RNA and DNA from soil

User manual

NucleoBond® RNA Soil

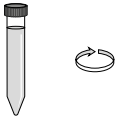
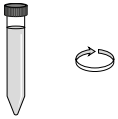
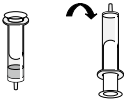
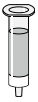
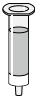
DNA Set for NucleoBond® RNA Soil

November 2017 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

RNA and DNA from soil

Protocol at a glance (Rev. 01)

	NucleoBond® RNA Soil	
1 Prepare sample		Fill tubes up to the 1 mL gradiuation mark
2 Add lysis buffers		800 µL Buffer E1 *Optional: 100 µL OPT* 100 µL Phenol:Chloroform:Isoamylalcohol Vortex 2 s
3 Bead beating		Vortex horizontally 5 min at RT
4 Lysate clearing		13,000 x *g*, 2 min Transfer the supernatants to a 15 mL tube
5 Adjust binding conditions		125 µL Buffer E2 per mL supernatant Vortex 5 s 2 min RT 13,000 x *g*, 2 min
6 Column setup and equilibration		12 mL Buffer EQU
7 Load column		Load supernatant on NucleoBond® RNA Column
8 Filter flush		6 mL Buffer E3 Discard Filter
9 Column wash		8 mL Buffer E4
10 RNA elution		5 mL Buffer ERNA

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Protocol at a glance (Rev. 01)

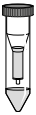
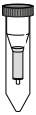
		NucleoBond® RNA Soil
11 Precipitate nucleic acids		3.5 mL Isopropanol Vortex 5 s
12 Bind nucleic acids		4,500 x *g* 2 min
13 Washing and drying		1 mL Buffer E5 4,500 x *g*, 2 min
14 Elute nucleic acids		100 µL RNase-free H_2O 4,500 x *g*, 2 min

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Table of contents

1 Components

1.1 Kit contents

	NucleoBond® RNA Soil	DNA Set for NucleoBond® RNA Soil
REF	**20 preps 740140.20**	**20 preps 740141.20**
Lysis Buffer E1	70 mL	-
Buffer OPT	10 mL	-
Equilibration Buffer EQU	250 mL	-
Binding Buffer E2	10 mL	-
Wash Buffer E3	125 mL	-
Wash Buffer E4	180 mL	-
RNA Elution Buffer ERNA	125 mL	-
DNA Elution Buffer EDNA	-	125 mL
Wash Buffer E5 (Concentrate)*	6 mL	6 mL
RNase-free H_2O	13 mL	13 mL
NucleoSpin® Bead Tubes Type A	80	-
NucleoBond® RNA Columns	20	-
Plastic Washer	20	-
NucleoSpin® Finisher Columns	20	20
User manual	1	-

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

1.2 Reagents, consumables and equipment to be supplied by user

Reagents

- Phenol:Chloroform:Isoamylalcohol (25:24:1 v/v), e.g., VWR Cat. No. 0883–100ML
- 96–100 % ethanol
- Isopropanol

Consumables

- 15 mL centrifuge tubes
- 50 mL centrifuge tubes
- RNase free pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Centrifuge for 15 mL and 50 mL tubes, capable of reaching 4,500 x *g*
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (e. g., labcoat, gloves, goggles)

2 Product description

2.1 Basic principle

The **NucleoBond® RNA Soil Kit** is designed for the purification of RNA from soil and sediment samples. In combination with the **DNA Set for NucleoBond® RNA Soil**, RNA and DNA can be isolated from the same soil sample. Thus, purified nucleic acids are suitable e.g., for metagenomic studies, where the genome and transcriptome are analyzed from the same sample.

Most soils contain relatively low amounts of RNA, compared to DNA. Therefore the sample input needs to be increased in order to gain acceptable RNA yields. The standard protocol of this kit uses four NucleoSpin® Bead Tubes Type A that can be filled with 250–500 mg of one soil sample each, resulting in a total sample amount of 1–2 g. This sample input amount will usually result in a RNA yield in the range from 1–10 µg. If working with four bead tubes per sample is too cumbersome, the content of four bead tubes can be combined into one 15 mL tube. This tube can be filled with 1–2 g of soil sample and four times the volume of Buffer E1, PCI and, if applicable, Buffer OPT. On the other hand, if a specific soil contains high amounts of RNA, the number of NucleoSpin® Bead Tubes Type A can also be reduced.

