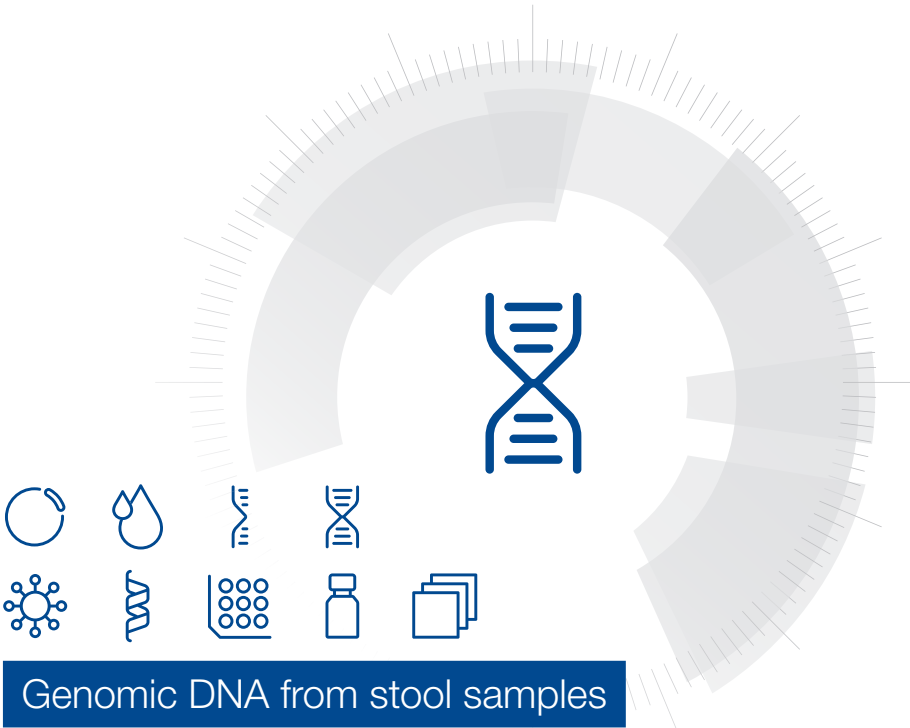


MACHEREY-NAGEL

User manual



Genomic DNA from stool samples

- NucleoSpin® 96 DNA Stool

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1 Components

1.1 Kit contents

NucleoSpin® 96 DNA Stool		
REF	1 × 96 preps 740473.1	4 × 96 preps 740473.4
Lysis Buffer ST1	125 mL	500 mL
Lysis Buffer ST2	25 mL	50 mL
Binding Buffer ST3	125 mL	500 mL
Wash Buffer ST4	75 mL	300 mL
Wash Buffer ST5 (Concentrate) ¹	50 mL	1 × 100 mL, 1 × 50 mL
Elution Buffer SE ²	30 mL	125 mL
MN Bead Tubes Type A	1 × 96	4 × 96
NucleoSpin® Stool Filter Plate (light blue rings)	1	4
NucleoSpin® Stool Binding Plate (light green rings)	1	4
MN Wash Plate ³	1	4
Square-well Block	1	4
Rack of Tube Strips ⁴	1	4
User manual	1	1

¹ For preparation of working solutions and storage conditions see section 3.

² Composition of Elution Buffer SE: 5 mM Tris/HCl, pH 8.5.

³ For use with vacuum only

⁴ Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

Kit contents continued

NucleoSpin® 96 DNA Stool Core Kit		
REF	4 × 96 preps 740457.4	24 × 96 preps 740457.24
Lysis Buffer ST1	500 mL	2 × 1000 mL, 1 × 500 mL, 1 × 125 mL
Lysis Buffer ST2	50 mL	6 × 50 mL
Binding Buffer ST3	500 mL	2 × 1000 mL, 1 × 500 mL
Wash Buffer ST4	300 mL	1 × 1000 mL, 1 × 500 mL, 1 × 150 mL
Wash Buffer ST5 (Concentrate) ¹	1 × 100 mL, 1 × 50 mL	4 × 200 mL
Elution Buffer SE ²	125 mL	1 × 500 mL, 1 × 60 mL
NucleoSpin® Stool Filter Plate (light blue rings)	4	24
NucleoSpin® Stool Binding Plate (light green rings)	4	24
User manual	1	1

¹ For preparation of working solutions and storage conditions see section 3.

