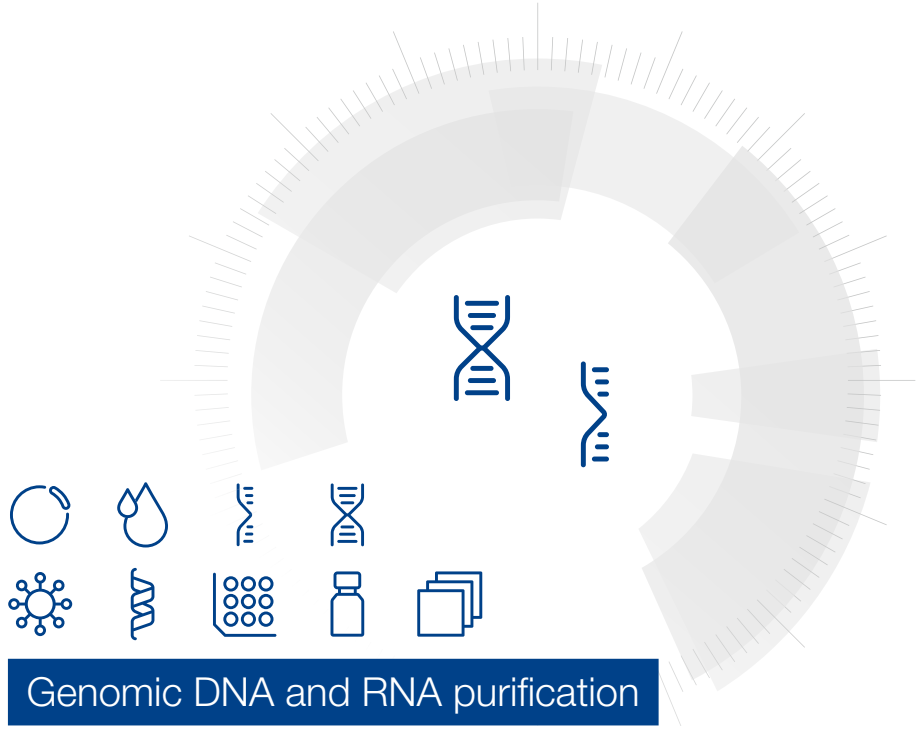
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA and RNA purification

- NucleoBond® RNA / DNA
- NucleoBond® CB
- NucleoBond® AXG Columns

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1 Components

1.1 Kit contents

	NucleoBond® RNA/DNA 80	NucleoBond® RNA/DNA 400
REF	**25 preps 740650**	**10 preps 740651**
Buffer W1	30 mL	30 mL
Buffer W3	10 mL	10 mL
Buffer W4	30 mL	30 mL
Buffer W5	80 mL	80 mL
Buffer W6	30 mL	30 mL
Buffer R0	2 × 125 mL	4 × 125 mL
Buffer R1	2 × 125 mL	2 × 125 mL
Buffer R2	2 × 125 mL	2 × 125 mL
Buffer R3	125 mL	125 mL
Buffer R4*	75 mL	75 mL
Buffer N5	125 mL	125 mL
Urea	36 g	36 g
NucleoBond® AXR 80 Columns	25	–
NucleoBond® AXR 400 Columns	–	10
Plastic Washer	10	5
User manual	1	1

* For preparation of working solutions and storage conditions see section 4.

1.1 Kit contents *continued*

	NucleoBond® CB 20	NucleoBond® CB 100	NucleoBond® CB 500
REF	**20 preps 740507**	**20 preps 740508**	**10 preps 740509**
Buffer G1*	125 mL	125 mL	2 × 125 mL
Buffer G2	125 mL	125 mL	125 mL
Buffer N2	70 mL	2 × 125 mL	2 × 125 mL
Buffer N3	125 mL	2 × 125 mL	2 × 125 mL
Buffer N5	32 mL	125 mL	125 mL
Saccharose*	15 g	15 g	2 × 15 g
Proteinase K (lyophilized)*	20 mg	40 mg	40 mg
Proteinase Buffer PB	8 mL	8 mL	8 mL
NucleoBond® AXG 20 Columns	20	–	–
NucleoBond® AXG 100 Columns	–	20	–
NucleoBond® AXG 500 Columns	–	–	10
Plastic Washer	10	10	5
User manual	1	1	1

* For preparation of working solutions and storage conditions see section 4.