Organisms in a soil sample are lysed by bead beating in the presence of Lysis Buffer E1 (check for precipitated SDS!) and Phenol:Chloroform:Isoamylalcohol. Buffer OPT reduces the adsorption of nucleic acids to clay and mineralic soil components, but will also increase the contamination with humic acids if present. Performance cannot be predicted and depends on the soil composition, but as a rule of thumb a sample with a high content of organic and **humic components**, e.g., forest soil, should be lysed **without Buffer OPT** while predominantly **mineralic soils**, e.g., river sediments and clay, should be lysed **with Buffer OPT**.

Unlysed components are sedimented by centrifugation. The supernatants of the four bead tubes are transferred and combined into one fresh 15 mL tube and mixed with Binding Buffer E2. The incubation and centrifugation steps that follow can be used to prepare the **NucleoBond® RNA Columns**. The columns including filters are fixed with the supplied Plastic Washers on top of a 50 mL tube or laboratory flask or any NucleoBond® Rack. Filters and columns are equilibrated by Buffer EQU to pre-wet the filters and to prepare the anion exchange chromatography columns.

Once the columns are equilibrated, the clear supernatant of the centrifuged samples is loaded onto the filters in the purification columns. Undissolved particles and some of the soluble macro molecules will be held back by the filters.

Wash Buffer E3 is used to flush the filter columns and to wash the nucleic acids from the filters onto the column matrix. Nucleic acids and soluble polyanionic molecules including some fraction of humic substances (if present) will bind to the anion exchange surface. A brown layer might be visible on top of the silica matrix. After the washing step the filter is removed and the anion exchange column is washed with Buffer E4 which removes contaminants.

RNA is eluted by Buffer ERNA. If isolation of RNA and DNA is desired, DNA is eluted by Buffer EDNA, contained in the DNA Elution Set (to be ordered separately, see section 6.2), from the same column.

Isopropanol is added to the eluates and the mixture is bound to NucleoSpin® Finisher Columns by centrifugation. The columns are washed with Buffer E5, dried and eluted in RNase-free H_2O.

Eluted RNA and DNA fractions might contain a fraction of the other nucleic acid. If necessary, digest DNA or RNA enzymatically. Contact MACHEREY-NAGEL (tech-bio@mn-net.com) for RNase A and DNase Buffer Sets and protocols if required.

2.2 Kit specifications

The **NucleoBond® RNA Soil** and **DNA Set for NucleoBond® RNA Soil** kits are designed for the isolation of pure RNA and DNA from soil.

Kit specifications at a glance

Parameter	NucleoBond® RNA Soil	DNA Set for NucleoBond® RNA Soil
Format	Anion exchange chromatography and silica spin purification	Elution Set (NucleoBond® RNA Soil kit required)
Processing	Manual	Manual
Sample material	1–2 g soil	NucleoBond® RNA Soil kit required
Elution volume	100 µL	100 µL
Preparation time	60 min/12 preps	15 min/ 12 preps
Typical yield	1–10 µg	5–50 µg

2.3 Bead tubes and lysis

Reasonable RNA yields in the lower microgram range can be purified from 1–2 g of soil sample. The supplied NucleoSpin® Bead Tubes Type A work best with a sample input of 250–500 mg for each tube, depending on the soil density and water content. Remove excess water by centrifugation if working with wet samples. For optimal lysis efficiency and to prevent overloading of the bead tubes it is recommended to fill the tubes with sample material up to the 1 mL mark, resulting in 250–500 mg sample weight. Thus an optimal ratio of beads:sample:lysis buffer is achieved. To gain the desired total amount of 1–2 g of sample material, four bead tubes are needed.

If using four bead tubes for one sample is inconvenient, e.g., due to bead beating capacities, or to prevent sample mix-up, the content of four NucleoSpin® Bead Tubes Type A can be filled into one 15 mL tube (not supplied). Consecutively, 1–2 g of soil sample can be filled into the 15 mL bead tubes and lysed with four times the volume of the lysis buffers. This procedure might be more suitable for larger sample amounts but it needs to be ensured that the 15 mL tubes close tightly!