² Composition of Elution Buffer SE: 5 mM Tris/HCl, pH 8.5.

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol

Consumables

- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (e.g., lab coat, gloves, goggles)
- NucleoVac 96 Vacuum Manifold (REF 740681, see ordering information, section 6.2)
- NucleoVac Vacuum Regulator (REF 740641, see ordering information, section 6.2)
- Swing mill; e.g., mixer mill MM200, MM300, MM400 (Retsch®) or comparable
Sample disruption device for lower sample numbers:
The MN Bead Tube Holder (REF 740469, see ordering information, section section 6.2) is recommended to be used in combination with the Vortex-Genie® 2 for cost-efficient and convenient disruption of stool samples.

The use of other disruption devices like FastPrep® System (MPBiomedicals), Precellys® (Bertin Technologies), MagNA™ Lyser (Roche), TissueLyser (QIAGEN), Bullet Blender® (Next Advance), Mini-Beadbeater™ (Biospec Products), Speed Mill (Analytik Jena), or similar devices might cause bead tube destruction. Such disruption devices can cause high mechanical stress on the bead tubes. Depending on bead tube type and content (beads like steel balls, liquid volume, sample type), especially high frequency of shaking and/or long shaking duration can cause destruction of the bead tubes. If using such a disruption device, it is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes during the individual experimental setup (e.g., intensity of agitation).

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® 96 DNA Stool** kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available online at www.mn-net.com.

2 Product description

2.1 The basic principle

The NucleoSpin® 96 DNA Stool kit is designed for the efficient isolation of both microbial and host genomic DNA from fresh and frozen stool samples.

The kit contains a special Lysis Buffer ST1 which, in combination with a mechanical lysis of the sample material using NucleoSpin® Beads Tubes Type A (containing ceramic beads) and a mechanical disruption device (see section 1.2), leads to a disruption of the sample material and contained microbes.

No enzymatic reactions like protease digestion are required to homogenize the sample material.

Undissolved sample material and the ceramic beads are subsequently removed by a short centrifugation. Proteins as well as PCR inhibitors present in the stool sample are precipitated by addition of Lysis Buffer ST2 and a short incubation at refrigerated temperatures, followed by an additional centrifugation step to remove all impurities.

The supernatant is finally cleared by passing it through a NucleoSpin® Stool Filter Plate that removes substances in stool samples that interfere with enzymatic reactions. Binding conditions are adjusted by addition of Binding Buffer ST3 to the flow-through of the NucleoSpin® Stool Filter Plate and the sample is loaded onto a NucleoSpin® Stool Binding Plate.

Residual contaminants such as complex polysaccharides, bile salts, and other PCR inhibitors are removed by an efficient washing procedure using Binding Buffer ST3 and Wash Buffers ST4 and ST5. After a drying step, ready-to-use DNA can be eluted with Elution Buffer SE

We can provide personalized support, protocol information, or verified scripts for numerous platforms. For more information, please contact our technical support or visit www.mn-net.com/automation

2.2 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® 96 DNA Stool
Technology	Silica-membrane technology
Format	96-well plates
Sample material	Stool samples (fresh or frozen)
Sample size	100–200 mg
Typical yield	3–15 µg (varies by sample and disruption device)
Elution volume	100–200 µL
Preparation time	90 min/48 samples
Binding capacity	50 µg
Use	For research use only

2.3 Amount of starting material

NucleoSpin® 96 DNA Stool is optimized for processing 100–200 mg of human stool. For stool samples from animals, lowering the sample amount may lead to better results.

For difficult stool samples like lipid, polysaccharide, or protein rich stool, a reduction of starting material might also improve the lysis efficiency and the purity of the DNA. It is recommended in such cases to start the extraction with 50–100 mg sample material.