1.1 Kit contents continued

	NucleoBond® Buffer Set III	NucleoBond® Buffer Set IV
Application	**Genomic DNA from bacteria and yeast**	**Genomic DNA from tissue**
REF	**740603**	**740604**
Buffer G2*	–	2 × 125 mL
Buffer G3*	125 mL	–
Buffer G4	60 mL	–
Buffer N2	2 × 125 mL	2 × 125 mL
Buffer N3	2 × 125 mL	2 × 125 mL
Buffer N5	125 mL	125 mL
RNase A (lyophilized)*	25 mg	2 × 25 mg
Proteinase K (lyophilized)*	2 × 50 mg	2 × 50 mg
Proteinase Buffer PB	8 mL	8 mL
User manual	1	1

* For preparation of working solutions and storage conditions see section 4.

1.1 Kit contents continued

	NucleoBond® AXG 20	NucleoBond® AXG 100	NucleoBond® AXG 500
REF	**740544**	**740545**	**740546**
NucleoBond® AXG 20 Columns	20	–	–
NucleoBond® AXG 100 Columns	–	20	–
NucleoBond® AXG 500 Columns	–	–	10
Plastic Washer	10	10	5
User manual	1	1	1

1.2 Reagents and equipment to be supplied by user

Reagents

- ß-mercaptoethanol
- Isopropanol (room-temperatured)
- 85 % or 70 % ethanol (room-temperatured; depending on protocol)
- Buffer for reconstitution of DNA, for example TE buffer or sterile H_2O
- Lysozyme (for the isolation of RNA / DNA from bacteria)
- Lyticase / zymolase (for the isolation of RNA / DNA from yeast)
- Please see the introduction of the related protocol for more detailed information.

Equipment

- Refrigerated centrifuge capable of reaching ≥ 5,000 x *g* with rotor for the appropriate centrifuge tubes or bottles
- Centrifugation tubes or vessels with suitable capacity for the volumes specified in the respective protocol
- NucleoBond® Rack Large, NucleoBond® Xtra Combi Rack (see ordering information), or equivalent holder

2 Introduction

2.1 Properties

NucleoBond® AX is a patented silica-based anion-exchange resin, developed by MACHEREY-NAGEL, for routine separation of different classes of nucleic acids. **NucleoBond® AX** Resin forms the basis for the entire line of nucleic acid purification products presented in this user manual. **NucleoBond® AX Resin** consists of hydrophilic, macro porous silica beads coupled to a methyl-ethylamine functional group. The functional group provides a high overall charge density that permits the negatively charged phosphate backbone of RNA or DNA to bind with high specificity to the resin. Due to a specialized manufacturing process that is rigorously controlled and monitored, the beads are uniform in diameter and contain particularly large pores. These special properties allow for optimum flow rates through the column and more efficient binding of nucleic acids to the matrix. Thus, using the matrix you can achieve sharp, well-defined elution profiles for individual nucleic acid species (see Figure 1). **NucleoBond® AX** can separate distinct nucleic acids from each other and from proteins, carbohydrates, and other unwanted cellular components. The purified nucleic acid products are suitable for use in the most demanding molecular biology applications, including transfection, in vitro transcription, automated or manual sequencing, cloning, hybridization, and PCR.

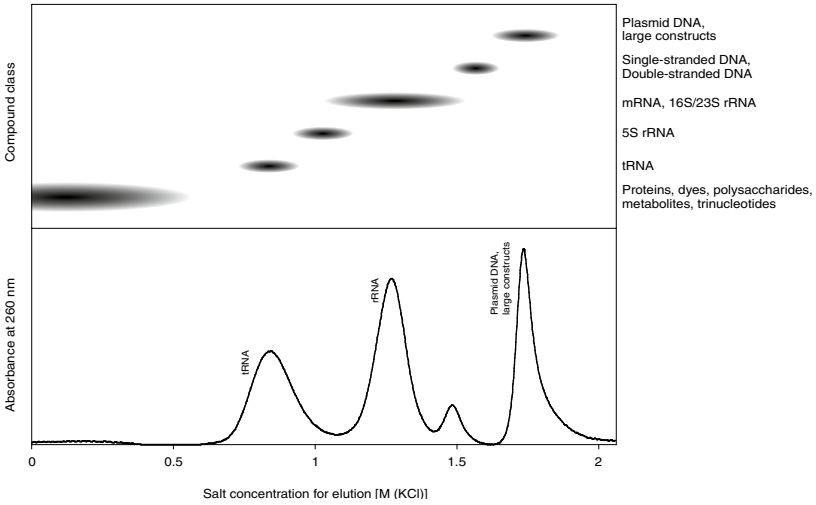

Figure 1:

