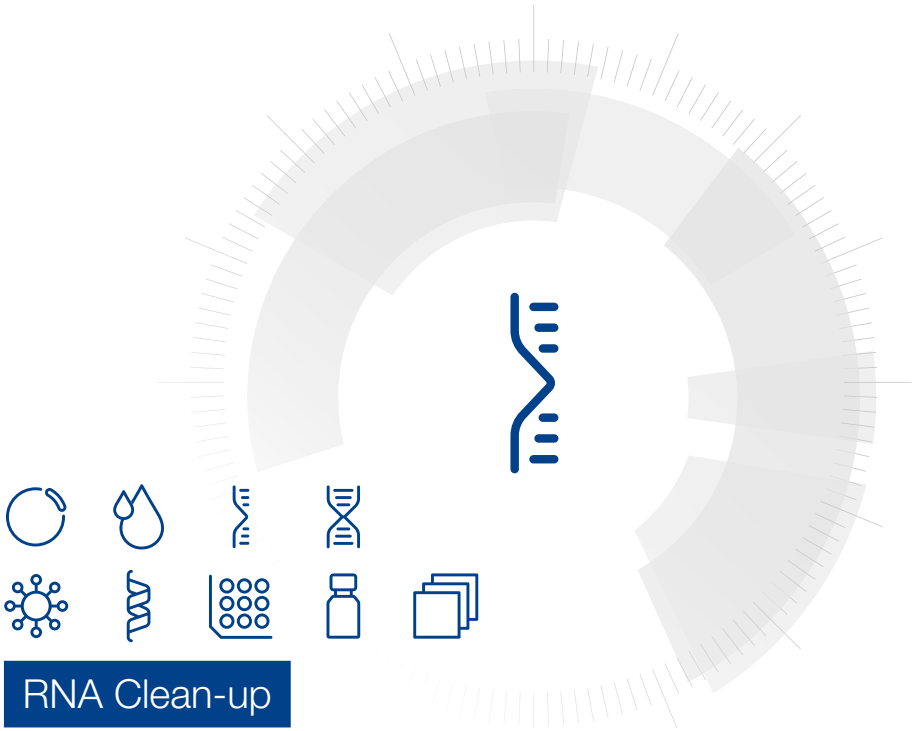
Bioanalysis

MACHEREY-NAGEL

User manual



RNA Clean-up

- NucleoBond® RS RNA Clean-up

October 2025 / Rev. 02

NucleoBond® RS RNA Clean-up

Protocol at a glance (Rev. 02)

	RS 10	RS 50	RS 100	RS 200
1 Sample preparation	Defrost sample if frozen at 4 °C or ice. Measure sample concentration and determine RNA amount/integrity. Dilute sample with RNase-free water to the recommended RNA input concentration range for optimal binding conditions: 0.5 – 4 mg/mL (higher for better binding conditions)			
Binding capacity	Up to 10 mg	Up to 45 mg	Up to 75 mg	Up to 140 mg
2 Adjustment of binding conditions	3 vol. parts (60 – 7.5 mL) NRB	3 vol. parts (270 – 33.75 mL) NRB	3 vol. parts (450 – 56.25 mL) NRB	3 vol. parts (840 – 105 mL) NRB
	Mix thoroughly Incubate 15 min at 4 °C			
3 Equilibration of the column	30 mL EQU @ 5 mL/min	120 mL EQU @ 10 mL/min	250 mL EQU @ 10 mL/min	400 mL EQU @ 10 mL/min
4 Binding RNA	Load the sample mixture onto the NucleoBond® RS column Recommended flow speeds: RS10 @ 1 mL/min RS50 – 200 @ 2 mL/min			
5 1st Wash	60 mL Buffer EQU @ 5 mL/min	225 mL Buffer EQU @ 10 mL/min	450 mL Buffer EQU @ 10 mL/min	850 mL Buffer EQU @ 10 mL/min
6 2nd Wash	60 mL Buffer NRW @ 5 mL/min	225 mL Buffer NRW @ 10 mL/min	450 mL Buffer NRW @ 10 mL/min	850 mL Buffer NRW @ 10 mL/min
7 Elution	40 mL Buffer NRE @ 5 mL/min	120 mL Buffer NRE @ 10 mL/min	250 mL Buffer NRE @ 10 mL/min	400 mL Buffer NRE @ 10 mL/min
8 Precipitation	40 mL Isopropanol	120 mL Isopropanol	250 mL Isopropanol	400 mL Isopropanol
	Mix thoroughly Incubate for 30 min at -20 °C 4,200 × g at 4 °C, 25 min			
9 Washing	5 mL 70 % EtOH 4,500 – 15,000 × g 4 °C, 15 min	15 mL 70 % EtOH 4,500 – 15,000 × g 4 °C, 15 min	25 mL 70 % EtOH 4,500 – 15,000 × g 4 °C, 15 min	40 mL 70 % EtOH 4,500 – 15,000 × g 4 °C, 15 min
10 Dry pellet	Dry approx. RT, 10 – 15 min	Dry approx. RT, 30 – 60 min	Dry approx. RT, 45 – 90 min	Dry approx. RT, 75 – 120 min
10 Reconstitution	Appropriate volume of H_2O-EF. It is recommended to choose the resuspension volume according to the requirements of the downstream application.			

