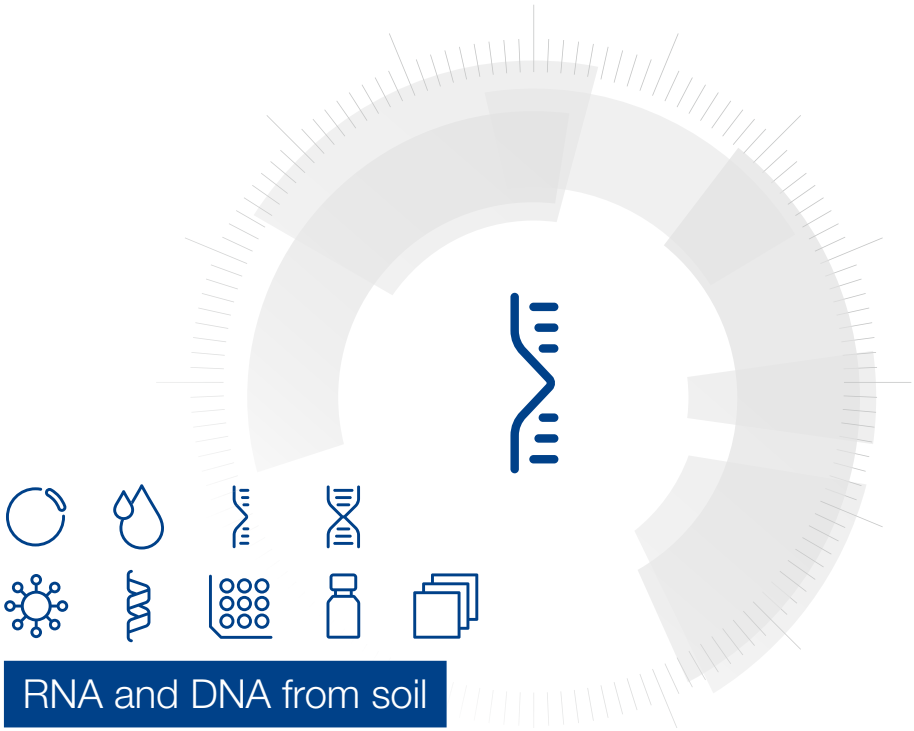
Bioanalysis

MACHEREY-NAGEL

User manual



RNA and DNA from soil

- NucleoBond® RNA Soil Mini
- DNA Set for NucleoBond® RNA Soil Mini

September 2023 / Rev. 03

RNA and DNA from soil

Protocol at a glance (Rev. 03)

	NucleoBond® RNA Soil
1 Prepare sample	 Fill tubes up to the 1 mL graduation 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	  20,000 × *g*, 2 min Transfer the supernatants to a 1.5 mL tube
5 Adjust binding conditions	  125 µL Buffer E2 per mL supernatant Vortex 5 s 2 min RT 20,000 × *g*, 2 min
6 Column setup and equilibration	 1.5 mL Buffer EQU
7 Load column	 Load supernatant on NucleoBond® RNA Mini Column
8 1st washing step	 250 µL Buffer E3
9 2nd washing step	 2 mL Buffer E4
10 RNA elution	 1 mL Buffer ERNA

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RNA and DNA from soil

Protocol at a glance (Rev. 03)

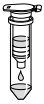
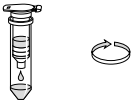
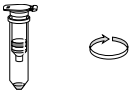
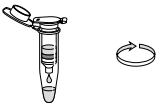
		NucleoBond® RNA Soil
11 Precipitate nucleic acids		700 µL Isopropanol Vortex 5 s
12 Bind nucleic acids		Load sample 11,000 × *g* 15 s
13 Washing		500 µL Buffer E5 11,000 × *g*, 15 s Discard flow through Repeat this step once.
14 Drying		11,000 × *g*, 1 min
15 Elute nucleic acids		50 – 100 µL RNase-free H_2O 11,000 × *g*, 1 min