Elution profile of NucleoBond® AX Resin at pH 7.0

The more interactions a nucleic acid can form between the phosphate backbone and the positively charged resin the later it is eluted with increasing salt concentration. Large nucleic acids carry more charges than short ones, double stranded DNA more than single stranded RNA.

2.2 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoBond® RNA/DNA** or **NucleoBond® CB** kits or **NucleoBond® AXG** in combination with a **NucleoBond® Buffer Set** is used for the first time.

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

The protocols in this manual (see overview in Table 1) are organized as follows:

The volumes of the respective buffers used for a particular column size are highlighted. Each procedural step is arranged like the following example (taken from section 8.3):

AXG 20	AXG 100	AXG 500

1 Cell dispruption

Thoroughly homogenize the tissue mechanically (Ultra Turrax) in Buffer G2. Alternatively, the tissue can be homogenized with a mortar and pestle under liquid nitrogen. The fine powder is dissolved in Buffer G2.

Note: Homogenize the tissue as good as possible. This step is very important for the lysis procedure as well as for a good flow rate of the NucleoBond® AXG Columns.

2 mL	10 mL	20 mL

For each step the name of the buffer, buffer volume, incubation times, repeats, or important handling steps are emphasized in **bold type** within the instruction. Additional notes or optional steps are printed in italic.

In the example shown above the tissue sample is mechanically disrupted in 2 mL (Mini prep with AXG 20 Columns), 10 mL (Midi prep with AXG 100 Columns) or 20 mL (Maxi prep with AXG 500 Columns) of Buffer G2.

Table 1: Protocol overview

Sample type	Sample size	Purification of	Section
NucleoBond® RNA/DNA			
Bacterial cells	0.5×10^8 (AXR 80) 2×10^9 (AXR 400)	RNA and DNA	6.1
Yeast	10^9 (AXR 80) $10^9 – 10^{10}$ (AXR 400)	RNA and DNA	6.1
Eukaryotic cells	$10^5 – 10^6$ (AXR 80) $10^6 – 5 \times 10^6$ (AXR 400)	RNA and DNA	6.1
	$10^6 – 5 \times 10^6$ (AXR 80) $5 \times 10^6 – 2 \times 10^7$ (AXR 400)	RNA and DNA	6.2
Tissue	20 mg (AXR 80) 100 mg (AXR 400)	RNA and DNA	6.2
Liquid samples, reaction mixtures	100 µL (AXR 80) 400 µL (AXR 400)	RNA and DNA	6.3
NucleoBond® CB			
Eukaryotic cells	5×10^6 (AXG 20) 2×10^7 (AXG 100) 10^8 (AXG 500)	DNA	7.1
Whole blood	0.1 – 1 mL (AXG 20) 2 – 5 mL (AXG 100) 5 – 20 mL (AXG 500)	DNA	7.1
Buffy coat	50 µL (AXG 20) 250 µL (AXG 100) 1 mL (AXG 500)	DNA	7.1
NucleoBond® AXG +NucleoBond® Buffer Set III			
Bacteria	2 – 4 mL (AXG 20) 15 – 20 mL (AXG 100) 60 – 80 mL (AXG 500)	DNA	8.1
Yeast	10^9 (AXG 20) $10^9 \times 10^{10}$ (AXG 100) $10^{10} \times 10^{11}$ (AXG 500)	DNA	8.2
NucleoBond® AXG +NucleoBond® Buffer Set IV			
Tissue	20 mg (AXG 20) 100 mg (AXG 100) 400 mg (AXG 500)	DNA	8.3