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1 Components

1.1 Kit contents

Table 1: NucleoBond® RS columns

REF	Name
743502	NucleoBond® RS 10 (5 columns)
743503	NucleoBond® RS 50 (1 column)
743504	NucleoBond® RS 100 (1 column)
743505	NucleoBond® RS 200 (1 column)

Every column package contains a leaflet that guides you to the latest version of the user manual.

1.2 Recommended reagents, consumables and equipment available for purchase from MN

Note: The following reagents are recommended for optimal purification and are available as separate components.

Table 2: Reagents and consumables

REF	Name	Function	Volume
740371.1000 740371.3000	NRB	Binding buffer	1000 mL 3000 mL
740317.1000 740317.3000	EQU	Equilibration and Wash 1 buffer	1000 mL 3000 mL
740372.1000 740372.3000	NRW	Wash 2 buffer	1000 mL 3000 mL
740373.1000 740373.3000	NRE	Elution buffer	1000 mL 3000 mL
740798.1 (optional)	H_2O-EF	Endotoxin-free water	1000 mL
740401.50 (optional)	MN Sterilizer CA	Sterile filtration of sample input	50 pieces
740553.5 (recommended)	NucleoBond® Bottle Top Filter Type 2	Lysate filtration	5 pieces
740641 (recommended)	NucleoVac 96 Vacuum Regulator	Vacuum regulator for controlling of vacuum	1 piece
743511 (recommended)	NucleoBond® RS FPLC Adapter Set	Adapter set for FPLC system operation	1 set

Table 2: Reagents and consumables			
743510 (recommended)	NucleoBond® RS Starter Set	Adapter set for peristaltic pump operation	1 set

1.2.1 Quantities of consumables required per preparation

The amount of buffer required for a preparation and the number of preparations per bottle of buffer are listed in the following tables. These tables are intended only as a rough guideline for the minimum amount of reagents that is required. Depending on culture characteristics and experimental setup (e.g. dead volume of tubing), a larger amount may be required.

Table 3: Required buffers in terms of possible preparations per bottle, as well as other necessary consumables for **NucleoBond® RS 10 column**.

Table 3: Required quantities of consumables for NucleoBond® RS 10 column (Standard protocol)

Buffer name	REF	Container volume	Required volume per preparation	Theoretical number of preps
NRB	740371.1000	1 L	7.5 mL	133
	740371.3000	3 L		400
EQU	740317.1000	1 L	90 mL	8,3
	740317.3000	3 L		25
NRW	740372.1000	1 L	60 mL	33,3
	740372.3000	3 L		100
NRE	740373.1000	1 L	40 mL	25
	740373.3000	3 L		75
H_2O-EF	740798.1	1 L	Depending on follow up application	
	740798.3	3 L		

Table 4: Required buffers in terms of possible preparations per bottle, as well as other necessary consumables for **NucleoBond® RS 50 column**.

Table 4: Required quantities of consumables for NucleoBond® RS 50 column (Standard protocol)

Buffer name	REF	Container volume	Required volume per preparation	Theoretical number of preps
NRB	740371.1000	1 L	33.75 mL	29
	740371.3000	3 L		88
EQU	740317.1000	1 L	345 mL	2,8
	740317.3000	3 L		8,6
NRW	740372.1000	1 L	225 mL	4,4
	740372.3000	3 L		13,3
NRE	740373.1000	1 L	120 mL	8,3
	740373.3000	3 L		25
H_2O-EF	740798.1	1 L	Depending on follow up application	
	740798.3	3 L		

Table 5: Required buffers in terms of possible preparations per bottle for **NucleoBond® RS 100 column**.

Table 5: Required quantities of consumables for NucleoBond® RS 100 column (Standard protocol)

Buffer name	REF	Container volume	Required volume per preparation	Theoretical number of preps
NRB	740371.1000	1 L	56.25 mL	17
	740371.3000	3 L		53
EQU	740317.1000	1 L	700 mL	1,4
	740317.3000	3 L		4,2
NRW	740372.1000	1 L	450 mL	2,2
	740372.3000	3 L		6,6
NRE	740373.1000	1 L	250 mL	4
	740373.3000	3 L		12
H_2O-EF	740798.1	1 L	Depending on follow up application	
	740798.3	3 L		

Table 6: Required buffers in terms of possible preparations per bottle for **NucleoBond® RS 200 column**.