Human stool samples may also contain undigested food matter (e.g., crop or fruit husks, undigested seeds). These particles should not be transferred to the MN Bead Tubes.

2.4 Sample lysis

A thorough sample lysis step is essential to achieve a high DNA yield and remove contaminants during the silica column purification procedure. As stool samples contain a complex mixture of food residues, lipids, proteins, bile salts, and polysaccharides, the chemical lysis by Buffer ST1 is supported by a mechanical disruption using NucleoSpin® Beads Tubes Type A (containing ceramic beads) and a mechanical disruption device (see recommendations for bead mills in section 1.2).

Ceramic beads have proven to be most effective in combination with a swing mill or an MN Bead Tube Holder (REF 740469) for Vortex-Genie® 2 (Scientific Industries Inc). See “User manual MN Bead Tube Holder” for handling of the MN Bead Tube Holder.

2.5 Lysate clearing and DNA binding

The lysate is cleared in two steps. In the first step, contaminants are precipitated by addition of Lysis Buffer ST2 and incubation at 2–8 °C to support the precipitation. In order to achieve an efficient temperature, transfer during this short incubation step, it is recommended to use a pre-cooled tube rack on crushed ice in a Styrofoam™ box or in a refrigerator.

A NucleoSpin® Stool Filter Plate is used for the final removal of all residual contaminants from the lysate. After addition of the Binding Buffer ST3 to the flow-through of the NucleoSpin® Stool Filter Plate, the DNA can be bound efficiently to NucleoSpin® Stool Binding Plate.

2.6 Washing procedure and membrane drying

The washing procedure performed in the NucleoSpin® 96 DNA Stool protocol is optimized to remove residual contaminating substances from the DNA bound to the silica membrane.

It starts with a washing step with Binding Buffer ST3, followed by a second washing step with Wash Buffer ST4 that also contains guanidinium salt.

The third and fourth washing steps are carried out with Wash Buffer ST5, which does not contain high salt. The Wash Buffer ST5 removes all residual contaminants as well as residual guanidinium salt.

Finally, the silica membrane is dried by applying vacuum for 15 min. The constant air stream passing the membrane leads to the evaporation of residual traces of ethanol.

2.7 Elution procedure

The recommended elution volume is 100–200 µL of Elution Buffer SE. The short incubation time after addition of the Elution Buffer SE of 1 min before applying vacuum should improve the release of DNA from the silica membrane.

If a lower volume than 100 µL is used for elution, the yield of DNA may be reduced.

2.8 Evaluation of DNA yield and quality

The most common method to determine the DNA yield is UV-VIS spectroscopy. The DNA concentration in the final eluate can be calculated from its absorption maximum at 260 nm (A_{260}). However, this calculation assumes the absence of any other compound that absorbs UV light at 260 nm. Some contaminations significantly contribute to the total absorption at 260 nm and can therefore lead to an overestimation of the actual DNA concentration.

Purity ratio A_{260}/A_{280}

The main indicator of DNA purity is the ratio A_{260}/A_{280} , which should be between 1.7 and 1.9. Values below 1.7 indicate protein contamination.

2.9 Automated processing on robotic platforms

NucleoSpin® 96 Stool DNA can be processed semi-automated on many common laboratory workstations. The bead beating lysis still has to be processed manually but the DNA clean-up can be automated using vacuum for loading, washing and elution. Please contact MN for scripts and general considerations on a specific robot.

The risk of cross-contamination is reduced by optimized vacuum settings, an improved shape of the NucleoSpin® Stool Binding Plate outlets, and application of the MN Wash Plate protecting the bottom and the binding plate outlets from lysate and wash buffer spray.

For the availability of scripts and general considerations about adapting NucleoSpin® 96 DNA Stool on a certain workstation, please contact MN. Visit MN online at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol.

3 Storage conditions and preparation of working solutions

Attention: Buffers ST3 and ST4 contain guanidinium thiocyanate and guanidine hydrochloride, respectively. Wear gloves and goggles!