Bead beating can be performed with any dedicated machine (e.g., FastPrep® Instrument - MP Biomedicals, Precellys – Bertin Corp., Retsch Mill or the like) which can accommodate 2 mL tubes or 15 mL tubes respectively. Alternatively the MN Bead Tube Holder can be used in combination with a Vortex-Genie 2 (Scientific Industries) for 2 mL tubes. Please see section 6.2 for MN Bead Tube Holder ordering information.

Bead beating for 5 minutes at full speed is sufficient to lyse the cells when using a Vortexer. Parameters for other beat beating machines need to be established individually (e.g., 5 m/s for 30 s on a FastPrep® Instrument). Feedback for working parameters with other machines is highly appreciated!

Extended lysis times may result in increased contamination of RNA eluates with sheared genomic DNA and should be avoided.

3 Storage conditions and preparation of working solutions

Attention: Buffer E4 contains guanidine thiocyanate! Wear gloves and goggles!

CAUTION: Buffer E4 contains guanidine thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochloride). DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Attention: Phenol:Chloroform:Isoamylalcohol is poisonous! Read the safety data sheet, supplied with the Phenol:Chloroform:Isoamylalcohol, and follow the instructions for use and disposal carefully! Take appropriate safety measures when working with Phenol:Chloroform:Isoamylalcohol and discard waste according to legal guidelines!

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.
- **Sodium dodecyl sulfate (SDS) in Buffer E1 may precipitate if stored at temperatures below 20 °C.** If a precipitate is observed in Buffer E1, incubate bottle at 30–40 °C for several minutes and mix well.

Before starting the **NucleoBond® RNA Soil** protocol prepare the following:

- Add 24 mL of 96–100 % ethanol to **Wash Buffer E5**.

4 Safety instructions

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***
Wash Buffer E4	ethanol 5–20 % *Ethanol 5–20 %* CAS 64-17-5d	 WARNING *ACHTUNG*	226	210
RNA Elution Buffer ERNA	ethanol 5–20 % *Ethanol 5–20 %* CAS 64-17-5d	 WARNING *ACHTUNG*	226	210

 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 226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.

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.

5 Protocol

Before starting the preparation:

- Check Lysis Buffer E1 for precipitated SDS according to section 3
- Check if Wash Buffer E5 was prepared according to section 3
- Check if Phenol:Chloroform:Isoamylalcohol (25:24:1 v/v) is available
- Equilibrate Phenol:Chloroform:Isoamylalcohol to room temperature and mix well!
- Check if isopropanol is available
- Check if 15 mL and 50 mL tubes are available

!

1 Prepare Sample

Fill **4 NucleoSpin® Bead Tubes Type A** with sample material up to the **1 mL graduation mark** each.

If the beads of 4 NucleoSpin® Bead Tubes Type A have been pooled into one 15 mL tube according to section 2.3, add up to 2 g of soil sample. For best results, a total sample mass of 1000–1500 mg is recommended.

fill tubes up to the 1 mL graduation mark

2 Add lysis buffers

Add **800 µL Lysis Buffer E1** to each NucleoSpin® Bead Tube Type A.

Optional: Add 100 µL Buffer OPT to each NucleoSpin® Bead Tube Type A.

Buffer OPT is recommended for soil with a predominantly mineralic matrix (e.g., clay, sediment) but negative for predominantly organic soil. It cannot be identified in advance, if addition of Buffer OPT increases yield or decreases purity.

Add **100 µL Phenol:Chloroform:Isoamylalcohol** (25:24:1 v/v) to each Bead Tube Type A.

Mix Phenol:Chloroform:Isoamylalcohol well before use! Take appropriate safety measures for working with Phenol:Chloroform:Isoamylalcohol!

If working with one 15 mL tube instead of four NucleoSpin® Bead Tubes Type A, add 3.2 mL Buffer E1, 0.4 mL Phenol:Chloroform:Isoamylalcohol and, if necessary, 0.4 mL Buffer OPT.

Vortex bead tubes for **2 s** to distribute beads, sample and buffers homogeneously.