Table 6: Required quantities of consumables for NucleoBond® RS 200 column (Standard protocol)				
Buffer name	**REF**	**Container volume**	**Required volume per preparation**	**Theoretical number of preps**
NRB	740371.1000	1 L	105 mL	9
	740371.3000	3 L		28
EQU	740317.1000	1 L	1650 mL	0,6
	740317.3000	3 L		1,8
NRW	740372.1000	1 L	850 mL	1,1
	740372.3000	3 L		3,5
NRE	740373.1000	1 L	400 mL	2,5
	740373.3000	3 L		7,5
H_2O-EF	740798.1	1 L	Depending on follow up application	
	740798.3	3 L		

1.3 Recommended reagents, consumables and equipment to be supplied by user

1.3.1 Reagents

- Isopropanol (room-temperatured) , ACS or molecular biology grade
- 96 – 100 % ethanol (room-temperatured), non-denatured, ACS or molecular biology grade

Minimum amount of reagents and consumables required per preparation (standard protocol)				
Name	**RS 10**	**RS 50**	**RS 100**	**RS 200**
Isopropanol	40 mL	120 mL	250 mL	400 mL
Ethanol	10 mL	30 mL	50 mL	80 mL

1.3.2 Equipment and consumables

Peristaltic pump with a pump head, suitable for tubing with a tubing wall thickness of 1.6 mm.

- Flow rate: Minimum up to 10 mL/min (1.6 mm inner diameter tubing)
- Pressure: up to 7.0 kg/cm^2 (100 psi)
- Examples of tested pump and pump head combinations:

  Lead Fluid BT100 L + YT15

  Integra Biosciences DOSE IT Peristaltic Pump

  Heidolph HeiFLOW Precision 01 + SP quick (SWS 1,6)

Alternatively, an FPLC or HPLC system can be used, e.g. ÄKTApure™ system. The specifications remain unchanged as for the peristaltic pump.

- Recommended: PharMed BPT tubing (Saint-Gobain or similar)

  Recommended tubing material is made of TPE, is pyrogen-free and sterile. Depending on the application, verify the tubing meets required regulatory compliances e.g. FDA and USP criteria.
- Tubing inner diameter: 1.6 mm (1/16") for NucleoBond® RS columns.
- Tubing wall thickness must match the requirements of the pump head. A 1.6 mm tubing wall thickness is recommended.
- If an FPLC or HPLC system is used, please use suitable supported tubing.
- We offer suitable adapter sets for peristaltic pump mode (REF 743510 NucleoBond® RS Starter Set) and FPLC mode (REF 743511 NucleoBond® RS FPLC Set).

Sample clarification :

Removing particles from the sample solution will prevent column blockage. If column clogging is an issue, achieve a clarification efficiency of sterile filtration with a pore size of at least 0.45 µm, while keeping shear forces as low as possible to avoid damaging the RNA and thereby compromising RNA integrity. Sterile filtration can be performed using our MN Sterilizer CA (REF 740401.50) or similar.

Connectors, supplementary material for preparation on a peristaltic pump:

- Barbed Male Luer (Lock) to tubing connectors 1/16
- Laboratory stand
- Waste containers for flow-through
- RNase-free containers or vessels for the collection of filtrates and eluates
- RNase-free plastic or glassware and pipette tips. If glassware or metalware is used, heat it overnight at 230 °C to inactivate potential RNases from surface.
- Wet ice or refrigerator
- Heating container for elution buffer (Thermomix, waterbath)

Connectors, supplementary material for use on a liquid chromatography system (e.g. ÄKTA™):

Please note that the NucleoBond® RS column connectors consist of a 4.26 mm female luer connector and a Luerlock male connector. For example, the following connections can be used to operate NucleoBond® RS columns on an ÄKTA™ system. For your convenience, we offer a suitable adapter set to purchase from MN directly (NucleoBond® RS FPLC Set REF 743511) or separate adapter can be purchased by Index-HS or other suppliers

Table 7: Three exemplary connection possibilities for NucleoBond® RS columns on FPLC systems

Pieces needed	Connector name	Catalogue No. of supplier Index-HS https://www.idex-hs.com/
2	1/16 Connector to 10 – 32 male	F-120
1	Luer Male to 10 – 32 female	P-656
1	Luer female to 10 – 32 female	P-659
OR		
2	1/16 Connector to ¼-28 male	XP-206
1	Luer Male to ¼-28 female	P-675
1	Luer female to ¼-28 female	P-658

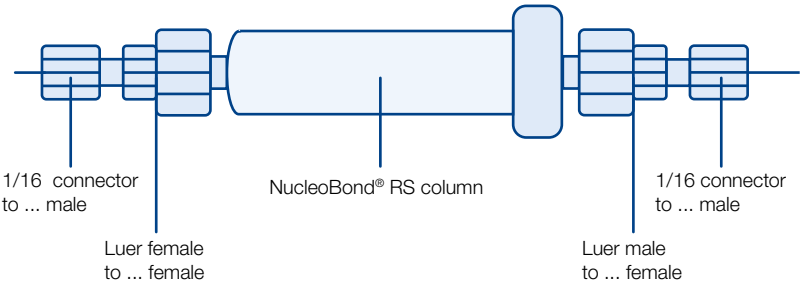

Figure 1 Example of NucleoBond® RS column setup on FPLC system.

