MACHEREY-NAGEL

NucleoMag® DNA Food

Purification of DNA from diverse stool samples

Introduction

Analysis of DNA from human and animal stool samples is an increasingly relevant topic. Due to changes in modern lifestyle and especially due to the increasing popularity of the western diet, health of the human digestive tract has been in focus of medical research. Recent research has included colorectal cancer as well as the state of gut microbiota in relation to conditions such as obesity, allergies and autoimmune diseases as well as diabetes mellitus and mental health issues. In addition to human health, fecal DNA analysis enables fast and reliable identification of animal species and individuals as well as tracking their dietary habits.

Despite being interesting and potentially very rewarding sources of biological information, fecal samples pose several obstacles for DNA extraction. Most importantly, stool material is rich in humic substances which have an inhibitory effect on many downstream reactions, such as PCR and sequencing. Further, sources of DNA in a stool sample are diverse, consisting of the host organism, its food and diverse microorganisms inhabiting its digestive tract. Achieving uniform DNA extraction from all these sources and avoiding downstream inhibition can be very challenging.

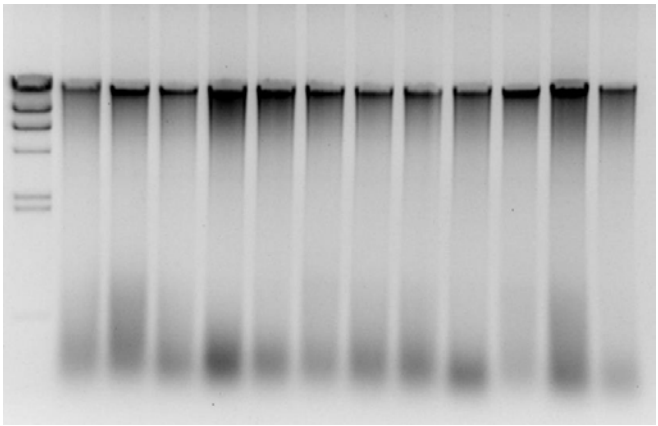
MACHEREY-NAGEL has developed the NucleoMag® DNA Food kit for DNA extraction from diverse and challenging samples. Due to its robust chemistry and extensive washing procedures, NucleoMag® DNA Food is well suited for extraction of clean DNA from contaminant rich, complex matrices such as stool.

In this note we describe a support protocol for purification of genomic DNA from stool samples using the NucleoMag® DNA Food kit. A combination of mechanical sample homogenization in ceramic bead tubes (NucleoSpin® Bead Tube Type A) and modified extraction with additional lysis buffers (Lysis Buffer T1 and buffer SL3) makes NucleoMag® DNA Food well suited for processing stool samples in particular for uniform extraction of both host genomic DNA as well as DNA from the food matrix and gut microbiota found in the sample. The extraction of DNA is followed by binding to magnetic beads and extensive washing steps, which act to remove humic substances from the beads and minimize downstream inhibition.

Material and methods

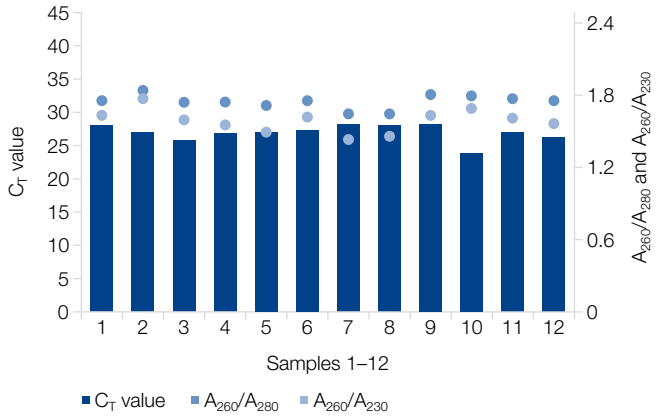
For each sample, 180–200 mg of stool material were transferred into a NucleoSpin® Bead Tube Type A. Upon addition of 850 µL of Lysis Buffer T1, each tube was closed, shaken horizontally for 2–3 seconds and subjected to a 5-minute incubation at 70°C. Following the incubation step, the bead tubes with the sample were agitated on a Vortex-Genie® 2 in the MN Bead Tube Holder for 10 min. After the agitation, the bead tubes were centrifuged for 3 min at 13.000 × *g*. From each tube 600 µL of supernatant were collected and transferred to a fresh 2 mL microcentrifuge tube. Upon addition of 100 µL of Buffer SL3, the tubes were agitated for 5 s on a Vortex-Genie® 2, incubated for 10 min on ice and subjected to another centrifugation step for 3 min at 13.000 × *g*. After centrifugation, 400 µL of clarified lysate were collected and transferred to a processing plate. From that point, the standard NucleoMag® DNA Food protocol was followed on a KingFisher™ Duo. The samples were processed as described in the manual, except for the repetition of step 5 (CQW wash) to facilitate contaminant removal.