+ 800 µL Buffer E1

optional: + 100 µL Buffer OPT

+ 100 µL Phenol:Chloroform:Isoamylalcohol

Vortex 2 s

3 Bead Beating

Lyse cells by **bead beating** for **5 min** at **maximum speed**.

See section 2.3 for details. Dedicated machines might require shorter lysis times (e.g., 5 m/s for 30 s) and could increase carry-over of sheared genomic DNA into the RNA eluates if activated for too long.

Recommended: Use the MN Bead Tube Holder in combination with a Vortex-Genie 2

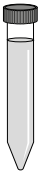
Vortex RT, 5 min

4 Lysate clearing

Centrifuge samples for **2 min** at **full speed**.

13,000 x *g* 2 min

Transfer and pool the supernatants of the **4 corresponding bead tubes** with one soil sample into a fresh 15 mL tube (not supplied) and determine the approximate volume.

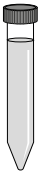


Transfer the supernatants to a 15 mL tube

Avoid transferring debris or the organic phase which is a thin layer between the aqueous phase and the solid debris and usually much darker than the aqueous supernatant!

5 Adjust binding conditions

For each mL of supernatant, add **125 µL Buffer E2**.



+ 125 µL E2 per mL supernatant

Example:

to 2.0 mL supernatant add 250 µL Buffer E2

to 2.5 mL supernatant add 313 µL Buffer E2

to 3.0 mL supernatant add 375 µL Buffer E2

Vortex for **5 s**.

Vortex 5 s 2 min RT

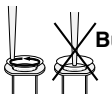
Incubate for **2 minutes** at **room temperature** and **centrifuge** for **2 min** at **full speed** afterwards.

13,000 x *g* 2 min

Proceed to column setup and equilibration during the incubation and centrifugation time.

6 Column setup and equilibration

For each soil sample, combine a **NucleoBond® RNA Column** (including a filter) with a **Plastic Washer** and arrange the combination on a 50 mL tube or laboratory flask (not supplied).



+ 12 mL Buffer EQU

Equilibrate filters and columns with **12 mL Buffer EQU**.

Add Buffer EQU on the upper rim of the filters.

All steps involving the NucleoBond® RNA Coulmn are performed with gravity flow. Do not use vacuum!

Discard flow through.

7 Load column

Load supernatant from centrifugation (step 5) into the **center of the filter**.

Load supernatant

Do not load sample on the rim of the filter column as this will decrease yield

Attention: Flow through contains remaining PCI!

Discard flow through according to legal guidelines.

8 Filter flush

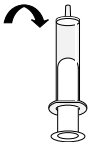
Wash filter with **6 mL Buffer E3**.

+ 6 mL Buffer E3

Wash the rim of the filter.

Discard flow through.

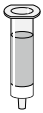
Discard filter.



Discard Filter

9 Column wash

Wash the NucleoBond® RNA Column without filter with **8 mL Buffer E4**.



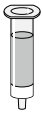
+ 8 mL Buffer E4

Discard flow through.

10 RNA elution

Transfer the column to a fresh 50 mL tube (not supplied).

Elute RNA with **5 mL Buffer ERNA**.



+ 5 mL Buffer ERNA

***Optional*: DNA elution (DNA Elution Set required)**

Transfer the column to a fresh 50 mL tube (not supplied).

Elute DNA with **5 mL Buffer EDNA**.

11 Precipitate nucleic acids

Add **3.5 mL isopropanol** (not supplied) to each eluate.

Vortex 5 s

Transfer mixture **into a NucleoSpin® Finisher Column** in a 50 mL centrifuge tube.

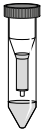
+ 3.5 mL Isopropanol

12 Bind nucleic acids

Centrifuge NucleoSpin® Finisher Column for **2 min** at **4,500 x *g***

If the mixture has not passed the silica completely, repeat centrifugation.

Discard flow through.



4,500 x *g*
2 min

13 Washing and drying

Add **1 mL Buffer E5** to the center of the silica.

Centrifuge for **2 min at 4,500 x *g***

Make sure the outlet of the NucleoSpin® Finisher Columns does not come in contact with the flow through.

Transfer NucleoSpin® Finisher Column to a fresh 50 mL centrifuge tube (not supplied).

