

# NucleoSpin® 96 DNA Stool

Purification of DNA from human and animal stool samples using the epMotion® 5075v platform



## Introduction

Since the advent of next generation sequencing technologies, medical, veterinary and nutritional sciences have increasingly turned to genomics for meaningful answers regarding the role of gut microbiota in health and disease, often requiring automated extraction of DNA from multiple samples in parallel. Silica membrane technology has proven itself as the technology of choice for delivering high quality DNA with minimized downstream inhibition. However, DNA extraction via a silica membrane comes with its own problems, such as column clogging during vacuum processing. A related problem is the ever present danger of contamination with humic acids, that can be detrimental to downstream analysis.

MACHEREY-NAGEL has developed a 96-well kit, NucleoSpin® 96 DNA Stool, for the reliable, automation-friendly isolation of high quality DNA from human and animal stool samples. The kit combines a robust filtration step, significantly reducing the chance of column clogging, with an efficient DNA extraction procedure based on silica membrane technology.

This application note describes the automated processing of human and animal (rat) fecal samples on the epMotion® 5075v liquid handling workstation using the NucleoSpin® 96 DNA Stool kit from MACHEREY-NAGEL. The novel optimized protocol allows for fully automated processing of samples following the initial disruption step.

The processing of 96 samples takes approximately 100 minutes (excluding sample preparation).

## Product at a glance

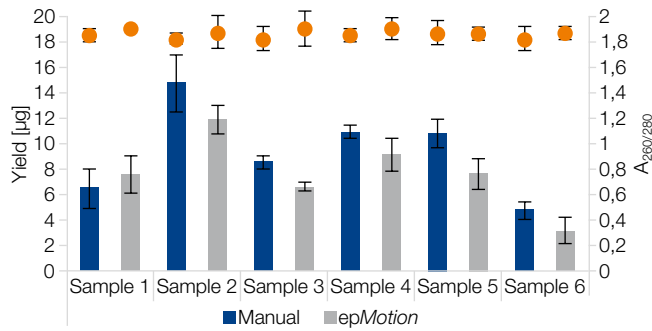
NucleoSpin® 96 DNA Stool	
Technology	Silica membrane technology
Format	96-well plate
Processing	Vacuum
Sample material	Human and animal stool samples
Starting material	< 200 mg human or animal stool
Elution volume	150 µL
Preparation time	~ 100 min for 96 samples (excluding lysis)

## Material and methods



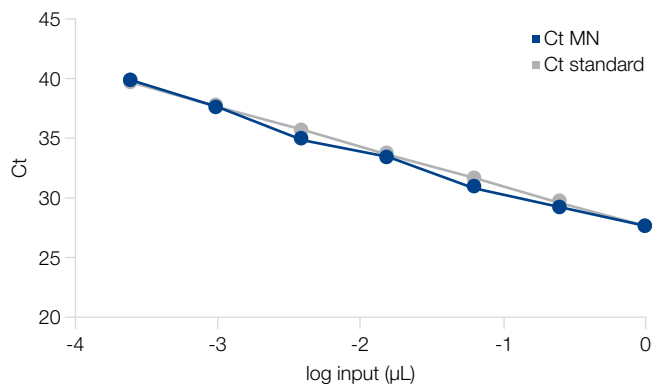
The optimized protocol is programmed to process up to 96 samples in parallel (variable sample number in multiples of 8) and developed for the epMotion® 5075v platform. Disruption of the stool sample should be performed according to the recommendations of the manual. Approximately 100–200 mg of human stool material should be transferred to an MN Bead Tube Type A (provided in the standard kit) followed by the addition of Buffer ST1. The samples are then disrupted by agitating the MN Bead Tubes (settings depend on the device, please consult manual). Following disruption and the addition of Buffer ST2 to each MN Bead Tube, the sample should be agitated again for 5 s on a Vortex-Genie® 2, followed by a 10 min incubation at 2–8 °C and a centrifugation for 3 min at 13,000 x g. Following the lysate transfer into the Square-well Block, all subsequent steps are performed on the epMotion® 5075v. Lysate clearance and DNA binding are performed by vacuum. The NucleoSpin® 96 Stool kit utilizes the NucleoSpin® Stool Filter Plate in order to achieve a reliable and robust filtration of the lysate. Following the filtration step, the DNA binds the NucleoSpin® Stool Binding Plate. Contaminants such as humic acids and salts are then removed from the silica membrane by four washing steps, and highly pure DNA is finally eluted under low ionic strength conditions in a slightly alkaline Elution Buffer SE.