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Table of contents

1 Components

1.1 Kit contents

	NucleoBond® RNA Soil Mini		DNA Set for NucleoBond® RNA Soil Mini	
REF	**10 preps 740142.10**	**50 preps 740142.50**	**10 preps 740143.10**	**50 preps 740143.50**
Lysis Buffer E1	13 mL	70 mL	-	-
Buffer OPT	3 mL	10 mL	-	-
Equilibration Buffer EQU	30 mL	100 mL	-	-
Binding Buffer E2	10 mL	10 mL	-	-
Wash Buffer E3	13 mL	30 mL	-	-
Wash Buffer E4	30 mL	125 mL	-	-
RNA Elution Buffer ERNA	13 mL	60 mL	-	-
DNA Elution Buffer EDNA	-	-	13 mL	60 mL
Wash Buffer E5 (Concentrate)*	6 mL	2 × 6 mL	6 mL	2 × 6 mL
RNase-free H_2O	13 mL	13 mL	13 mL	13 mL
NucleoSpin® Bead Tubes Type A	10	50	-	-
NucleoBond® RNA Mini Columns	10	50	-	-
NucleoSpin® RNA Columns	10	50	-	-
NucleoSpin® DNA Columns	-	-	10	50
Collection Tubes	-	-	10	50
Plastic Washers	10	10		
User manual	1	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. 0883-00ML
- 96-100 % ethanol
- Isopropanol

Consumables

- 1.5 mL centrifuge tubes
- 2.0 mL centrifuge tubes
- RNase-free pipette tips
- NucleoBond® Rack Small, alternatively, 50 mL centrifuge tubes or standard laboratory flask

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (e.g., labcoat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoBond® RNA Soil Mini** or **DNA Set** for **NucleoBond® RNA Soil** Mini is used for the first time.

All technical literature is available on the internet at ***www.mn-net.com***.

2 Product description

2.1 Basic principle

The **NucleoBond® RNA Soil Mini Kit** is a downscaled version of the NucleoBond® RNA Soil Kit (740140.20) and the corresponding DNA Elution Set (740141.20). A smaller anion exchange column reduces both, capacity and dead volume, resulting in a decreased elution volume. The final purification, concentration and desalting step can be performed in centrifuge for microcentrifuge tubes.

For downstream applications like PCR or RT-PCR, high yields of nucleic acid might not be necessary. In contrast to the larger NucleoBond® RNA Soil kit a single NucleoSpin® Bead Tube Type A, filled to the 1 mL mark with 250 to 500 mg of soil sample is sufficient and will result in ¼ of the larger kit's yield (0.25 – 2.5 µg RNA). Nucleic acid yield is mainly influenced by the type of soil and varies strongly from soil to soil.

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 supernatant of the bead tube is transferred into one fresh 1.5 mL tube and mixed with Binding Buffer E2. The incubation and centrifugation steps that follow can be used to prepare the NucleoBond® RNA Mini Columns. The columns are fixed on a NucleoBond® Rack Small (not supplied, see ordering information) or by the use of supplied Plastic Washers on top of a standard laboratory flask or a 50 mL centrifuge tube. Columns are equilibrated by Buffer EQU to prepare the anion exchange chromatography columns.

Once the columns are equilibrated, the clear supernatant of the centrifuged samples is loaded onto the purification columns.

Wash Buffer E3 is used as a first washing buffer for the column matrix. Nucleic acids and soluble polyanionic molecules including some fraction of humic substances (if present) will bind to the anion exchange surface, resulting in a brown silica matrix. Buffer E4 removes contaminants in a second washing step.

RNA is eluted by Buffer ERNA and afterwards DNA can be eluted by Buffer EDNA, supplied in the additional **DNA Set for NucleoBond® RNA Soil Mini**. If DNA isolation is not necessary, the DNA elution step may be skipped.

Isopropanol is added to the eluates and the mixture is bound to NucleoSpin® RNA Columns or NucleoSpin® DNA Columns by centrifugation. The columns are washed with Buffer E5, dried and eluted in RNase-free H_2O. Elution with 50 µL of RNase-free H_2O will result in highly concentrated nucleic acids but total yield might be decreased. Elution with 100 µL of RNase-free H_2O will result in a high total yield but decreased concentration.