2 About this user manual

General information about RNA can be found in section 3. A detailed description of the NucleoBond® RS Clean-up workflow and important information about sample preparation and clean-up steps are provided in section 4.

Section 5 provides information on storage and buffer preparation, and section 6 provides information on safety instructions and disposal of the kit.

First-time users are strongly advised to read these sections thoroughly before using this kit. Experienced users may proceed directly to the clean-up protocol (section 7) or use the Protocol at a Glance for a quick reference.

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the Internet at ***www.mn-net.com***.

Please contact Technical Support regarding information about changes of the current user manual compared to previous or updated revisions.

If additional information are required, such as regulatory support files, please contact our Technical Support.

3 Product description

3.1 The basic principle

The NucleoBond® RS Column RNA Clean-up Workflow is designed to clean large amounts of RNA from either pre-purified RNA (e.g. phenol-purified RNA) and RNA from reaction mixtures (e.g. DNase-treated samples, in vitro transcribed samples) or from enzyme reactions.

One of the most important aspects during handling of RNA is to prevent degradation of the RNA. With the Clean-up workflow, samples containing RNA are mixed with a solution to create appropriate binding conditions that allow RNA to be adsorbed onto the anion exchange resin. Washing steps with two different wash buffers remove impurities. Finally, pure RNA is eluted under high-salt conditions and by a shift of pH. Under these alkaline conditions the positive charge of the anion exchange resin is neutralized and RNA is released. For any downstream application it is necessary to precipitate the RNA and to remove salt and all traces of alcohol since they disturb or inhibit follow-up applications.

The RNA clean-up using NucleoBond® RS columns can be performed at room temperature. However, for best performance it is recommended to keep the sample cool until binding and to elute at elevated temperatures.

The eluate should be treated with care since RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints and dust. Keep RNA frozen at -20 °C for short-term or -70 °C for long-term storage to ensure RNA stability.

3.2 RNase-free working environment

An RNase-free working environment is a critical factor for performing successful RNA clean-up and handling. The sources of RNase contamination are diverse and can be found in tips, tubes, water, buffers, enzymes, RNA isolation method, laboratory surfaces and many others. Therefore, general recommendations to avoid RNase contamination should be followed:

- Maintain a separate area, dedicated pipettes and materials when working with RNA.
- Wear gloves when handling RNA and reagents to avoid contact with skin, which is a source of RNases. Change gloves frequently.
- Use RNase decontamination solution for working spaces.
- Use sterile RNase-free vessels.
- Use RNase-free water for resuspension.
- Keep all components sealed when not in use and all vessels tightly closed when possible.

3.3 Kit specifications

- **NucleoBond® RS RNA Clean-up** is recommended for the clean-up and concentration of prepurified RNA samples. Typical sample material covers milligram amounts of prepurified RNA (e.g. phenol-purified RNA) and RNA from reaction mixtures (e.g. DNase-treated samples, in-vitro transcribed samples).
- The isolated RNA is ready to use in diverse downstream applications.
- RNA isolated with the NucleoBond® RS columns is of high integrity. Obtained RIN (RNA Integrity Number) or RQN (RNA Quality Number) is predominantly determined by the integrity of the RNA within the sample.
- RNA molecules longer than approximately 200 nucleotides will typically recovered with rates of > 90 %. RNA molecules shorter than approximately 200 bp show lower recovery rates.

NucleoBond® RS columns are designed for large-scale isolation of plasmid DNA and RNA clean-up using peristaltic pumps or FPLC systems. In combination with the optimized NucleoBond® buffer chemistry, resembling the NucleoBond® RS workflow the NucleoBond® RS columns are able to achieve clean RNA samples. After two efficient washing steps removing contaminants, the RNA is eluted, precipitated, and easily reconstituted in H_2O-EF, TE-EF, or any other suitable buffer for further use.

- All **NucleoBond® RS columns** are resistant to organic solvents such as alcohol, chloroform & phenol and are suitable for buffers containing denaturing agents such as formamide, urea, or detergents such as Triton X-100 or NP-40.
- **The resin of all NucleoBond® RS columns** can be used over a wide pH range and can remain in contact with buffers for several hours without changing its chromatographic properties.
- For research use only (RUO).

NucleoBond® RS columns specifications at a glance	
Technology	Anion exchange technology
Format	RS column
Sample material	1 to 200 mg crude RNA
Typical recovery	≥ 80 %
$A_{260/280}$	≥ 2.0
$A_{260/230}$	≥ 2.0
Typical RIN (RNA Integrity Number)	equal to integrity of RNA input ± 1
Binding capacity	10 – 140 mg depending on column size
Use	For research use only

NucleoBond® RS column binding capacity of RNA		
REF	**Name**	**Theoretical binding capacity**
743502	NucleoBond® RS 10 (5 columns)	up to 10 mg
743503	NucleoBond® RS 50 (1 column)	up to 45 mg
743504	NucleoBond® RS 100 (1 column)	up to 75 mg
743505	NucleoBond® RS 200 (1 column)	up to 140 mg

Note: Compatibility with other buffers, than specifically optimized NucleoBond® RS buffers has not been tested.

