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RNA from stool samples

User manual

NucleoSpin® RNA Stool

October 2017 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

RNA from stool samples

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

		NucleoSpin® RNA Stool	
1	Prepare sample		NucleoSpin® Bead Tube Type A 180–220 mg sample material 200 µL NucleoZol 660 µL Buffer RST1
2	Sample lysis	 	Horizontally vortex 10 min at RT or use other homogenizers according to manufacturers protocol 13,000 x g, 5 min Transfer 510 µL supernatant
3	Precipitate contaminants	 	140 µL Buffer RST2 Vortex 5 s 13,000 x *g*, 3 min
4	Filter lysate	 	Load supernatant on NucleoSpin® Inhibitor Removal Column (red ring) 13,000 x *g*, 1 min
5	Adjust binding conditions		180 µL Buffer RST2 *or* 120 µL Buffer RST2 Vortex 5 s
6	Bind RNA	 	Load 600 µL sample on NucleoSpin® NucleoSpin® RNA Stool Column (light blue ring) 13,000 x *g*, 1 min Load remaining sample 13,000 x *g*, 1 min
8	Wash silica membrane and digest DNA	 	1st: 600 µL Buffer RST3 11,000 x *g*, 30 s 80 µL rDNase reaction mixture RT, 15 min 2nd: 600 µL Buffer RST4 13,000 x *g*, 1 min 3rd: 600 µL Buffer RST2 13,000 x *g*, 1 min 4th: 600 µL Buffer RST5 13,000 x *g*, 1 min
9	Dry silica membrane	 	11,000 x *g*, 2 min
10	Elute RNA	 	100 µL RNase free H_2O 11,000 x *g*, 30 s

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Stool	
REF	**10 preps 740130.10**	**50 preps 740130.50**
NucleoZol	6 mL	12 mL
Lysis Buffer RST1	10 mL	40 mL
Binding Buffer RST2	13 mL	50 mL
Wash Buffer RST3	6 mL	36 mL
Wash Buffer RST4	10 mL	35 mL
Wash Buffer RST5 (Concentrate)*	6 mL	12 mL
RNase free H_2O	13 mL	13 mL
Reaction Buffer for rDNase	7 mL	7 mL
rDNase, RNase free (lyophilized)*	1 vial (size D)	3 vials (size D)
NucleoSpin® Bead Tubes Type A	10	50
NucleoSpin® Inhibitor Removal Columns (red ring)	10	50
NucleoSpin® RNA Stool Columns (light blue ring)	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.

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

Reagents

- 96–100 % ethanol

Consumables

- 1.5 mL nuclease-free microcentrifuge tubes with lid
- 2.0 mL nuclease-free 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).

2 Product description

2.1 The basic principle

The **NucleoSpin® RNA Stool** kit is designed for the efficient isolation of RNA including small RNA species from fresh and frozen stool samples.

The kit combines NucleoZol and a specific Lysis Buffer RST1 for a chemical disruption of the stool sample and the containing microbes with a mechanical lysis. The mechanical lysis is performed using NucleoSpin® Bead Tubes Type A (containing ceramic beads) in combination with 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. A next lysate clearing step comprises addition of Binding Buffer RST2, followed by a short centrifugation.

The supernatant is finally cleared by passing it through a NucleoSpin® Inhibitor Removal Column that removes substances in stool samples that interfere with RT-PCR.

Binding conditions are adjusted by adding additional Binding Buffer RST2 to the flow-through of the NucleoSpin® Inhibitor Removal Column. Next the sample is loaded onto a NucleoSpin® RNA Stool Column.

Residual contaminants such as complex polysaccharides, bile salts, and other PCR inhibitors are removed by an efficient washing procedure using Buffer RST2 as well as Wash Buffers RST3, RST4 and RST5. In between the different washing steps, residual DNA is removed via an on column DNA digestion step using rDNase. After a drying step, ready-to-use RNA can be eluted with RNase free H_2O.

The **NucleoSpin® RNA Stool** kit also offers the option to isolate different nucleic acid compositions from stool samples. The standard protocol will isolate total RNA including small RNA species from stool samples. If the DNA from the stool sample is required too, the on column DNA digestion step should be omitted. If less of the small RNA species should be isolated, the total amount of Binding Buffer RST2 for the binding step should be reduced.

The RNA preparation using the NucleoSpin® RNA Stool kit can be performed at room temperature. The eluate, however, should be treated with care because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints and dust. To ensure RNA stability keep RNA frozen at -20 °C for short-term or -70 °C for long-term storage.

