Genomic DNA from stool samples

User manual

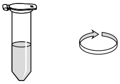
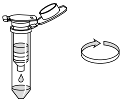
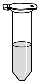
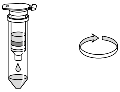
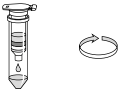
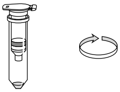
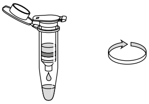
NucleoSpin® DNA Stool

April 2016 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

Genomic DNA from stool samples

Protocol-at-a-glance (Rev. 01)

		NucleoSpin® DNA Stool
1 Prepare sample		NucleoSpin® Bead Tube Type A 180–220 mg sample material 850 µL ST1, shake horizontally 2–3 s
2 Lyse sample		70 °C, 5 min Vortex 10 min at RT using MN Bead Tube Holder on Vortex-Genie® 2 at max. speed
3 Precipitate contaminants		13,000 x *g*, 3 min Transfer 600 µL supernatant 100 µL ST2 Vortex 5 s 5 min, 2–8 °C 13,000 x *g*, 3 min
4 Filter lysate		Transfer 550 µL cleared lysate on NucleoSpin® Inhibitor Removal Column 13,000 x *g*, 1 min
5 Adjust binding conditions		200 µL ST3 Vortex 5 s
6 Bind DNA		Load 700 µL sample on NucleoSpin® DNA Stool Column 13,000 x *g*, 1 min
7 Wash silica membrane		**1st** 600 µL ST3 13,000 x *g*, 1 min **2nd** 550 µL ST4 13,000 x *g*, 1 min **3rd** 700 µL ST5 Vortex 2 s 13,000 x *g*, 1 min **4th** 700 µL ST5 13,000 x *g*, 1 min
8 Dry silica membrane		13,000 x *g*, 2 min
9 Elute DNA		30–100 µL SE 13,000 x *g*, 1 min Vortex 2 s

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1 Components

1.1 Kit contents

	NucleoSpin® DNA Stool	
REF	**10 preps** **740472.10**	**50 preps** **740472.50**
Lysis Buffer ST1	20 mL	50 mL
Lysis Buffer ST2	10 mL	10 mL
Binding Buffer ST3	10 mL	60 mL
Wash Buffer ST4	6 mL	30 mL
Wash Buffer ST5 (Concentrate)*	6 mL	25 mL
Elution Buffer SE**	13 mL	13 mL
NucleoSpin® Bead Tubes Type A	10	50
NucleoSpin® Inhibitor Removal Columns (red rings)	10	50
NucleoSpin® DNA Stool Columns (green rings)	10	50
Collection Tubes (2 mL)	10	50
Collection Tubes (2 mL, lid)	10	50
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* For preparation of working solutions and storage conditions see section 3.

** 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

- 1.5 mL microcentrifuge tubes with lid
- 2.0 mL microcentrifuge tubes with lid
- Additional Collection Tubes (2 mL) (optional)
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (e.g., lab coat, gloves, goggles)
- Sample disruption device:
  The MN Bead Tube Holder (REF 740469, see ordering information, 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 Vortex Adapter (MoBio) for Vortex-Genie® 2 X is also suitable.

Alternatively, a swing mill can be used (e.g., mixer mill MM200, MM300, MM400 (Retsch®).

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® 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® 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 5 minute heating step, leads to a chemical disruption of membranes prior to 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).

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® Inhibitor Removal Column that completely 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® Inhibitor Removal Column and the sample is loaded onto a NucleoSpin® DNA Stool Column.

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.

2.2 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® DNA Stool
Technology	Silica-membrane technology
Format	Mini spin column
Sample material	Stool samples (fresh or frozen)
Sample size	Approx. 200 mg (180–220 mg)*
Typical yield	2–10 µg (varies by sample and disruption device)
Elution volume	30–100 µL
Preparation time	60 min/10 preps
Binding capacity	50 µg

2.3 Amount of starting material

NucleoSpin® DNA Stool is optimized for processing 200 mg (180–220 mg) of human stool. For stool samples from animals, lowering the sample amount may lead to better results.