3 Product description

3.1 The basic principle

NucleoBond® RNA/DNA and **NucleoBond® CB**, as well as **NucleoBond® AXG** in combination with a **NucleoBond® Buffer Set** employ chaotropic salt or enzymatic lysis procedures to prepare a variety of sample materials for genomic DNA and RNA purification. After equilibrating the appropriate **NucleoBond® Column**, RNA and/or DNA are bound to the anion-exchange resin under low-salt conditions at an acidic pH of 6.3. Whereas RNA is digested for the purification of genomic DNA with NucleoBond® AXG and NucleoBond® Buffer Set, several different RNA species can be specifically washed out or eluted with NucleoBond® RNA/DNA using Buffers R1-R4 which contain increasing amounts of KCl at pH 6.3 (Table 2).
Finally high molecular weight DNA can be eluted after efficient washing of the column at a slightly alkaline pH. The RNA or DNA is then precipitated to remove the salt and dissolved in TE buffer or water for further use.

Table 2: Elution conditions for different RNA species

Compound	KCl salt concentration for elution	Wash with	Elute with
tRNA	0.45 – 0.65 M	Buffer R1	Buffer R2, R4
5S rRNA	0.65 – 0.85 M	Buffer R1/R2 (1 :1)	Buffer R3, R4
mRNA	0.70 – 1.15 M	Buffer R1/R2 (1 :1)	Buffer R3, R4
rRNA	0.95 – 1.10 M	Buffer R1/R2 (1 :1), Buffer R2	Buffer R3, R4
tRNA, 5S rRNA, mRNA, rRNA	0.45 – 1.15 M	Buffer R1	Buffer R3, R4

3.2 Kit specifications

NucleoBond® RNA/DNA purification kits contain **NucleoBond® AXR Columns** and appropriate buffers to purify high molecular weight RNA and DNA from eukaryotic cells, bacteria (up to 5×10^7 cells), tissue (up to 20 mg), yeast, liquid samples, and reaction mixtures. Kits are available with two column sizes (AXR 80 and 400) for 80 µg (NucleoBond® RNA/DNA 80) and 400 µg RNA (NucleoBond® RNA/DNA 400).

NucleoBond® CB purification kits contain **NucleoBond® AXG Colums** and appropriate buffers to purify high molecular weight genomic DNA from cell cultures (up to 2×10^7 cells) and blood (2 – 5 mL). Kits are available with three column sizes (AXG 20, 100, and 500) for 20 µg (NucleoBond® CB 20), 100 µg (NucleoBond® CB 100) and 500 µg DNA (NucleoBond® CB 500).

NucleoBond® AXG Columns (AXG 20, AXG 100, AXG 500) are available separately as well and can be combined with a **NucleoBond® Buffer Set III** to purify high molecular weight genomic DNA from bacteria and yeast or with **NucleoBond® Buffer Set IV** to purify genomic DNA from tissue.

NucleoBond® AXR and **AXG Columns** are polypropylene columns containing **NucleoBond® AX Silica Resin** packed between two inert filter elements. The columns are available in several sizes to accommodate a wide range of purification needs (see Table 3).

Table 3: NucleoBond® Column binding capacities

NucleoBond® Columns	Binding capacity
AXR 80	80 µg RNA
AXR 400	400 µg RNA
AXG 20	20 µg genomic DNA
AXG 100	100 µg genomic DNA
AXG 500	500 µg genomic DNA

All **NucleoBond® Columns** are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of DNase and RNase.

NucleoBond® AX Resin can be used over a wide pH range, from pH 2.5 – 8.5, and can remain in contact with buffers for up to three hours without any change in its chromatographic properties. After three hours, nucleic acids will begin to elute at increasingly lower salt concentrations. Normally, the resin remains functional in buffers containing up to 2 M salt. It remains intact in the presence of denaturing agents like formamide, urea, or common detergents such as Triton X-100 and NP-40.

4 Storage conditions and preparation of working solutions

All kit components can be stored at room temperature (15 – 25 °C) and are stable until: see package label.

Before starting the first **NucleoBond® RNA/DNA** purification, prepare the following:

- **Buffer R4:** Add 75 mL of Buffer R4 to 36 g Urea and mix thoroughly. Transfer all of the resulting Buffer R4 with Urea back to the Buffer R4 bottle. Indicate date of Urea addition. The solution will be stable at this temperature for at least 6 months.