2.2 Kit specifications

Table 1: Kit specifications at-a-glance

Parameter	NucleoSpin® RNA 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	10–30 µg (varies by sample and protocol used)
Elution volume	100 µL
Preparation time	70 min / 10 preps
Binding capacity	200 µg

* 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.

2.3 Amount of starting material

NucleoSpin® RNA 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., 30–80 mg and to increase the volume of the lysis reagents (see also section 2.4 for detailed information about input material).

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 RNA. It is recommended to start the extraction with 50–100 mg sample material in these cases.

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.

Optimal results will be achieved with fresh sample material, kept at 2–8 °C after collection until the RNA extraction, which should take place within 24 hours after stool collection. If this is not possible, freezing of the stool sample at -20 °C as soon as possible after stool collection is recommended. Thawing of the sample material should be performed at room temperature immediately prior to the extraction or over night on ice. Multiple freeze-thaw cycles of stool samples prior to the extraction are not recommended and can lead to degradation of the RNA.

2.4 Sample lysis

A thorough sample lysis step is essential to achieve a high RNA 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 NucleoZol and Lysis Buffer RST1 is supported by the mechanical disruption in the NucleoSpin® Bead Tube to solubilize the sample material completely. During the 10 minute shaking on a Vortex, even solid stool samples like mouse droppings will be suspended and host cells and microbial cells will break up. If a bead mill is used, the best conditions for disruption have to be adjusted, e.g. for the Retsch 300 MM bead mill, homogenization for 1–2 minutes at a frequency of 30/s is recommended.

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.

For animal stool samples, the standard protocol may not lead to optimal results. A general recommendation for all animal stool samples is to use 150 µL NucleoZol and depending on the water content of the stool sample 700 to 800 µL Lysis Buffer RST1 for the sample lysis step, so that at least 500 µL lysate can be transferred after the first centrifugation step. In addition, the amount of starting material should be reduced compared to human stool samples. For carnivores (e.g. feline) and birds (e.g. chicken) 100 mg stool sample should be used as the maximum. For dry and fiber rich stool from herbivores (e.g. rabbit or sheep) 50–80 mg should be used and for very hard and dry samples like dried mouse droppings only 30–40 mg of stool should be used as starting material.

2.5 Lysate clearing and RNA binding

The lysate is cleared in three steps. In the first step, only the coarse particles are removed by centrifugation. In a second step contaminants are precipitated by addition of Binding Buffer RST2 and a short centrifugation step. In the standard protocol, 140 µL Binding Buffer RST1 are used to generate a cleared lysate.

A NucleoSpin® Inhibitor Removal Column is used for the removal of residual contaminants from the lysate. After addition of a second volume of Binding Buffer RST2 to the flow-through of the NucleoSpin® Inhibitor Removal Column, the RNA can be bound efficiently to the NucleoSpin® RNA Stool Column.

In the standard protocol, 180 µL Binding Buffer RST1 are used to adjust binding conditions. Under these conditions total RNA (large and small RNA species) are purified. If only the large RNA should be bound to the NucleoSpin® RNA Stool Column, the amount of binding buffer needs to be reduced to 120 µL.

For animal stool samples, different conditions for lysate clearing and RNA binding were adjusted to get optimal RNA yield and purity. See Table 2 for recommendations for selected species.

Table 2: Recommended RST2 volumes for lysate clearing and RNA binding steps

Stool sample	RST2 volume for lysate clearing	RST2 volume binding step
Feline, sheep, rabbit	200 µL	170 µL
Mouse, chicken	140 µL	240 µL

2.6 Washing procedure and DNase digestion

The washing procedure performed in the NucleoSpin® RNA Stool protocol is optimized to remove residual contaminating substances that are bound to the silica membrane.

It starts with a wash step with Wash Buffer RST3, followed by the on column DNase digestion step. The DNase digest should be omitted in case the DNA needs to be isolated too. The second wash step is carried out with Wash Buffer RST4, followed by two additional wash steps, using Binding Buffer RST2 and the final Wash Buffer RST5. After an additional centrifugation to dry the silica membrane within the NucleoSpin® RNA Stool Column, the RNA can be eluted using RNase-free H_2O.

2.7 Elution procedures

The recommended elution volume is 100 µL of RNase-free water.

If a lower volume than 100 µL is used for elution, it is important to pipette the RNase-free water onto the center of the NucleoSpin® RNA Stool Column in order to moisten the silica membrane completely. The overall yield may be reduced when less than 100 µL volume are used for the elution step. Using less than 30 µL RNase free water for elution is not recommended.

If less than 60 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 eluate once again from the elution tube onto the membrane of the NucleoSpin® RNA Stool Column and centrifuge again for 1 minute at 13,000 x *g*.