Storage conditions:

All kit components should be stored at room temperature (15–25 °C) and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts. If precipitation occurs, incubate the bottle for several minutes at about 30–40 °C and mix well until the precipitate is dissolved.

Before starting the first NucleoSpin® 96 DNA Stool procedure, prepare the following:

Wash Buffer ST5: Add the indicated volume of ethanol (96–100 %) to Buffer ST5 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Buffer ST5 is stable at 15–25 °C for at least one year.

NucleoSpin® 96 DNA Stool		
REF	1 × 96 preps 740473.1	4 × 96 preps 740473.4
Buffer ST5 (Concentrate)	50 mL Add 200 mL ethanol	50 mL Add 200 mL ethanol 100 mL Add 400 mL ethanol

NucleoSpin® 96 DNA Stool Core Kit		
REF	4 × 96 preps 740457.4	24 × 96 preps 740457.24
Buffer ST5 (Concentrate)	50 mL Add 200 mL ethanol 100 mL Add 400 mL ethanol	200 mL Add 800 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® 96 DNA Stool** kits wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at www.mn-net.com/msds).



Caution: Guanidine hydrochloride in buffer ST4, and guanidinium thiocyanate in buffer ST3 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® 96 DNA Stool** kits has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements

5 Protocol for fresh or frozen stool samples

The NucleoSpin® 96 DNA Stool kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information, section 6.2). When processing less than 96 samples, Self-adhering PE Foil (see ordering information, section 6.2) should be used to close and protect unused wells to ensure proper vacuum.

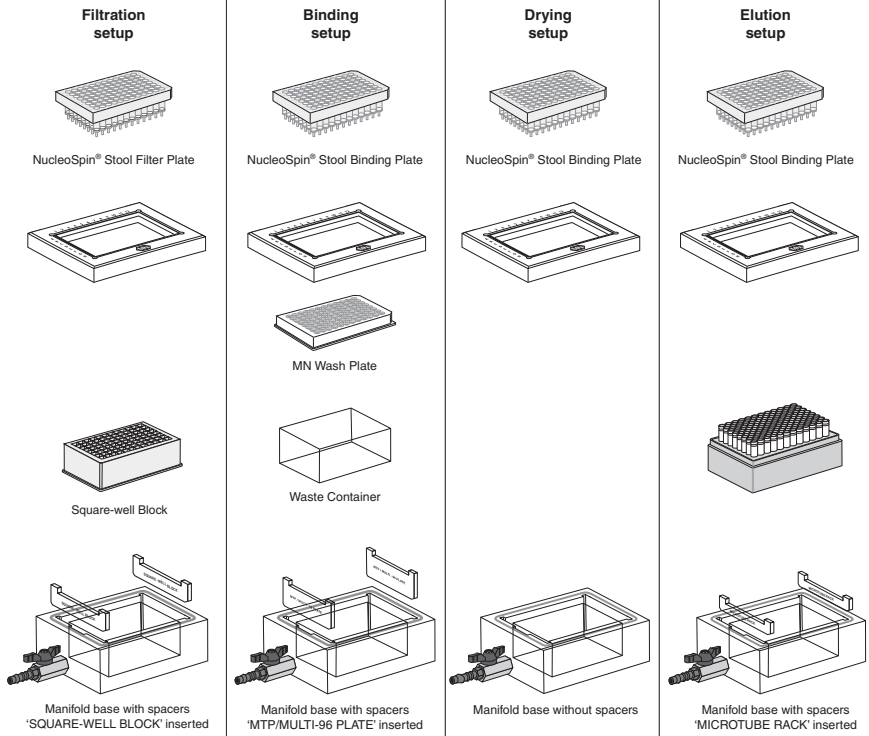
Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. It can be used in combination with a vacuum pump, house vacuum, or a water aspirator. We recommend a vacuum of -0.4 to -0.6 bar (reduction of atmospheric pressure) that can be adjusted by the NucleoVac Vacuum Regulator (see ordering information, section 6.2).