Before starting the first **NucleoBond® CB** purification, prepare the following:

- **Buffer G1:** Add Saccharose to Buffer G1. After addition of Saccharose to Buffer G1 the buffer has to be stored at 4 °C and is stable for at least 3 months.
- **Proteinase K:** Add the indicated volume of **Proteinase Buffer PB** to dissolve lyophilized Proteinase K. Proteinase K solution is stable at -20 °C for at least 6 months.

Before starting the first **NucleoBond® Buffer Set III / IV** purification, prepare the following:

- **Buffer G2 (NucleoBond® Buffer Set IV):** Add 1 mL Buffer G2 to an **RNase A** vial and vortex. Transfer the resulting solution back into the Buffer G2 bottle and mix thoroughly. Indicate date of RNase A addition. Store Buffer G2 containing RNase A at 4 °C. The solution will be stable at this temperature for at least 6 months.
- **Buffer G3 (NucleoBond® Buffer Set III):** Add 1 mL Buffer G3 to an **RNase A** vial and vortex. Transfer the resulting solution back into the Buffer G3 bottle and mix thoroughly. Indicate date of RNase A addition. Store Buffer G3 containing RNase A at 4 °C. The solution will be stable at this temperature for at least 6 months.
- **Proteinase K:** Add the indicated volume of **Proteinase Buffer PB** to dissolve lyophilized Proteinase K. Proteinase K solution is stable at -20 °C for at least 6 months.

	NucleoBond® RNA/DNA 80	NucleoBond® RNA/DNA 400
REF	**25 preps 740650**	**10 preps 740651**
Buffer R4	75 mL Add 36 g Urea	75 mL Add 36 g Urea

	NucleoBond® CB 20	NucleoBond® CB 100	NucleoBond® CB 500
REF	**20 preps 740507**	**20 preps 740508**	**10 preps 740509**
Buffer G1	125 mL Add 15 g Saccharose	125 mL Add 15 g Saccharose to each bottle	2 × 125 mL Add 15 g Saccharose to each bottle
Proteinase K	20 mg Add 1 mL Proteinase Buffer PB	40 mg Add 2 mL Proteinase Buffer PB	40 mg Add 2 mL Proteinase Buffer PB

	NucleoBond® Buffer Set III	NucleoBond® Buffer Set IV
REF	**740603**	**740604**
Buffer G2	–	2 × 125 mL Add 25 mg RNase A to each bottle
Buffer G3	125 mL Add 25 mg RNase A	–
Proteinase K	2 × 50 mg Add 2.5 mL Proteinase Buffer PB to each vial	2 × 50 mg Add 2.5 mL Proteinase Buffer PB to each vial

5 Safety instructions

When working with the **NucleoBond®** kit 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 *www.mn-net.com/msds*).

Caution: Guanidine hydrochloride in buffer G4 and guanidinium thiocyanate in buffer W1 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®** 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 and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

5.1 Disposal

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

6 Protocols for NucleoBond® RNA/DNA 80 / 400

6.1 Isolation of RNA and genomic DNA from bacteria, yeast, and small amounts of eukaryotic cells

Before starting the preparation:

- Check if Buffer R4 was prepared according to section 4.
- Prepare lysozyme solution for RNA / genomic DNA from bacteria (250 – 1000 µg/mL TE, pH 8.0).
- Prepare lyticase/zymolase solution for RNA / genomic DNA from yeast (1 M sorbitol, 100 mM EDTA, 14 mM ß-mercaptoethanol, 50 – 100 U/mL lyticase or zzymolase pH 7.4).
- Check that isopropanol, ß-mercaptoethanol, and 85 % ethanol is available.

AXR 80	AXR 400

1 Sample preparation

Note: During the homogenization process the isolated genomic DNA can be sheared into small fragments and may partially appear in the RNA fraction. If a complete removal of genomic DNA is required, a DNase treatment after the precipitation of RNA or for example a subsequent LiCl precipitation of the isolated nucleic acids is recommended.

A For the isolation of **RNA from bacteria**:

An enzymatic treatment before starting the isolation is recommended. For this purpose add for example **lysozyme** to the bacterial cell pellet, resuspend it and incubate for **10 min** at **room temperature**. Depending on the bacterial strain other appropriate enzymes are also compatible with this method.