Very dry stool samples like rabbit or mouse feces may absorb lysis buffer, resulting in an insufficient sample volume after the first centrifugation step. In these cases it is recommended to reduce the amount of stool material to e.g., 60–80 mg and to increase the total lysis volume to 1 mL. A one to one mixture of Buffer ST1 and nuclease free water is recommended for these stool samples (see also section 2.4 for detailed information about input material and lysis conditions).

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 60–80 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 NucleoSpin® Bead Tubes.

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* For human stool samples approx. 200 mg should be used. For animal stool samples – depending on the species – a lower amount of sample material may be required for optimal results.

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 heating step at 70 °C for 5 minutes. This heating step improves lysis and solubilization of the stool compounds. It is necessary to shake each sample horizontally for 2–3 seconds after addition of Buffer ST1 before placing it in the heat incubator in order to mix the stool material and the buffer (take two NucleoSpin® Bead Tubes between thumb and index finger and shake vigorously for 2–3 s).

For some animal stool samples, e.g., feces from herbivores like rabbit and sheep, the heat incubation step at 70 °C can be omitted. It is sufficient to perform the bead-beating lysis step with such stool samples only.

The subsequent homogenization step in the NucleoSpin® Bead Tube completely dissolves the stool sample in the lysis buffer and breaks up host and microbial cells. Even solid stool samples such as dried mouse droppings will be suspended after 10 minutes shaking on a Vortex or on a bead mill like Retsch® 300 MM for 30–60 seconds at a frequency of 30/s (the best condition for the mechanical lysis on different disruption devices has to be adjusted). Ceramic beads have proven to be most effective in combination with 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.

Please refer to the following recommendations to achieve optimal lysis conditions:

Table 2: Recommended sample input and lysis conditions

Stool sample	**Starting amount**	**Buffer volumes**	**Heat incubation**
Omnivore and carnivore, e.g., human or feline (Medium to elevated water content, sometimes viscous)	180–220 mg	850 µL ST1	Yes
Herbivore, e.g., sheep or rabbit (Medium to low water content, fiber rich)	60–80 mg	500 µL ST1 plus 500 µL water*	No
Very hard and dry stool, e.g., dried mouse feces (Very low water content)	60–70 mg	500 µL ST1 plus 500 µL water*	Yes

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* Use nuclease free water to dilute Buffer ST1.

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® Inhibitor Removal Column 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® Inhibitor Removal Column, the DNA can be bound efficiently to the NucleoSpin® DNA Stool Column.

2.6 Washing procedure

The washing procedure performed in the NucleoSpin® 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 short vortex step in the protocol after the first addition of Buffer ST5 to the NucleoSpin® DNA Stool Column aims to remove all potential guanidinium salt residues from the inside of the column body and the column lid. As guanidinium salt absorbs at 230 nm, this vortex step also helps to improve the A_{260}/A_{230} ratio. The second washing step with Buffer ST5 can be carried out without prior vortexing of the spin column.

2.7 Elution procedures

It is possible to adapt the volume of elution buffer used for the subsequent application of interest. In addition to the standard method, an increase of DNA concentration can be achieved by reducing the elution volume from 100 µL to 30 µL.

If a lower volume than 100 µL is used for elution, it is important to pipette the elution buffer onto the center of the NucleoSpin® DNA Stool Column in order to moisten the silica membrane completely.

Incubating the NucleoSpin® DNA Stool Column for 1 minute at room temperature after applying the elution buffer may also improve the elution efficiency when using less volume than 100 µL.

If 30 µL are used for elution, the yield can be improved by loading the elution buffer twice onto the spin column. After the first elution step, pipette the 30 µL elution buffer once again from the elution tube onto the membrane of the NucleoSpin® DNA Stool Column and centrifuge again for 1 minute at 13,000 x *g*.