4 NucleoBond® RS Clean-up workflow

4.1 Schematic setup and procedure of NucleoBond® RS columns

The **NucleoBond® RS column** is mounted on a clamp that is attached to a laboratory stand. Adjust the height of the column as required. The setup is shown in Figure 2. The column can be oriented vertically with a flow direction from top to bottom or bottom to top.

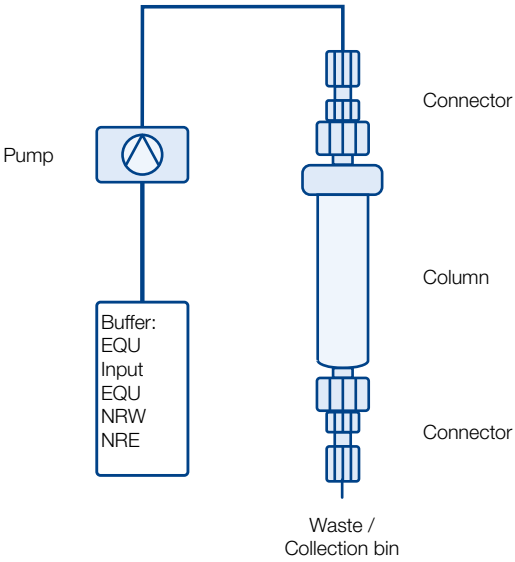

Figure 2 NucleoBond® RS RNA Clean-up workflow can be roughly divided into 7 steps. Sample preparation, Column Equilibration (EQU), Sample Binding (Input), Wash 1 (EQU), Wash 2 (NRW) and Elution (NRE), Desalting eluate.

4.2 Sample clarification

After sample preparation, it is recommended to clear the sample from particles and precipitates to ensure high RNA purity and a fast column flow rate. This can be achieved by using MN Sterilizer CA or any other filtration method that results in a filtration performance equivalent to a pore size of at least 0.45 µm. A gentle filtration procedure is recommended in order to minimize shear forces acting on the vulnerable RNA.

After clarification, the sample material can be loaded onto the **NucleoBond® RS column** without the risk of a gradual decrease in flow rate or column blockage. However, the maximum recommended flow rate of the column should be respected, especially during the binding step.

4.3 Washing of the column

To remove all traces of contaminants, like proteins and nucleotides off the **NucleoBond® RS column,** it is essential to wash the column in two subsequent wash steps. This ensures the highest yield with highest attainable purity.

4.4 Elution and concentration of RNA

Elution is performed under high salt conditions and by shifting the pH. Under these alkaline conditions, the positive charge of the anion exchange resin is neutralized and the RNA is released. For most subsequent applications, it is necessary to precipitate the RNA and remove salt and any traces of alcohol. Failure to remove will result in interference or inhibition of these applications.

All **NucleoBond® RS column** eluates already contain sufficient salt for isopropanol precipitation of RNA. Therefore, the precipitation can be performed by direct addition of 1 (v/v) volume of isopropanol. Use only chilled **isopropanol and ethanol.** Do not add the RNA solution into a vial containing isopropanol. Instead, **add isopropanol to the final eluate and mix immediately**. Follow the steps 8 – 11 in the **NucleoBond® RS** Clean-up workflow for desalting and concentration in section 7.2. Reconstitution of RNA is performed using RNase-free H_2O or slightly alkaline low salt buffer like TE-EF. Do not use pure water unless pH is definitely higher than 7.0.

4.5 Convenient stopping points

For optimal performance the clean-up should not be interrupted. The buffer flow can be interrupted for several hours as the column will not run dry. However, this may result in loss of RNA yield and decrease in RNA integrity.

The eluate should be processed immediately but can be stored at -20 °C for several days. Salt may precipitate during storage. If this occurs, resuspend the salt by warming the eluate to room temperature before starting the RNA precipitation.

5 Storage conditions and preparation of working solutions

All components can be stored at 15 – 25 °C and are stable until: see component/package label.

RNA intended to be used as sample for the **NucleoBond® RNA Clean-up** procedure should be handled with the same care as any RNA sample. The stability of prepurified RNA depends very much on the performed procedure. RNA in biological samples is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Similarly, prepurified RNA should be kept cold or frozen up to the point it is mixed with Binding Buffer NRB.

Before using of the **NucleoBond® RS column,** prepare the following:

- Prepare a 70 % ethanol solution with 96 – 100 % ethanol and endotoxin-free water for isopropanol precipitation.
- Precool Binding Buffer NRB in the refrigerator before adding to the sample material.

5.1 Stability of isolated RNA

Eluted RNA should immediately be put and always kept on ice during work for optimal stability! Contamination with almost omnipresent RNases (general lab ware, fingerprints, dust) may be a risk for isolated RNA. For short-term storage freeze at -20 °C, for long-term storage freeze at -70 °C.