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 Mini** kit is designed for the isolation of pure RNA from soil, the additional **DNA Set for NucleoBond® RNA Soil Mini** for the isolation of pure 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 Mini kit required)
Processing	Manual	Manual
Sample material	0.25 – 0.5 g soil	NucleoBond® RNA Soil Mini kit required
Elution volume	50 – 100 µL	50 – 100 µL
Preparation time	60 min/12 preps	15 min/12 preps
Typical yield	0.25 – 2.5 µg	1.25 – 12.5 µg
Use	For research use only	For research use only

2.3 Bead tubes and lysis

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. Alternatively, the MN Bead Tube Holder can be used in combination with a Vortex Genie II – Scientific Industries for 2 mL tubes.

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. The following parameters are a good starting point for further optimization:

- 5 m/s for 30 s on a FastPrep® instrument (twice to three times)
- 30 Hz for 5 min on a Retsch mill
- 2700 rpm for 5 min on a vortexer (horizontally)

Do not overfill the NucleoSpin® Bead Tubes! Lysis is most effective when having a ratio of one part beads to one part sample to two parts of lysis buffer in a 2 mL tube. Further increase of sample material will result in a reduced lysis activity and therefore in a reduced yield. For convenient processing the 1 mL mark of the skirted bead tubes can be used as a fill marking. NucleoSpin® Bead Tubes Type A filled to the 1 mL mark will contain the optimal range of 250 – 500 mg of sample, depending on soil density and water content.

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 15 – 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 each bottle of **Wash Buffer E5**.

4 Safety instructions

When working with the NucleoBond® RNA Soil Mini kit or DNA Set for NucleoBond® RNA Soil Mini 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 http://***www.mn-net.com/msds***).

Caution: Guanidin thiocyanate in Buffer E4 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 **NucleoBond® RNA Soil Mini** kit or **DNA Set for NucleoBond® RNA Soil Mini** kit 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 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

Before starting the preparation:

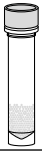
- Check Lysis Buffer E1 for precipitated SDS according to section 3.
- Check if Wash Buffer E5 was prepared according to section 3.
- Check if Phenol:Chloroform:Isoamylalcohol (25:24:1 v/v) is available.
- Equilibrate Phenol:Chloroform:Isoamylalcohol to room temperature and mix well!

!

1 Prepare Sample

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

Usually: 250 – 500 mg sample per tube.



fill tubes up to the 1 mL graduation mark

2 Add lysis buffers

Add **800 µL Lysis Buffer E1**.

Optional: Add 100 µL Buffer OPT.

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).

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

Vortex bead tube 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**

- **5 m/s** for **30 s** on a FastPrep® Instrument (twice to three times)
- **30 Hz** for **5 min** on a Retsch mill
- **2700 rpm** for **5 min** on a vortexer (horizontally)

See section 2.3 for details.

Recommended: use MN Bead Tube Holder in combination with a Vortex-Genie II (Scientific Industries Inc.).

Vortex RT, 5 min

4 Lysate clearing

Centrifuge samples for **2 min** at **full speed** (best: 20,000 × *g*).

20,000 x *g*
2 min

Transfer the supernatant into a fresh 1.5 mL tube (not supplied) and determine the approximate volume.

Transfer the supernatants to a 1.5 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 0.6 mL supernatant add 75 µL Buffer E2

to 0.7 mL supernatant add 87.5 µL Buffer E2

to 0.8 mL supernatant add 100 µL Buffer E2

Vortex for 5 s.

Vortex 5 s
2 min RT

Incubate for **2 min** at **room temperature** and **centrifuge** for **2 min** at **full speed** (best: 20,000 × *g*) afterwards.

20,000 x *g*
2 min

Proceed to step 6 (column setup and equilibration) during the incubation and centrifugation time.

6 Column setup and equilibration

For each soil sample, combine a **NucleoBond® RNA Mini Column** with a Plastic Washer (reusable) and place the combination on top of a laboratory flask or 50 mL centrifuge tube.

+ 1.5 mL Buffer EQU

Equilibrate columns with **1.5 mL Buffer EQU**.

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

See ordering information for a convenient NucleoBond® Rack Small instead of Plastic Washers and centrifuge tubes or flasks.

7 Load column

Load supernatant from centrifugation (step 5) into the column.

Load supernatant

Avoid transferring debris.

Attention: Flow through contains remaining PCI! Discard separately according to local guidelines!

8 **1st washing step**

Wash column with **250 µL Buffer E3**.

+ 250 µL Buffer E3

9 **2nd washing step**

Wash the NucleoBond® RNA Mini Column with **2 mL Buffer E4**.