100 µL	400 µL

Afterwards, add **Buffer W1** and mix carefully.

400 µL	1.6 mL

B For the isolation of **RNA from yeast**:

Resuspend the yeast cell pellet in **lyticase / zymolase solution** and incubate for **30 min** at **30 °C**.

1 mL lyticase / zymolase	4 mL lyticase / zymolase

AXR 80	AXR 400

Centrifuge for **10 min** at **1.000 x *g*** to pellet the spheroblasts.

Remove the supernatant, add **Buffer W1** to the cell pellet and homogenize the lysis mixture by vortexing.

500 µL	2 mL

C For the isolation of **RNA from eukaryotic cells**:

Add **Buffer W1** to the cells. Homogenize the lysis mixture by pipetting up and down, vortexing by using a machanical disruption device (e.g., a PTFE homogenizer).

500 µL	2 mL

2 **Cell lysis and separation of proteins**

Add **ß-mercaptoethanol** to the solution and homogenize by vortexing. In order to reduce the viscosity pass the lysate 3 times through a sterile plastic syringe fitted with a 20 gauge needle.

0.5 µL	2 µL

Add **Buffer W3**, mix the sample and incubate for **5 min** at **4 °C**.

50 µL	200 µL

Add **Buffer W4**, mix the sample carefully and incubate for **5 min** at **room temperature**. Centrifuge the mixture at **10,000 x *g*** for **20 min** at **4 °C** in order to separate cellular debris and proteins.

500 µL	2 mL

Add **Buffer R0** to the supernatant and mix carefully.

10 mL	36 mL

Optional: If necessary, centrifuge the solution (10 min, 12,000 x g, 4 °C) in order to remove insoluble particles and to avoid clogging of the column, and collect the clear supernatant.

3 **Equilibration**

Equilibrate a NucleoBond® AXR Column with **Buffer R1**.

1 mL	3 mL

AXR 80	AXR 400

4 Binding

Transfer the clear supernatant of step 2 to the column. Collect the flow-through containing genomic DNA.

5 Wash

Wash the NucleoBond® AXR Column with:

Buffer R1 for purification of tRNA or tRNA / mRNA / rRNA

or **Buffer R1 / 2 (1:1)** for purification of rRNA / mRNA

or **Buffer R2** for purification of rRNA or viral RNA (stringent wash)

6 mL	12 mL

6 Elution

Elute the RNA with **Buffer R4** (preheating to 50 °C improves the yield of RNA but may increase DNA contamination). Alternatively, use Buffer R3 for elution to reduce DNA contamination. Yield, however, can be reduced as well.

3 mL	6 mL

***Optional* subsequent isolation of genomic DNA:**

Apply the flow-through (step 4) to the column and wash the column with ***Buffer R3*** *to remove residual RNA.*

3 mL	6 mL

Elute DNA with ***Buffer N5*** *preheated to 50 °C.*

3 mL	6 mL

7 Precipitation

Add **isopropanol** to the RNA (DNA) eluate, mix, incubate for **15 min on ice**, and centrifuge for **25 min** at **10,000 x *g*** and **4 °C**.

2.5 mL	5 mL

AXR 80	AXR 400
Wash the RNA (DNA) pellet with **85 % ethanol**, dry the pellet for **5 – 10 min**, and dissolve it in an appropriate buffer for further use.	
1 mL	**1 mL**
If complete removal of DNA (or RNA) is necessary for subsequent reactions an additional enzymatic treatment with DNase (or RNase) is recommended (see ordering information for rDNase Set or RNase). Alternatively, a further purification with NucleoBond® or NucleoSpin® kits is recommended.	

6.2 Isolation of RNA and genomic DNA from eukaryotic cells and tissue

Before starting the preparation:

- Check if Buffer R4 was prepared according to section 4.
- Check that isopropanol, ß-mercaptoethanol, and 85 % ethanol is available.

AXR 80	AXR 400

1 Cell lysis and separation of proteins

Sample preparation

Note: During the homogenization process the isolated genomic DNA can be sheared into small fragments and may partially appear in the RNA fraction. If a complete removal of genomic DNA is required, a DNase treatment after the precipitation of RNA or for example a subsequent LiCl precipitation of the isolated nucleic acids is recommended.