6 Safety instructions

Wear appropriate protective clothing (e.g., lab coat, disposable gloves, and safety glasses) when working with the **NucleoBond® RS columns**. For more information, refer to the appropriate Material Safety Data Sheet (MSDS available online at ***www.mn-net.com/msds***).

NucleoBond® RS column waste has not been tested for residual infectious material. Contamination of liquid waste with residual infectious material is unlikely, but it cannot be completely excluded. Therefore, liquid waste must be considered infectious and should be handled and disposed of according to local safety regulations.

6.1 Disposal

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

7 Protocol

7.1 Analytical check of sample material

Before starting the **NucleoBond® RS RNA Clean-up**, it is recommended to check the sample material (concentration & integrity). Dilute the sample or reduce the volume to within the RS column's specifications. The concentration determined in the analytical check can be used to calculate the amount of sample required to optimize the utilization of the **NucleoBond® RS column**. The amount of sample used must be adjusted according to the maximum binding capacity of each column (see section 3.3).

7.2 NucleoBond® RS RNA Clean-up workflow

The following **NucleoBond® RS RNA Clean-up workflow** is based on the use of IVT RNA, but can be applied for pre-purified RNA or enzyme reactions as well. **NucleoBond® RS columns** are intended for single use only. The pressure within the column should not exceed 7 kg/cm^2 (100 psi) during use. Use only oven-baked glassware or pyrogen-free plasticware to avoid contamination with RNase. This is especially important during the elution, precipitation, and reconstitution steps.

	RS 10	RS 50	RS 100	RS 200
1	**Sample preparation** Defrost sample if frozen at 4 °C or ice. Dilute sample with RNase-free water to the recommended RNA input concentration range of 0.5 – 4 mg/mL for establishing optimal binding conditions. Provide at least 2.5/11.25/18.75/35.0 **mL sample volume** containing 10/45/75/140 mg crude RNA in a 50 mL tube or glass flasks (not provided).			
2	**Adjustment of binding conditions** Add **3 parts of chilled Binding Buffer NRB** to the sample and mix well by gentle vortexing or by pipetting several times up and down.			
	7.5 – 60 mL NRB	33.75 – 270 mL NRB	56.25 – 450 mL NRB	105– 840 mL NRB
	Incubate the sample mix on ice or 4 °C for at least 15 minutes. During the incubation period, continue with step 3.			
	> 15 min			

	RS 10	RS 50	RS 100	RS 200

3 Equilibration of the column

Mount the column upright on a laboratory frame. Equilibrate the NucleoBond® RS column in an upward direction with buffer EQU at a flow rate of 5 – 10 mL/min at room temperature.

Check all fitting connections for tightness and use cable ties to prevent leakage.

! *Note: If the column runs dry at any step during use, rehydrate the column bed by re-equilibrating the column with buffer EQU.*

30 mL EQU	120 mL EQU	250 mL EQU	400 mL EQU
5 mL / min	10 mL / min	10 mL / min	10 mL / min

4 Binding RNA

Load the sample onto the NucleoBond® RS column (equilibrated with buffer EQU) at the appropriate flow rate. Check the actual flow rate of the pump. Depending on the volume of the cleared lysate loaded on the NucleoBond® RS column, the flow rate may reduce which is a common observation during the binding phase. However, if the flow rate drops below half of the set rate stop the pump and refer to the troubleshooting in section 8.2.

! *Note: You may wish to save some or all of the flow through for analysis.*

Note: If the column runs dry, rehydrate the column bed by re-equilibrating the column with EQU buffer and continue with the protocol as normal.

1 mL / min	2 mL / min	2 mL / min	2 mL / min

5 1st Wash with Buffer EQU

Wash the NucleoBond® RS column with **Wash 1 buffer EQU**.

! *Note: If the column runs dry, rehydrate the column bed by re-equilibrating the column with EQU buffer and continue with the protocol as normal.*

60 mL EQU	225 mL EQU	450 mL EQU	850 mL EQU
5 mL / min	10 mL / min	10 mL / min	10 mL / min

6 2nd Wash with Buffer NRW

Wash the NucleoBond® RS column with **Wash 2 buffer NRW**.

! *Note: If the column runs dry, rehydrate the column bed by re-equilibrating the column with EQU buffer and continue with the protocol as normal.*

	RS 10	RS 50	RS 100	RS 200
	60 mL NRW	225 mL NRW	450 mL NRW	850 mL NRW
	5 mL / min	10 mL / min	10 mL / min	10 mL / min

7 Elution (Buffer NRE)

Elute the RNA with **Elution Buffer NRE** pre-heated to 80 °C. Collect the eluate in an oven-baked glass or RNase-free plastic vessel.

Depending on the size of the vessel, preheating can be performed in a Thermomix or in a water bath.

It is recommended to precipitate the eluate as soon as possible (step 8 – 11). However, the eluate can be stored in a closed container on ice for several hours.

Note: If possible, continuously check the RNA concentration at A_{260} during elution to obtain maximum yield and concentration by adjusting the elution volume. Store the eluate on ice during elution.