## Application data



### Comparable performance both manually and on epMotion® 5075v

Six individual human stool samples were processed manually and on epMotion® 5075v in quadruplicates. Yield (blue/grey bars) and purity (A<sub>260/280</sub> nm, orange dots) were measured for all of the preparations. The manual and automated extractions delivered comparable yields with high purity (A<sub>260/280</sub> > 1.7 in all samples).



### No downstream inhibition

DNA was purified from a human stool sample on epMotion® 5075v using NucleoSpin® 96 DNA Stool. A series of 1:4 dilutions, ranging from the undiluted eluate to a 4096fold dilution was analyzed by qPCR using an *E.coli* specific target and compared against a theoretical standard in a logarithmic plot. The slope of the regression curve (-3.4106) shows an excellent qPCR-performance without PCR inhibition.

## Automate your DNA purification from stool samples

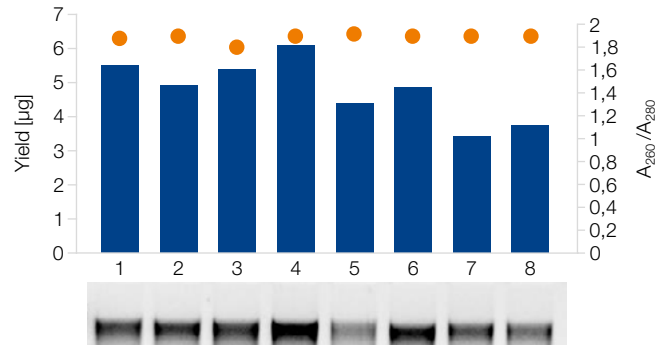
MACHERY-NAGEL and Eppendorf® deliver a fully automated solution for your high throughput DNA extraction from human and animal stool samples. We have adapted the NucleoSpin® 96 DNA Stool kit on the epMotion® 5075v to ensure a robust workflow with reliable results.

- Lysate clearing with minimal risk of clogging using the NucleoSpin® Stool Filter Plate
- Reliable performance and excellent yields using NucleoSpin® 96 DNA Stool kit on the epMotion® 5075v

## Ordering information

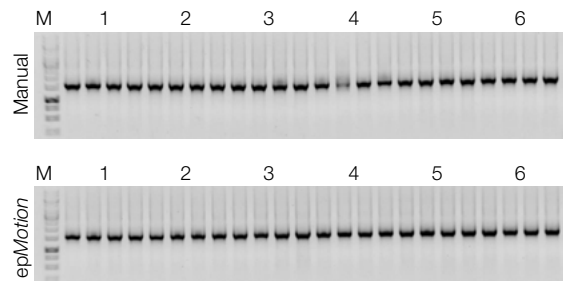
Product	Specifications	Preps	REF
NucleoSpin® 96 DNA Stool	NucleoSpin® Stool Binding Plates, MN Wash Plates, NucleoSpin® Bead Tubes, NucleoSpin® Stool Filter Plates, buffers, Square-well Blocks, Racks of Tube Strips	1 × 96/4 × 96	740473.1/4
NucleoSpin® 96 Stool (4x96) Core Kit / NucleoSpin® 96 Stool (24x96) Core Kit	NucleoSpin® Stool Binding Plates, NucleoSpin® Stool Filter Plates, buffers	4 × 96/24 × 96	740457.4/.24
MN Bead Tubes Type A (50)	2 mL tubes with ceramic beads for sample homogenization	50	740786.50
MN 96 Bead Plate Type A	Pack of 1 set consists of 1 rack, 12 strips with 8 tubes each. Tubes are pre-filled with ceramic beads. Tubes are sealed with 12 cap strips	1 × 96/4 × 96/24 × 96	740850.1/4/.24
epMotion® 5075v	epMotion® 5075v, basic device incl. vacuum system, gripper, vac frame 2, vac frame holder, epBlue™ software, waste box, 100 – 240 V ±10%/50 – 60 Hz ±5%, 0.2 µL – 1 mL		5075000303

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### High quality DNA from rat stool samples

Eight individual rat stool samples were processed on epMotion® 5075v. Yield (blue bars), purity (A<sub>260/280</sub> nm, orange dots) and analysis of DNA on the gel indicate the reliably high quality of the extracted DNA.



### Consistently excellent PCR performance

Six individual human stool samples were processed manually and on epMotion® 5075v in quadruplicates. From each eluate, 2.5 µL were used in a 25 µL endpoint PCR reaction, amplifying a 1,5 kb fragment of the bacterial 16SrRNA gene. Three µL from each PCR reaction were analyzed on a 1% agarose/TAE gel. In each case, the samples were successfully amplified, providing a good indication for the robust performance of the DNA in downstream applications.