The risk of cross-contamination is reduced by optimized vacuum settings, an improved shape of the NucleoSpin® Stool Binding Plate outlets, and application of the MN Wash Plate protecting the bottom and the binding plate outlets from lysate and wash buffer spray.

Before starting the preparation:

- Check Lysis Buffer ST1 for precipitates. Dissolve any precipitate by incubating the buffer at 30–40 °C for 10 min and shaking the bottle every 2 min.
- Put a tube rack in a Styrofoam™ box with crushed ice or in a refrigerator for the precipitation of contaminants at 2–8 °C.
- *It is recommended to wear lab coat, goggles and gloves throughout the whole procedure.*

Setup of vacuum manifold



Protocol-at-a-glance

1	Prepare sample	100–200 mg sample to MN Bead Tube Type A 950 µL ST1
2	Lyse sample	Mechanically homogenize
3	Precipitate contaminants	100 µL Buffer ST2 Vortex 5 s 10 min at 2–8 °C 13,000 x g, 3 min
4	Filter lysate	Assemble filtration setup Load samples -0.6 bar
5	Adjust binding conditions	250 µL ST3 Mix
6	Bind DNA	Assemble binding setup Load samples -0.4 to -0.6 bar
7	Wash silica membrane	600 µL ST3 -0.4 to -0.6 bar 600 µL ST4 -0.4 to -0.6 bar 700 µL ST5 -0.4 to -0.6 bar 700 µL ST5 -0.4 to -0.6 bar
8	Dry silica membrane	Assemble drying setup Full vacuum 15 min
9	Elute viral RNA and DNA	Assemble elution setup 100–200 µL SE 1 min -0.4 to -0.6 bar

Detailed protocol

1 Prepare sample

Transfer **100 – 200 mg** of human **stool material** to a **MN Bead Tube Type A**.

Important: Do not overload the bead tube as this may lead to reduced yield and purity.

Add **950 µL Buffer ST1**.

Note: For animal stool samples it may be advantageous to use less starting material.

Close the MN Bead Tube.

2 Lyse sample

Different disruption methods can be used to homogenize the sample material. See section 2.4 for more information about recommendations for different disruption devices.

Agitate the MN Bead Tube in the Retsch® swing mill MM300 for **5 min at 30 Hz**.

Alternatively, other disruption devices can be used (see section 1.2).

Note: If another disruption device is used, optimal agitation time and frequency may differ from the parameters defined for the Retsch® MM300 swing mill.

MN Bead tubes can also be agitated on a MN Bead Tube Holder mounted on a Vortex-Genie® 2. Vortex the samples at full speed and room temperature for 10 min.

Centrifuge for **3 min at 13,000 x g**.

3 Precipitate contaminants

Add **100 µL Buffer ST2** to the MN Bead Tube Type A , close the lid and vortex for **5 s**.

Incubate for **10 min at 2–8 °C**.

Centrifuge for **3 min at 13,000 x g**.

4 Filter lysate

Assemble filtration setup: Insert spacers labeled /SQARE-WELL BLOCK/ with the notched side up. Place a MN Square-well Block onto the spacers.

Close the manifold with the manifold lid and place the NucleoSpin® Stool Filter Plate on top of the lid (see Filtration setup, page 14).

Seal unused wells using self adhering PE Foil (see ordering information, section 6.2).

Load **650 µL of clear supernatant** from step 3 into each well of the plate. Apply vacuum (-0.6 bar) until all liquid has passed the plate. Switch off vacuum and ventilate the manifold.

Discard NucleoSpin® Stool Filter Plate.

5 Adjust binding conditions

Add **250 µL Buffer ST3** to the flow-through in each well of the Square-well Block.

Mix by pipetting up and down. Adjust the pipette to at least 700 µL to ensure efficient mixing.

6 Bind DNA

Assemble binding setup: Place the waste container into vacuum manifold base. Insert spacers labeled /MTP/MULTI-96 PLATE/ with the notched side up. Place the MN Wash Plate onto the spacers. Close the manifold with the manifold lid and place the NucleoSpin® Stool Binding Plate on top of the lid (see Binding setup, page 14).