2.8 Evaluation of RNA yield and quality

The most common method to determine the RNA yield is UV-VIS spectroscopy. The RNA 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. Any contaminations, e.g., proteins or DNA (in case the on column DNA digestion was not performed) significantly contribute to the total absorption at 260 nm and can therefore lead to an overestimation of the actual RNA concentration. If RNA and DNA were isolated, specific dye based quantification methods may be better suited for quantification.

Purity ratio A_{260}/A_{280}

The main indicator of DNA purity is the ratio A_{260}/A_{280}, which should be between 1.8 and 2.2. Values below 1.8 indicate protein contamination.

Purity ratio A_{260}/A_{230}

Another indicator of RNA 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 RNA and can be accepted down to about 1.5. Ratios around or even below 1.0 indicate impurities, which could be of variable nature as several compounds absorb at this wavelength.

3 Storage conditions and preparation of working solutions

Attention: NucleoZOL contains phenol (corrosive liquid/poison) and guanidium thiocyanate (irritant). Wear gloves, eye protection, and goggles!

CAUTION: Read the warning note on the container and MSDS. NucleoZOL contains phenol and guanidinium thiocyanate which CAUSES BURNS and can be fatal. When working with NucleoZOL, use gloves and eye protection (face shield, safety goggles). Do not get the reagent on skin or clothing. Avoid breathing fumes. In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and if necessary seek medical attention. Store lyophilized rDNase (RNase-free) at 4 °C on arrival (stable up to 1 year). Wear gloves and goggles!

CAUTION: Buffer RST4 contains guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Storage conditions:

- Store lyophilized rDNase (RNase-free) at 4 °C on arrival (stable up to 1 year).
- All other 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® RNA Stool procedure, prepare the following:

- Wash Buffer RST5: 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 RST5 is stable at room temperature (18–25 °C) for at least one year.

	NucleoSpin® RNA Stool	
REF	**10 preps 740130.10**	**50 preps 740130.50**
Buffer RST5 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol

rDNase (RNase-free): Add 1.4 mL Reaction Buffer for rDNase to each rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots in nuclease-free 1.5 mL microcentrifuge tubes (not provided) and store at -20 °C. The frozen working solution is stable for at least 6 months. Do not freeze / thaw the aliquots more than three times. (Be careful when opening the vial as some particles of the lyophilisate may be attached to the lid.)

In some cases the vial of rDNase may appear empty. This is due to lyophilized enzyme sticking to the septum. To avoid loss of rDNase, make sure to collect rDNase on the bottom of the vial before removing the plug. Alternatively, inject RNase-free water into the vial using a needle and syringe, invert the vial to dissolve the rDNase, and remove the dissolved rDNase using syringe and needle.

4 Safety instructions

The following components of the **NucleoSpin® RNA 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*
NucleoZol	phenol 30–60 % + guanidinium thiocyanate 30–45 % *Phenol 30–60 % + Guanidinthiocyanat 30–45 %* CAS 108-95-2, 593-84-0	 DANGER *GEFAHR*	301, 311, 314, 331, 341, 373, 412	201, 260sh, 273, 280sh, 303+361+353, 305+351+338, 311, 405
RST2	1,4-Dioxan 40–90 % *1,4-Dioxan 40–90 %* CAS 123-91-1	 DANGER *GEFAHR*	225, 319, 335, 351, EUH019, EUH066	201, 210, 261sh, 280sh, 403+233
RST3	guanidine hydrochloride 24–36 % + ethanol 35–55 % *Guanidinhydrochlorid 24–36 % + Ethanol 35–55 %* CAS 50-01-1, 64-17-5	 WARNING *ACHTUNG*	226, 302	210, 264W, 301+312, 330
RST4	ethanol 35–55 % *Ethanol 35–55 %* CAS 64-17-5	 DANGER *GEFAHR*	226	210
rDNase	rDNase 90–100 % *rDNase 90–100 %* CAS 9003–98-9	 DANGER *GEFAHR*	317, 334	261sh, 280sh, 342+311

 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.