+ 2 mL Buffer E4

10 **RNA elution**

Place a fresh 2.0 mL tube below the NucleoBond® RNA Mini Column.

Elute RNA with **1 mL Buffer ERNA**.

+ 1 mL Buffer ERNA

***Optional*: DNA elution** (DNA Set for NucleoBond® RNA Soil Mini required)

Place a fresh 2.0 mL tube below the NucleoBond® RNA Mini Column.

Elute DNA with **1 mL Buffer EDNA**.

11 **Precipitate nucleic acids**

Add **700 µL isopropanol** (not supplied) to each eluate.

Vortex 5 s.

+ 700 µL Isopropanol

12 **Bind nucleic acids**

Load **600 µL** of the resulting mixture into a **NucleoSpin® RNA Column** (blue, RNA) or a **NucleoSpin® DNA Column** (green, DNA) in a Collection Tube.

Centrifuge NucleoSpin® Columns for **15 s at 11,000 × *g***

Discard flow through and place the NucleoSpin® Columns back into the Collection tubes.

Repeat this step until all of the mixture has passed the silica of the NucleoSpin® Columns.

11,000 × *g* 15 s

13 Washing

Add 500 µL Buffer E5 to the center of the silica.

Centrifuge NucleoSpin® Columns for **15 s** at **11,000 × *g***

Discard flow through, place the NucleoSpin® Columns back into the Collection tubes.

Repeat this step once.

+ 500 µL Buffer E5

11,000 x *g* 15 s

14 Drying

Centrifuge empty NucleoSpin® Columns for **1 min** at **11,000 × *g***.

Transfer NucleoSpin® Columns to a fresh 1.5 mL centrifuge tube (not supplied).

Avoid contact between the outlet of the columns and the flow through.

11,000 x *g* 1 min

15 Elute nucleic acids

Add **50 µL** (high concentration) to **100 µL** (high yield) **RNase-free H_2O** to the center of the silica.

Centrifuge NucleoSpin® Columns for **1 min** at **11,000 × *g***

+ 100 µL RNase-free H_2O

11,000 x *g* 1 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 is mandatory to isolate long, intact RNA.
	Storage of soil sample • Storage conditions might alter RNA integrity and total amount of nucleic acids.
	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!
	Sample material • Usually the RNA content in a soil sample is much lower than the DNA content. DNA might originate from dead organic material as well. If the isolated RNA concentration is too low, try the larger version of this kit (see ordering information: REF 740140 and 740141).

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 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. Degraded DNA from dead cells will elute with intact RNA of living cells. 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.

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
NucleoBond® RNA Soil Mini	740142.10 / .50	10 / 50 preps
DNA Set for NucleoBond® RNA Soil Mini	740143.10 / .50	10 / 50 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
NucleoBond® Rack Small	740562	1 piece

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 costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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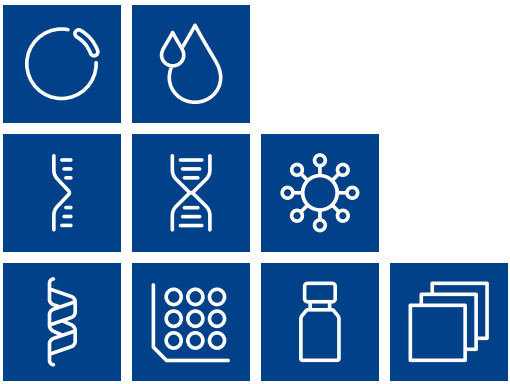
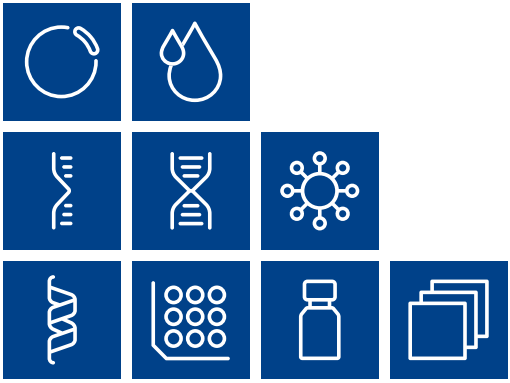


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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Management System
EN ISO 13485:2016
ISO 9001:2015

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