Seal unused wells using self adhering PE Foil (see ordering information, section 6.2).

Load **binding mixtures** from step 5 onto the binding plate.

Apply vacuum (-0.4 to -0.6 bar) until all liquid has passed the plate. Switch off vacuum and ventilate the manifold.

7 Wash silica membrane

1st wash

Load **600 µL Buffer ST3**.

Apply vacuum (-0.4 to -0.6 bar) until all liquid has passed the plate. Switch off vacuum and ventilate the manifold.

2nd wash

Load **600 µL Buffer ST4**.

Apply vacuum (-0.4 to -0.6 bar) until all liquid has passed the plate. Switch off vacuum and ventilate the manifold.

3rd wash

Load **700 µL Buffer ST5**.

Apply vacuum (-0.4 to -0.6 bar) until all liquid has passed the plate. Switch off vacuum and ventilate the manifold.

4th wash

Load **700 µL Buffer ST5**.

Apply vacuum (-0.4 to -0.6 bar) until all liquid has passed the plate. Switch off vacuum and ventilate the manifold.

8 Dry silica membrane

Assemble drying setup: Put NucleoSpin® Stool Binding Plate on a clean paper towel to remove residual wash buffer from plate outlets. Discard MN Wash Plate and remove waste container from vacuum manifold. Close the manifold with the manifold lid and place the NucleoSpin® Stool Binding Plate back on top of the lid (see Drying setup, page 14).

Apply maximum vacuum for 15 min to dry membrane and to eliminate last traces of ethanol.

Note: Ethanol in Buffer ST5 inhibits enzymatic reactions and has to be removed completely before eluting the DNA.

9 Elute DNA

Assemble elution setup: Insert spacers/MICROTUBE RACK/into the vacuum manifold base. Place the Rack of Tube Strips onto the spacers. Close the manifold with the manifold lid and place the NucleoSpin® Stool Binding Plate back on top of the lid (see Elution Setup on page 14).

Load **100–200 µL Buffer SE** directly into the center of the silica membrane of each well.

Incubate for 1 min.

Apply vacuum (-0.4 to -0.6 bar) until all liquid has passed the plate.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no DNA yield	<i>Suboptimal lysis conditions</i> <ul style="list-style-type: none">• Too much sample material was filled into the MN Bead Tube. Too little head space does not allow the necessary motion of the beads to disrupt the sample and concentration of the lysis solution may be too low to chemically disrupt the sample material. Use less sample material.
	<i>Insufficient disruption and/or homogenization of starting material</i> <ul style="list-style-type: none">• Shaking of the MN Bead Tube was too weak or not long enough. Increase shaking time and velocity or use another shaking device (see section 2.4 for more information).
	<i>Reagents not applied or stored properly</i> <ul style="list-style-type: none">• Always dispense exactly the buffer volumes given in the protocol!• Always follow closely the given instructions with regard to order and mode of mixing (shaking, vortexing etc.).• Add the indicated volume of ethanol (96–100%) to Wash Buffer ST5 Concentrate and mix thoroughly (see section 3 for more information).• Store kit components at room temperature. Storage at lower temperatures may cause salt precipitation. Check Lysis Buffer ST1 for white precipitate. If precipitation occurred, incubate the bottle at 40–50 °C until all precipitate is dissolved.• Keep bottles tightly closed in order to prevent evaporation or contamination.
	<i>Sample material not stored properly</i> <ul style="list-style-type: none">• Stool samples should be kept at 2–8 °C after collection. If the DNA is not extracted from the stool sample within the same day, it should be frozen at -20 °C as soon as possible after collection and kept at -20 °C until processing. Stool samples should be thawed at room temperature immediately before extraction or overnight at 2–8 °C.