Hazard phrases

H 225	Highly flammable liquid and vapour. *Flüssigkeit und Dampf leicht entzündbar.*
H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 301	Toxic if swallowed. *Giftig bei Verschlucken.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 311	Toxic in contact with skin. *Giftig bei Hautkontakt.*
H 314	Causes severe skin burns and eye damage. *Verursacht schwere Verätzungen der Haut und schwere Augenschäden.*
H 317	May cause an allergic skin reaction. *Kann allergische Hautreaktionen verursachen.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H 331	Toxic if inhaled. *Giftig bei Einatmen.*
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. *Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
H 335	May cause respiratory irritation. *Kann die Atemwege reizen.*
H 341	Suspected of causing genetic defects <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>. *Kann vermutlich genetische Defekte verursachen (Expositionsweg angeben, sofern schlüssig belegt ist, dass diese Gefahr bei keinem anderen Expositionsweg besteht).*
H 351	Suspected of causing cancer <state route of exposure if it is conclusively proven that no other routs of exposure cause the hazard>. *Kann vermutlich Krebs erzeugen (Expositionsweg angeben, sofern schlüssig belegt ist, dass diese Gefahr bei keinem anderen Expositionsweg besteht).*
H 373	May cause damage to organs <or state all organs affected, if known> through prolonged or repeated exposure <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>. *Kann die Organe schädigen (alle betroffenen Organe nennen) bei längerer oder wiederholter Exposition (Expositionsweg angeben, wenn schlüssig belegt ist, dass diese Gefahr bei keinem anderen Expositionsweg besteht).*
H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH 019	May form explosive peroxides. *Kann explosionsfähige Peroxide bilden.*
EUH 031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*
EUH 066	Repeated exposure may cause skin dryness or cracking. *Wiederholter Kontakt kann zu spröder oder rissiger Haut führen.*

Precaution phrases

P 201	Obtain special instructions before use. *Vor Gebrauch besondere Anweisungen einholen.*
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 260sh	Do not breathe dust / vapors. *Staub / Dampf nicht einatmen.*
P 273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P 280sh	Wear protective gloves / eye protection / face protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER / doctor / … / if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt / … anrufen.*
P 303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. *BEI BERÜHRUNG MIT DER HAUT (oder dem Haar): Alle kontaminierten Kleidungsstücke sofort ausziehen. Haut mit Wasser abwaschen / duschen.*
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 311	Call a POISON CENTER / doctor / …. *GIFTINFORMATIONSZENTRUM*
P 330	Rinse mouth. *Mund ausspülen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / … *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM/Arzt / … anrufen.*
P 403+235	Store in a well-ventilated place. Keep cool. *An einem gut belüfteten Ort aufbewahren. Kühl halten.*
P 405	Store locked up. *Unter Verschluss aufbewahren.*

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

Before starting the preparation:

- Check Lysis Buffer RST1 for precipitates. Dissolve any precipitate by incubating the buffer at 40–50 °C.
- Prepare rDNase reaction mixture with rDNase reconstituted in Reaction Buffer for rDNA (see section 3 for details) or thaw appropriate aliquots from -20 °C freezer and keep rDNase on ice until use.
- For each RNA extraction, 80 µL of reconstituted rDNase is used.

It is recommended to wear lab coat, goggles and gloves throughout the whole procedure.

5.1 Protocol for fresh or frozen stool samples

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.

180–220 mg sample

+ 200 µL NucleoZol

+ 660 µL Buffer RST1

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

Important: Do not overload the bead tube as this may lead to reduced yield and purity. It is recommended to use an appropriate balance to weigh in the sample material.

Add **200 µL NucleoZol** and **660 µL Buffer RST1**.

Note: For animal stool samples it is recommended to use less sample material, 150 µL NucleoZol and 700–800 µL RST1. See section 2.3 and 2.4 for details.

Close the NucleoSpin® Bead Tube before putting it onto the MN Bead Tube Holder.

2 Lyse sample

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

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

Vortex RT, 10 min

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

Centrifuge for **5 min** at **13,000 x *g***.

13,000 x *g*, 5 min

Transfer **510 µL** of the supernatant to a fresh **2 mL microcentrifuge tube** with lid (not provided).

Transfer 510 µL supernatant

Note: If the volume of clear supernatant is less than 510 µL, transfer as much lysate as possible to the 2 mL microcentrifuge tube without aspirating the pellet. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

3 Precipitate contaminants

Add **140 µL Buffer RST2**, close the lid and vortex for **5 s**.

+ 140 µL Buffer RST2

Vortex 5 s

Note: For animal stool samples different RST2 volumes may be used to clear the lysate. See Table 2 in section 2.5 for details.

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

13,000 x *g*, 3 min

4 Filter lysate

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

Load supernatant

Avoiding the pellet, transfer **600 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***.

13,000 x *g*, 1 min

Discard the NucleoSpin® Inhibitor Removal Column.

5 Adjust binding conditions

+ 180 µL Buffer RST2 or 120 µL Buffer RST2

Add **180 µL Buffer RST2** for isolating **total RNA** or **120 µL Buffer RST2** for isolating only **large RNA** from the sample and close the lid.

See section 2.5 for more details on isolating different RNA species.