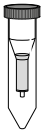
+1 mL Buffer E5

4,500 x *g*
2 min

14 Elute nucleic acids

Add **100 µL RNase-free H_2O** to the center of the silica.

Centrifuge for **2 min** at **4,500 x *g***



+ 100 µL RNase-free H_2O

4,500 x *g*
2 min

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or low nucleic acid yield	*Nucleic acid adsorbed to soil matrix* • Use Buffer OPT or increase volume of Buffer OPT. Reduce volume of Buffer E1 when increasing volume of Buffer OPT • Increase volume of Phenol:Chloroform:Isoamylalcohol. Some soils do not require Phenol:Chloroform:Isoamylalcohol, but in most cases addition of Phenol:Chloroform:Isoamylalcohol is mandatory to gain long, intact RNA.
	Storage of soil sample • Storage conditions might alter RNA integrity and total amount of nucleic acids. Especially mRNA profiles might change and total yield might reduce during storage at room temperature or depending on humidity.
	Sample amount • Do not fill the NucleoSpin® Bead Tubes Type A higher than the 1 mL mark as this will result in decreased bead beating efficiency. “Less is more”. • Check if the beads are moving vigorously in the tubes during the bead beating.
	Room temperature • Best performance is achieved at 18–25 °C. Do not store buffers in the fridge or on ice!

Problem	Possible cause and suggestions
Poor downstream performance	*Humic acid contamination* • To verify PCR inhibition by humic substances as root cause for poor downstream application results, check if a dilution series (e.g., 1:10, 1:100, 1:1000) of the eluates with water will restore functionality in downstream application. If not, other reasons must be taken into account. • Buffer OPT will release PCR inhibitors from soils. Skip addition or decrease volume. • Reduce bead beating time.
	DNA contamination • Separation of DNA and RNA is not complete. If residual DNA in the RNA fraction or RNA in the DNA fraction is influencing downstream application results, digest RNA and DNA enzymatically. Contact MACHEREY-NAGEL (techbio@mn-net.com) for enzymes and protocols if necessary.
Low nucleic acid integrity	*Degraded nucleic acids* • Degraded DNA from dead cells will also elute in the RNA eluate fraction and might be detected as low molecular weight smear. Usually this nucleic acid will not interfere with downstream applications. Digest residual DNA if necessary.
	Ambient temperature • Elution profiles strongly depend on the pH value of the elution buffers. Changes in room- or buffer temperature might change the pH value, resulting in a decreased rRNA yield (temperatures above 25 °C) or increased DNA contamination in the RNA fraction (temperatures below 18 °C). Always keep buffers at room temperature (18–25 °C). The pH value of buffer ERNA should be at pH 7.1 +/- 0.1, the pH value of buffer EDNA at pH 8.0 +/- 0.1. If not, do not adjust buffer pH value but buffer temperature!

6.2 Ordering information

Product	REF	Pack of
NucleoBond® RNA Soil	740140.20	20 preps
DNA Set for NucleoBond® RNA Soil	740141.20	20 preps
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® RNA Stool	740130.10 / .50	10 / 50 preps
NucleoSpin® DNA Stool	740472.10 / .50/ / .250	10 / 50 / 250 preps
rDNase Set	740963	500 reactions
Liquid RNase A	740397	500 reactions
MN Bead Tube Holder	740469	1 piece
NucleoSpin® Bead Tubes Type A (0.6–0.8 mm ceramic beads)	740786.50	50 pieces

6.3 Product use restriction / warranty

NucleoBond® RNA Soil Kit and **NucleoBond® DNA Elution Set** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

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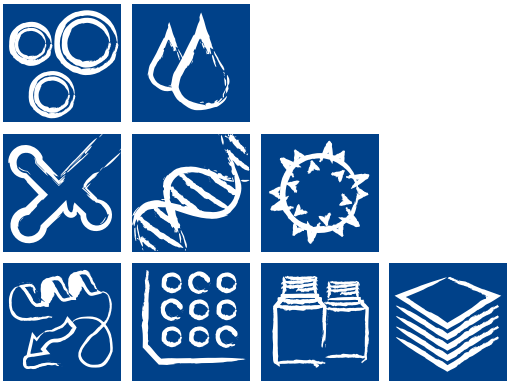

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