Problem	Possible cause and suggestions
Suboptimal performance of DNA in downstream experiments	<i>DNA yield was overestimated</i>
	<ul style="list-style-type: none"> If DNA eluates are not completely free of contaminants (e.g., proteins) UV-VIS quantification based on A_{260} is not reliable due to the contribution of the contaminants to the absorption at 260 nm.
	<i>Carryover of ethanol or salt</i>
	<ul style="list-style-type: none"> Make sure to dry the silica membrane and the NucleoSpin® Stool DNA Plate completely before elution to avoid carry-over of ethanol containing Wash Buffer ST5. Check if Buffer ST5 has been equilibrated to room temperature before use. Washing at lower temperatures decreases the efficiency of salt removal.
	<i>Contamination with PCR inhibitors</i>
	<ul style="list-style-type: none"> The DNA purity can be increased by lowering the amount of starting material (see section 2.3 for more information.) Make sure to carefully follow the washing instructions. Dilute DNA 1:10 to reduce concentration of inhibitors.

Visit www.mn-net.com for more detailed product information.

6.2 Ordering information

Product	REF	Pack of
MN Bead Tube Holder	740469	1
NucleoSpin® DNA Stool	740472.10 / .50	10 / 50 preps
NucleoSpin® DNA Insect	740470.10 / .50	10 / 50 preps
NucleoSpin® Microbial DNA	740235.10 / .50	10 / 50 preps
NucleoSpin® RNA Stool	740130.10 / .50	10 / 50 preps
MN Bead Tubes Type A (0.6–0.8 mm ceramic beads; recommended for stool, soil, and sediments)	740786.50	50
MN 96 Bead Plate Type A (0.6–0.8 mm ceramic beads; recommended for stool, soil, and sediments)	740850.1 / .4 / .24	1 / 4 / 24

Product	REF	Pack of
MN Bead Tubes Type B (40–400 µm glass beads; recommended for bacteria)	740812.50	50
MN Bead Tubes Type C (1–3 mm corundum; recommended for yeast)	740813.50	50
MN Bead Tubes Type D (3 mm steel balls; recommended for insects)	740814.50	50
MN Bead Tubes Type E (40–400 µm glass beads and 3 mm steel balls; recommended for hard-to-lyse bacteria within insect or tissue samples)	740815.50	50
MN Bead Tubes Type F (1–3 mm corundum + 3 mm steel balls, recommended for challenging tissues in combination with the NucleoSpin® RapidLyse Kit) - use only with MN Bead Tube Holder	740816.50	50
MN Bead Tubes Type G (5 mm steel balls, recommended for plant material)	740817.50	50
MN Square-well Block	740476 740476.24	4 24
Square-well Block	740481	4
MN Wash Plate	740479 740479.24	4 24
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 24
Cap Strips	740478 740478.24	48 288
Self-adhering PE Foil	740676	50
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the customer and are not a part of a contract of sale or of this warranty.

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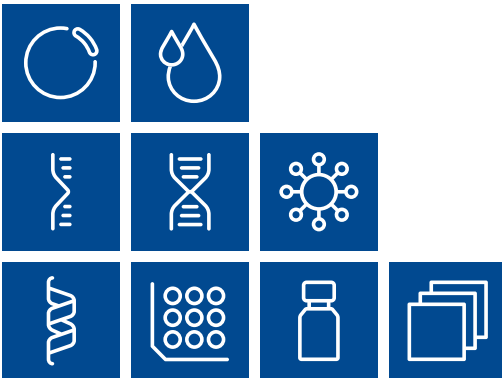
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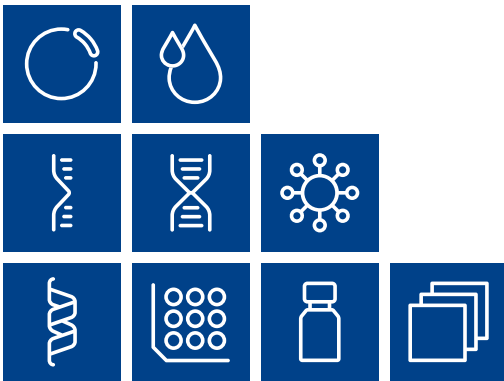
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