Close the lid and vortex for **5 s**.

Vortex 5 s

Note: For animal stool samples different RST2 volumes may be used to adjust binding conditions. See Table 2 in section 2.5 for details.

6 Bind RNA

Load 600 µL sample

13,000 x *g*, 1 min

Place a **NucleoSpin® RNA Stool Column** (light blue ring) in a Collection Tube (2 mL).

Load 600 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 remaining sample

13,000 x *g*, 1 min

Load the **residual sample** and centrifuge again for **1 min** at **13,000 x *g***.

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

7 Wash silica membrane and digest DNA

1st wash

+ 600 µL Buffer RST3

Add **600 µL Buffer RST3** to the NucleoSpin® RNA Stool Column.

13,000 x *g*, 1 min

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

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

Digest DNA

+ 80 µL rDNase reaction mixture

RT, 15 min

Apply **80 µL rDNase reaction mixture** directly onto the center of the silica membrane of the column. Incubate at **room temperature** for **15 min**.

Note: If DNA should be co-purified together with the RNA, omit the DNA digestion step and continue with 2nd wash.

2nd wash

Add **600 µL Buffer RST4** to the NucleoSpin® RNA Stool Column.

+ 600 µL Buffer RST4

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

13,000 x *g*, 1 min

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

3rd wash

Add **600 µL Buffer RST2** to the NucleoSpin® RNA Stool Column.

+ 600 µL Buffer RST2

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

13,000 x *g*, 1 min

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

4th wash

Add **600 µL Buffer RST5** to the NucleoSpin® RNA Stool Column.

+ 600 µL Buffer RST5

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

13,000 x *g*, 1 min

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

<u>Note:</u> 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.

8 Dry silica membrane

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

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

13,000 x *g*, 2 min

9 Elute RNA

Place the **NucleoSpin® RNA Stool Column** into a new nuclease-free 1.5 mL microcentrifuge tube (not provided).

+ 100 µL RNase free H_2O

Add **100 µL RNase free H_2O** to the column.

<u>Note:</u> If a lower volume is used for elution, read the recommendations in section 2.7.

13,000 x *g*, 1 min

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

Discard the NucleoSpin® RNA Stool Column.

Vortex each microcentrifuge tube for 2 s.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no RNA 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. Additionally the concentration of the lysis solution may be too diluted by the sample volume to chemically disrupt the sample material. 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 for 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 RST5 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 RST1 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. *Sample material not stored properly* • Stool samples should be kept at 2–8 °C after collection. If the RNA 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 (18–25 °C) immediately before extraction or over night at 2–8 °C.

RNA is degraded	*Stool sample was not stored correct before extraction* • Use fresh stool samples that were stored cooled (2–8 °C) and extract RNA within 24 hours after collection or freeze stool sample as soon as possible after collection at -20° and thaw at room temperature (18–25 °C) immediately before extraction or over night at 2–8 °C. *RNase contamination* • Create an RNase free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use.
Suboptimal performance of RNA in downstream experiments	*RNA yield was overestimated* • If RNA 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. • If the on column DNA digestion was omitted, copurified DNA leads to an overestimation of RNA yield in the UV based quantification. *Carryover of ethanol or salt* • Make sure to dry the silica membrane and the NucleoSpin® RNA Stool Column completely before elution to avoid carry-over of ethanolic Wash Buffer RST5. *Contamination with PCR inhibitors* • The RNA 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 RNA 1:10 to reduce concentration of inhibitors.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Stool	740130.10 / .50	10 / 50 preps
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® Soil	740780.10 / .50	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; recommended for challenging tissues in combination with the NucleoSpin® RapidLyse Kit) - use only with MN Bead Tube Holder	740816.50	50
NucleoSpin® Bead Tubes Type G (5 mm steel balls; recommended for plant material)	740817.50	50
Collection Tubes (2 mL)	740600	1000

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

6.3 Product use restriction / warranty

NucleoSpin® RNA 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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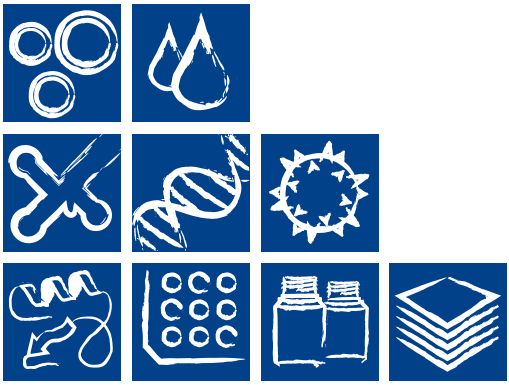

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