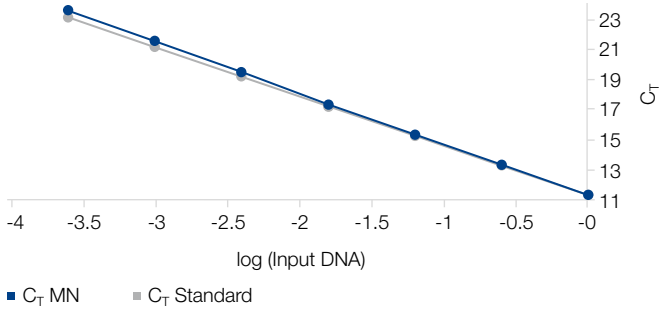
Application data



Successful extraction of good quality DNA from different stool samples

DNA was extracted from 12 different stool samples originating from individual donorus. An analysis via agarose gel electrophoresis demonstrates the consistent sharp peaks and good quality of the extracted genomic DNA. If RNA-free DNA is desired, an optional RNase treatment is possible. Please check the protocol for details.





Consistently pure DNA from different samples

Different stool samples deliver varying yields of DNA, depending on the properties of the starting material. However, consistent qPCR amplification results and purity levels (in terms of A_{260}/A_{280} and A_{260}/A_{230}) indicate high reliability of NucleoMag DNA Food for purification of DNA from fecal samples.

Minimal qPCR inhibition

Although stool samples are rich in humic substances, which exhibit strong inhibitory effects on PCR, the carryover of inhibitors during the purification procedure of NucleoMag® DNA Food is minimal, resulting in a dilution curve very similar to a theoretical curve with no qPCR inhibition.

Product at a glance

General properties of NucleoMag® DNA Food

NucleoMag® DNA Food	
Technology	Magnetic bead technology
Format	Scalable
Sample material	≤ 200 mg
Fragment size	300 bp–approx. 50 kbp
Typical yield	0.1–10 µg (depending on sample quality)
A_{260}/A_{280}	1.6–1.9
Elution volume	50–200 µL
Preparation time	40–120 min/96 preps (excl. lysis)*
Theoretical binding capacity	0.4 µg/µL beads

*Depending on instrument type, setup, and configuration

Ordering information

Product	Specifications	Preps	REF
NucleoMag® DNA Food	Kit based on magnetic bead technology for the isolation of genomic DNA from food and feed samples including NucleoMag® B-Beads, buffers, Liquid Proteinase K	1 x 96 4 x 96	744945.1 744945.4
NucleoSpin® Bead Tubes Type A	2 mL tubes with ceramic beads for sample homogenization	5 50	740786.SAMPLE 740786.50
NucleoSpin® Bead Tubes Type A 5 mL	5 mL tubes with ceramic beads for sample homogenization	8 50	740799.SAMPLE 740799.50
Buffer T1	Lysis Buffer T1	50 mL 100 mL 1000 mL	740940.25 740940.100 740940.1000
Buffer SL3	Lysis Buffer SL3	50 mL	740783.50
Buffer CQW	Wash Buffer CQW	125 mL	740313.125
MN Bead Tube Holder	Blue rubber-foam adapter for processing Bead Tubes with Vortex-Genie 2	1	740469
MN Bead Tube Holder 5 mL	Blue rubber-foam adapter for processing 5 mL Bead Tubes with Vortex-Genie 2	1	740459
NucleoMag® SEP	Magnetic separator, for use with 96-well plates	1	744900
NucleoMag® SEP Mini	Magnetic separator, for use with 12 x 1.5 mL or 2 mL reaction tubes	1	744901