	40 mL NRE	120 mL NRE	250 mL NRE	400 mL NRE
	5 mL / min	10 mL / min	10 mL / min	10 mL / min

8 Precipitation (Isopropanol)

Note: It is highly recommended to determine the RNA yield by measuring A_{260} before precipitating the RNA. This helps to select the best buffer volume in step 16 and allows calculating the recovery after precipitation.

Add 1.0 volume of **room temperature isopropanol** to precipitate the eluted RNA. **Mix thoroughly by shaking.**

Incubate for 30 minutes at -20 °C temperature and pellet the RNA by centrifugation (15,000 x *g* for 30 min at 4 °C).

Carefully discard the supernatant. Take care not to discard the RNA pellet.

	1 .0 (v/v) volumes of isopropanol			
	40 mL	120 mL	250 mL	400 mL

9 Washing (70 % EtOH)

Add **70 % ethanol** at **room temperature** to the pellet.

Mix briefly and centrifuge (≥ 4,500 x *g*, preferably ≥ 15,000 x *g* for 15 min at 4 °C)**.**

Carefully remove ethanol completely from the container with a pipette tip.

Note: To reduce the risk of isopropanol carry-over, this step may be repeated.

Allow the pellet to dry at **room temperature or 37 °C.**

Note: RNA might be harder to dissolve when over-dried.

	5 mL	15 mL	25 mL	40 mL

	RS 10	RS 50	RS 100	RS 200

10 Dry pellet

Allow the pellet to dry at room temperature or 37 °C.

Note: RNA might be harder to dissolve when over-dried.

11 Reconstitution (Buffer TE-EF or H_2O-EF)

Dissolve the RNA pellet in an appropriate volume of RNase-free buffer for subsequent use.

Determine RNA yield by UV spectrophotometry.
Confirm RNA integrity by agarose gel electrophoresis or Bioanalyzer.

Note: It is recommended to choose the resuspension volume according to the requirements of the downstream application. Depending on the type of container, dissolve by gentle pipetting up and down or by constant spinning in a sufficient volume of buffer for 10 – 180 min (3D shaker).

8 Appendix

8.1 rDNase digestion in solution

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase and digested DNA) are required.

In order to obtain good results, i.e. keep the RNA integrity, the RNA has to be provided in an RNase-free solution, preferably water. Then, the high quality, RNase-free rDNase (REF 740963) can be used for DNA digestion in solution in order to remove even traces of contaminating DNA. Subsequently, RNA can be cleaned-up with NucleoBond® RS RNA Clean-up.

1. Digest DNA (Reaction setup)

Add 100 µL Reaction Buffer for rDNase and 10 µL rDNase per 1000 µL RNA solution.

Gently swirl the tube in order to mix the solution. Spin down gently (1 s at 1,000 x *g*) to collect every droplet of the solution at the bottom of the tube.

2. Incubate sample

Incubate for 10 min at 37 °C.

3. Purify RNA

Purify RNA with a NucleoBond® RS RNA Clean-up following section 7.

8.2 Troubleshooting

If you are experiencing problems with reduced yield or purity, it is recommended to sequentially check which clean-up step of the procedure is causing issues.

First, check the sample material. Second, aliquots of the binding flow-through, wash steps and eluate can be retained for further analysis by agarose gel electrophoresis or Bioanalyzer.

The following table describes some problems that may occur during preparation, explains the cause, and suggests how to resolve these issues:

Problem	Possible cause and suggestions
Low RNA recovery	*Highly degraded RNA* • Some RNA types, e.g. toroula yeast RNA, is commonly highly degraded with fragment length of < 50 nt. Such highly degraded RNA might cause reduced recovery rate if less than 1/3 theoretical binding capacity is used as sample. Use more sample material for such RNA types. *Low elution efficiency* • Ensure that the elution buffer NRE is pre-heated to 80 °C. Elution efficiency may be reduced to 20 % if the buffer temperature is too low.
Unexpected ratio $A_{260/280}$	*RNA type* • Ratio $A_{260/280}$ is base dependent. E.g. Poly-A+ RNA has a $A_{260/280}$ ratio of 3.3 – 3.7 and a $A_{260/230}$ ratio of 3.5 – 4.1. Consider the RNA base composition for interpretation of absorbance ratios.
Discrepancy between spectrophotometric and fluorescent dye based quantification methods	• Some RNA types (e.g. highly fragmented RNA like commercial toroula yeast RNA and homopolymeric RNA like poly-A+ RNA) have a reduced affinity to fluorecent dye based quantification methods like RiboGreen assays or Bioanalyzer assays. Consider this.

