL is continuously expanding on its collaborations with automation partners in order to offer more support to high throughput customers. We now present the first automation of the NucleoMag® DNA FFPE kit on the ep*Motion*® 5075t system including the time consuming deparaffinization and sample lysis procedure. The unique method eliminates a maximum of hands on time, by automating the deparaffinization, sample lysis, DNA decrosslinking and the DNA isolation. Our optimized protocol requires only one defined manual intervention, allowing the full processing of 24 samples, including deparaffinization and lysis within approximately 5 h 50 min.

Products at a glance

NucleoMag® DNA FFPE	
Technology	Magnetic beads
Sample material	≤ 5 mg tissue, < 15 mg paraffin
Preparation time	Approx. 5 h 50 min including sample preparation and extraction for 24 samples. (Note: Preparation time can be shortened.)
Typical yield	Strongly depended on sample type, quality, and amount
Elution volume	> 25 µL

Material and methods

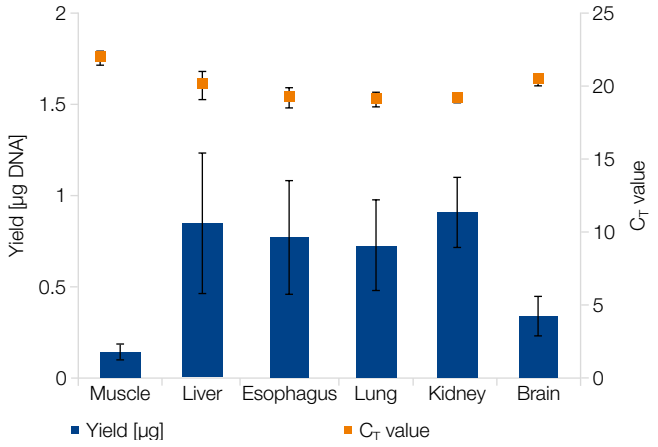
The paraffin is removed from the tissue with the Paraffin Dissolver following by sample lysis in a convenient two phase system with an effective lysis buffer. The released DNA in the lower aqueous phase is still highly crosslinked. Therefore, heat incubation in a special Decrosslinking Buffer is carried out to separate the DNA (see the NucleoMag® DNA FFPE kit protocol for more detailed information). The lower aqueous phase is transferred manually to a fresh 1.5 mL reaction tube in order to ensure a reliable DNA extraction procedure. Subsequent DNA isolation is performed on the automation platform ep*Motion*® 5075t.



Automated purification workflow of FFPE samples

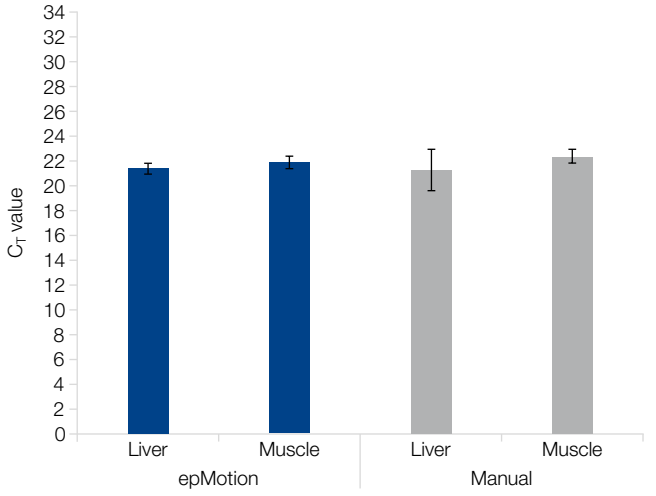
The isolation principle is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions during the binding step. Contaminants, such as salts or lipids, are then removed by three washing steps, while nucleic acids are reversible bound to the paramagnetic beads. Pure DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Application data



Automated isolation of DNA from various mouse FFPE samples

DNA was isolated from various mouse FFPE samples (n = 4; approximate section size muscle: 1 mm²; liver: 12 mm²; esophagus: 3 mm²; lung: 5 mm²; kidney: 8 mm²; brain: 4,5 mm²) using the NucleoMag® DNA FFPE kit on an ep*Motion*® 5075t system. The total yield was determined by UV spectrometry (dark blue bars). A subsequent qPCR analysis was performed with a Taqman® Probe for a GADPH 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 mouse FFPE samples.



Comparison of automated and manual processing

DNA was isolated from mouse FFPE samples (n = 4; approximate 10 mg paraffin each) using the NucleoMag® DNA FFPE kit in an automated manner on an ep*Motion*® 5075t system (dark blue bars) or manually (grey bars). A subsequent qPCR analysis was performed with a Taqman® Probe for a GADPH amplicon using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. The results demonstrate a reliable performance of the established, automated method.

Automate your FFPE sample handling

MACHEREY-NAGEL delivers a ready to go solution for your automated FFPE sample handling. We adapted the NucleoMag® DNA FFPE procedure on the ep*Motion*® 5075t system to facilitate your DNA extraction workflow.

- Automated sample preparation and sample extraction with only one manual user intervention
- Reliable performance using NucleoMag® DNA FFPE on the ep*Motion*® 5075t system
- Excellent recovery and easy handling due to paraffin removal buffer

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® DNA FFPE	Kit based on magnetic bead technology for the isolation of genomic DNA from formalin-fixed paraffin-embedded samples	1 x 96 4 x 96	744320.1 744320.4
Eppendorf ep*Motion*® 5075t	Made by order automated liquid handling system	1	5075000302 www.eppendorf.com/epMotion