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.

Purity ratio A_{260}/A_{230}

Another indicator of DNA purity is the ratio of the absorption at 260 nm and 230 nm. A_{260}/A_{230} should be higher than 2.0 for pure DNA and can be accepted down to about 1.5. Ratios around or even below 1.0 indicate impurities in the DNA eluate, which could be of different nature as several compounds absorb at these wavelengths.

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 (18–25 °C) and are stable for at least one year. 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® 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 room temperature (18–25 °C) for at least one year.

	NucleoSpin® DNA Stool	
REF	**10 preps** **740472.10**	**50 preps** **740472.50**
Buffer ST5 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® DNA Stool** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**GHS symbol** ***GHS-Symbol***	**Hazard phrases** ***H-Sätze***	**Precaution phrases** ***P-Sätze***
ST3	Guanidinium thiocyanate 30–60 % *Guanidinthiocyanat 30–60 %* CAS 593-84-0	 WARNING *ACHTUNG*	302, 412, EUH031	260, 273, 301+312, 330
ST4	Guanidine hydrochloride 36–50 % and 2-propanol 20–50 % *Guanidinhydrochlorid 36–50 % und 2-Propanol 20–50 %* CAS 50-01-1, 67-63-0	 WARNING *ACHTUNG*	226, 302, 319, 336	210, 233, 264, 280, 301+312, 305+351+338, 330, 337+313, 370+378, 403+235

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H 336	May cause drowsiness or dizziness. *Kann Schläfrigkeit und Benommenheit verursachen.*
H 412	Harmful in contact with skin. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH 031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*

Precaution phrases

P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. *Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
P 233	Keep container tightly closed. *Behälter dicht verschlossen halten.*

P 260	Do not breathe dust / fume / gas / mist / vapours / spray. *Staub / Rauch / Gas / Nebel / Dampf / Aerosol nicht einatmen.*
P 264	Wash … thoroughly after handling. *Nach Handhabung … gründlich waschen.*
P 273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P 280	Wear protective gloves / protective clothing / eye protection / face protection. *Schutzhandschuhe / Schutzkleidung / Augenschutz / Gesichtsschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER / doctor / … / if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt / … anrufen.*
P 305+351+338	IF IN EYES: Rinse cautiously with water for several minuts. Remove contact lenses, if present and easy to do. Continue rinsing. *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 337+313	If eye irritation persists: Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.*
P 370+378	In case of fire: Use … to extinguish. *Bei Brand: … zum Löschen verwenden.*
P 403+235	Store in a well-ventilated place. Keep cool. *An einem gut belüfteten Ort aufbewahren. Kühl halten.*

⚠ The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

For further information please see Material Safety Data Sheets (***www.mn-net.com***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**www.mn-net.com**).*

5 Protocol for fresh or frozen stool samples

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.
- Adjust a heating block to 70 °C for the initial heat incubation step.
- 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.

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1 Prepare sample

See sections 2.3 *and* 2.4 *for more information about the amount of starting material and the recommended lysis procedure for stool samples from different species.*

Transfer **180–220 mg** of human **stool material** to a **NucleoSpin® Bead Tube Type A**.

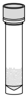
<u>Important:</u> Do not overload the bead tube as this may lead to reduced yield and purity. It is recommended to use an appropriate balance to portion the sample material.

Add **850 µL Buffer ST1**.

<u>Note:</u> For very dry or fiber rich animal stool samples it is recommended to increase the total lysis volume to 1 mL by adding 0.5 mL ST1 Buffer and 0.5 mL nuclease-free water to the sample, as the stool material will take up part of the lysis buffer volume.

Close the NucleoSpin® Bead Tube and **shake** horizontally for **2–3 seconds** to mix stool sample and lysis buffer before putting it onto a heat incubator.

<u>Note:</u> Take two NucleoSpin® Bead Tubes between thumb and index finger and shake vigorously for 2–3 seconds to mix stool sample and lysis buffer.