Problem	Possible cause and suggestions
NucleoBond® RS Column is blocked or very slow	*Sample is too viscous* • Dilute sample further with Binding Buffer NRB *Sample material contains particulate matter* • Filtrate sample material with MN Sterilizer or similar *High back pressure during clean-up* • Sample material contains particulate matter. Particulate matter may clog the inline filters. If necessary, perform a filtration of sample material. Be sure to equilibrate and use the column in the correct direction (see protocol and scheme). Make sure to use fresh or clean tubes and connectors. • If the flow rate decreases dramatically over time (to less than half of the set flow rate), the column is on the verge of blockage. In this case please stop the binding step immediately and continue with the next step of the protocol.
Genomic DNA or Plasmid DNA contamination of sample	• Perform a DNase digestion before applying the sample to the column.
Low purity (A_{260}/A_{280} < 2.0)	*Only minimal amounts of RNA were loaded onto the column* • Too much free binding capacity requires more extensive washing – double washing step with Buffer EQU.
No nucleic acid pellet formed after precipitation	*Pellet was lost* • Handle the precipitate with care. Decant solutions carefully. Determine RNA yield in Buffer NRE in order to estimate the bound RNA amount. *RNA might be smeared over the wall of the tube* • Dissolve RNA with an appropriate volume of RNase-free reconstitution buffer by rolling the container for at least 30 min. *RNA did not precipitate* • Check type and volumes of precipitating solvent. Make sure to use at least 1.0 volumes of isopropanol and mix thoroughly. • Centrifuge for longer periods of time at higher speed

Problem	Possible cause and suggestions
Nucleic acid pellet is opaque or white instead of clear and glassy	*Co-precipitation of salt* • Add another ethanol wash step to the precipitation procedure in step 9 to remove residual salt. • Check isopropanol purity, and perform precipitation at room temperature but centrifuge at 4 °C. • Do not let the eluate drip from the column into isopropanol but add isopropanol to the final eluate and mix immediately.
Nucleic acid pellet does not resuspend in buffer	*Pellet was over-dried* • Try to dissolve at higher temperatures for a longer period of time (e.g., 2 h at 37 °C or overnight at RT), preferably under constant spinning (3D-shaker). *Co-precipitation of salt or residual alcohol* • Wash the pellet again with 70 % ethanol, or increase the reconstitution buffer volume. *Insoluble particles in redissolved RNA* • Centrifuge the redissolved RNA to pellet the insoluble particles and transfer supernatant to a new tube. Insoluble particles do not affect RNA quality.
Purified RNA does not perform well in subsequent reactions	*RNA is contaminated with chromosomal DNA or plasmid DNA* • Refer to the detailed troubleshooting above. *RNA is contaminated with residual alcohol* • RNA was not dried completely before redissolving. Precipitate RNA again by adding 1/4 volume of Elution buffer and 1.0 volumes of isopropanol. Proceed with the precipitation protocol in this manual und dry RNA pellet completely. *RNA is degraded* • Make sure that your entire equipment (pipettes, centrifuge tubes, etc.) is clean and RNase-free.

8.3 Ordering information

Product	REF	Pack of
NucleoBond® RS 10	743502	5 columns
NucleoBond® RS 50	743503	1 column
NucleoBond® RS 100	743504	1 column
NucleoBond® RS 200	743505	1 column
Buffer NRB	740371.1000 / 740371.3000	1000 mL / 3000 mL
Buffer EQU	740317.1000 / 740317.3000	1000 mL / 3000 mL
Buffer NRW	740372.1000 / 740372.3000	1000 mL / 3000 mL
Buffer NRE	740373.1000 / 740373.3000	1000 mL / 3000 mL
H_2O-EF (nuclease free)	740798.1	1000 mL
MN Sterilizer CA	740401.50	50 pieces
NucleoBond® Bottle Top Filter Type 2	740553.5	5 pieces
NucleoVac 96 Vacuum Regulator	740641	1 piece
NucleoBond® RS FPLC Adapter Set	743511	1 set
NucleoBond® RS Starter Set	743510	1 set

8.4 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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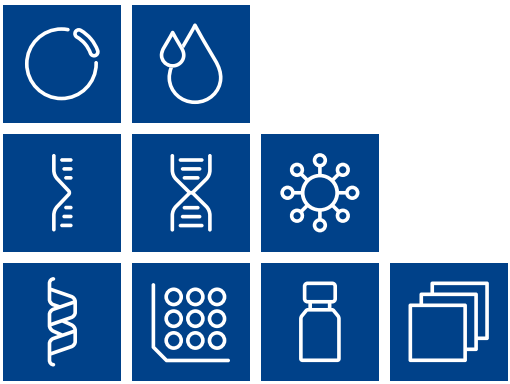
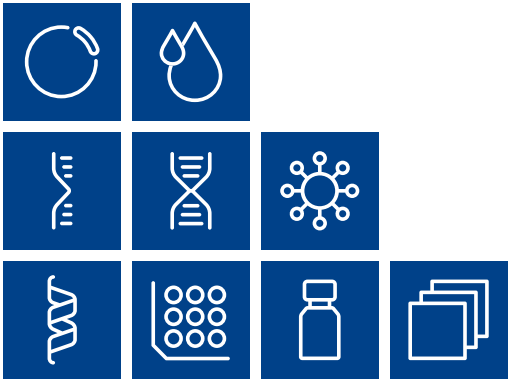


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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