180–220 mg sample

+ 850 µL ST1

Shake horizontally 2–3 s

2 Lyse sample

See section 2.4 for more information about recommended lysis and homogenization conditions for different sample materials.

70 °C, 5 min

Incubate NucleoSpin® Bead Tubes for **5 min** at **70 °C**.

<u>Note:</u> For some animal stool samples that contain mostly fibers, e.g., feces from herbivores such as rabbit and sheep, the heat incubation step at 70 °C can be omitted. It is sufficient to perform the bead-beating lysis step (see section 2.4 for more information).

Agitate
RT, 10 min

Agitate the NucleoSpin® Bead Tube in the MN Bead Tube Holder on a Vortex-Genie® 2. Vortex the samples at full speed and room temperature (18–25 °C) for 10 min.

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

3 Precipitate contaminants

13,000 x *g*,
3 min

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

Transfer
600 µL
supernant

Transfer **600 µL** of the supernatant to a fresh **2 mL microcentrifuge tube** with lid (not provided).

<u>Note:</u> If less volume is available, transfer as much lysate as possible to the 2 mL microcentrifuge tube. Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

+ 100 µL ST2

Vortex 5 s

2–8 °C, 5 min

13,000 x *g*,
3 min

Add **100 µL Buffer ST2**, close the lid and vortex for **5 s**.

Incubate for **5 min** at **2–8 °C**.

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

4 Filter lysate

Place a **NucleoSpin® Inhibitor Removal Column** (red ring) in a Collection Tube (2 mL, lid).

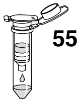
Avoiding the pellet, transfer **550 µL** of the **cleared lysate** onto the NucleoSpin® Inhibitor Removal Column.

Note: If less volume is available, transfer as much cleared lysate as possible to the filter column. Avoid transferring material from the pellet or material, which floats on top of the lysate onto the column.

Centrifuge for **1 min** at **13,000 x *g***.

Discard the NucleoSpin® Inhibitor Removal Column.

Note: If a pellet is visible in the flow-through, transfer the clear supernatant to a new 2 mL microcentrifuge tube (not provided).



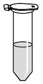
Transfer 550 µL cleared lysate

13,000 x *g*, 1 min

5 Adjust binding conditions

Add **200 µL Buffer ST3** and close the lid.

Vortex for **5 s**



+ 200 µL ST3

Vortex 5 s

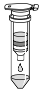
6 Bind DNA

Place a **NucleoSpin® DNA Stool Column** (green ring) in a Collection Tube (2 mL).

Load 700 µL sample onto the column.

Centrifuge for **1 min** at **13,000 x *g***.

Discard flow-through and place the column back into the collection tube.



Load 700 µL sample

13,000 x *g*, 1 min

7 Wash silica membrane

1st wash

Add **600 µL Buffer ST3** to the NucleoSpin® DNA Stool Column.

Centrifuge for **1 min at 13,000 x *g***.

Discard flow-through and place the column back into the collection tube.

+ 600 µL ST3

13,000 x *g*, 1 min

2nd wash

Add **550 µL Buffer ST4** to the NucleoSpin® DNA Stool Column.

Centrifuge for **1 min at 13,000 x *g***.

Discard flow-through and place the column back into the collection tube.

+ 550 µL ST4

13,000 x *g*, 1 min

3rd wash

Add **700 µL Buffer ST5** to the NucleoSpin® DNA Stool Column.

Close the lid and **vortex** for **2 s**. Centrifuge for **1 min** at **13,000 x *g***.

Discard flow-through and place the column back into the collection tube.

+ 700 µL ST5

Vortex 2 s

13,000 x *g*, 1 min

4th wash

Add **700 µL Buffer ST5** to the NucleoSpin® DNA Stool Column.

Centrifuge for **1 min** at **13,000 x *g***.

Discard flow-through and place the column back into the collection tube.

Note: The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If new collection tubes are used for each step, see section 6.2 for ordering information.

+ 700 µL ST5

13,000 x *g*, 1 min

8 Dry silica membrane

Centrifuge for **2 min** at **13,000 x *g***.

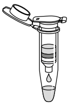
Note: If for any reason, the liquid in the collection tube has touched the NucleoSpin® DNA Stool Column after the drying step, discard flow-through and centrifuge again.

13,000 x *g*, 2 min

9 Elute DNA

Place the **NucleoSpin® DNA Stool Column** into a new 1.5 mL microcentrifuge tube (not provided).

Add **30 µL** (for high concentration), **50 µL** (for medium concentration and yield), or **100 µL** (for high yield) **Buffer SE** to the column.



30–100 µL SE

Note: If a lower volume is used for elution, yield may be improved by following the recommendations in section 2.7.

Close the lid and centrifuge for **1 min** at **13,000 x *g***.

Discard the NucleoSpin® DNA Stool Column.

Vortex each microcentrifuge tube for **2 s**.

13,000 x *g*, 1 min

Vortex 2 s

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no DNA yield	*Suboptimal lysis conditions* • Too much sample material was filled into the NucleoSpin® Bead Tube. Too little head space does not allow the necessary motion of the beads to disrupt the sample. Use less sample material (see section 2.3 and 2.4 for more information).
	Insufficient disruption and/or homogenization of starting material • Shaking of the NucleoSpin® 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).
	Reagents not applied or stored properly • 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 (18–25 °C). Storage at lower temperatures may cause salt precipitation. Check Lysis Buffer ST1 for white precipitate. If precipitation occurred, incubate the bottle for 10 min at 30–40 °C and shake every 2 minutes until all precipitate is dissolved. • Keep bottles tightly closed in order to prevent evaporation or contamination.
	Sample material not stored properly • 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 until processing. Stool samples should be thawed at room temperature (18–25 °C) immediately before extraction or over night in a Styrofoam™ box with crushed ice.

Problem	**Possible cause and suggestions**
DNA is degraded	*Too harsh mechanical sample disruption* • Reduce intensity or incubation time of mechanical sample lysis.
Suboptimal performance of DNA in downstream experiments	*DNA yield was overestimated* • If DNA eluates are not completely free of contaminants, UV-VIS quantification based on A_{260} is not reliable due to the contribution of the contaminants to the absorption at 260 nm. *Carryover of ethanol or salt* • Make sure to dry the silica membrane and the NucleoSpin® DNA Stool Column completely before elution to avoid carry-over of ethanolic Wash Buffer ST5. • Check if Buffer ST5 has been equilibrated to room temperature (18–25 °C) before use. Washing at lower temperatures decreases the efficiency of salt removal. *Contamination with PCR inhibitors* • 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.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® DNA Stool	740472.10 / .50	10 / 50 preps
MN Bead Tube Holder	740469	1
NucleoSpin® DNA Insect	740470.10 / .50	10 / 50 preps
NucleoSpin® Microbial DNA	740235.10 / .50	10 / 50 preps
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® Bead Tubes Type A (0.6–0.8 mm ceramic beads; recommended for stool, soil, and sediments)	740786.50	50
NucleoSpin® Bead Tubes Type B (40–400 µm glass beads; recommended for bacteria)	740812.50	50
NucleoSpin® Bead Tubes Type C (1–3 mm corundum; recommended for yeast)	740813.50	50
NucleoSpin® Bead Tubes Type D (3 mm steel balls; recommended for insects)	740814.50	50
NucleoSpin® 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
NucleoSpin® Bead Tubes Type F (1–3 mm corundum + 3 mm steel balls; use only with MN Bead Tube Holder!)	740816.50	50
Collection Tubes (2 mL)	740600	1000

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

6.3 Product use restriction / warranty

NucleoSpin® DNA Stool kit components were developed, designed and sold for research purposes only. They are suitable for in vitro uses only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the NucleoSpin® DNA Stool kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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