Add **Buffer W1** to the cells or tissue.

500 µL	2 mL

Add **ß-mercaptoethanol** to the solution and homogenize 3 – 4 times for each 20 s using a commercial homogenizer (e.g., Polytron, Dounce). Alternatively, other homogenization tools like mortar and pestle in the presence of liquid nitrogen may be used. In order to reduce the viscosity pass the lysate 3 times through a sterile plastic syringe fitted with a 20 gauge needle.

0.5 µL	2 µL

Add **Buffer W3**. Mix the sample and incubate for **15 min** at **4 °C**.

50 µL	200 µL

Add **Buffer W4**. Mix the sample carefully and incubate it for 15 min at 4 °C.

500 µL	2 mL

Centrifuge the mixture at **10,000 x *g*** for **20 min** at **4 °C** in order to separate cellular debris.

2 Precipitation of nucleic acids

Add **isopropanol** to the supernatant, mix carefully and incubate for **10 min on ice.**

850 µL	3.4 mL

AXR 80	AXR 400

Centrifuge the mixture at **10,000 x *g*** for **20 min** at **4 °C**.

Discard the supernatant and dissolve the RNA pellet in **Buffer W1** very carefully.

200 µL	800 µL

Note: If dissolution in Buffer W1 is not possible, try one of the following options:

a) Incubate at 65 °C for 1 – 3 min. Note that RNA might be damaged.

b) Dissolve pellet in Buffer W5 and Buffer W6 according to section 6.3*, step 1B*.

Optional: If total removal of DNA is necessary for subsequent reactions, an additional enzymatic treatment with DNase is recommended (see ordering information for rDNase Set). Dissolve the pellet in Reaction Buffer for rDNase and follow the instructions given in the rDNase Set leaflet. Finally add Buffer W1 and proceed as described above.

50 µL	100 – 200 µL

Optional: Remove insoluble particles by centrifugation (10 min, 12,000 x g, 4 °C) and collect the supernatant.

Add **Buffer R0** to supernatant and mix.

2 mL	8 mL

3 Equilibration

Equilibrate a NucleoBond® AXR Column with **Buffer R1**.

1 mL	3 mL

4 Binding

Transfer the clear supernatant of step 2 to the column. Collect the flow-through containing genomic DNA.

5 Wash

Wash the NucleoBond® AXR Column with:

Buffer R1 for purification of tRNA or tRNA / mRNA / rRNA

or **Buffer R1 / 2 (1:1)** for purification of rRNA / mRNA

or **Buffer R**2 for purification of rRNA or viral RNA (stringent wash)

AXR 80	AXR 400
6 mL	12 mL

6 Elution

Elute the RNA with **Buffer R4** (preheating to 50 °C improves the yield of RNA but may increase DNA contamination). Alternatively, use Buffer R3 for elution to reduce DNA contamination. Yield, however, can be reduced as well.

AXR 80	AXR 400
3 mL	6 mL

***Optional* subsequent isolation of genomic DNA:**

*Apply the flow-through (step 4) to the column and wash the column with **Buffer R3** to remove residual RNA.*

AXR 80	AXR 400
3 mL	6 mL

*Elute DNA with **Buffer N5** preheated to 50 °C.*

AXR 80	AXR 400
3 mL	6 mL

7 Precipitation

Add **isopropanol** to the RNA (DNA) eluate, mix, incubate for **15 min on ice**, and centrifuge for **25 min** at **10,000 x *g*** and **4 °C**.

AXR 80	AXR 400
2.5 mL	5 mL

Wash the RNA (DNA) pellet with **85 % ethanol**, dry the pellet for **5 – 10 min**, and dissolve it in an appropriate buffer for further use.

AXR 80	AXR 400
1 mL	1 mL

If complete removal of DNA (or RNA) is necessary for subsequent reactions an additional enzymatic treatment with DNase (or RNase) is recommended (see ordering information for rDNase Set or RNase). Alternatively, a further purification with NucleoBond® or NucleoSpin® kits is recommended.

6.3 RNA clean-up of liquid samples and reaction mixtures

AXR 80	AXR 400

1 Sample preparation

A For RNA-containing fluid samples or reaction mixtures:

Use the indicated sample volumes. If doubled volumes have to be processed use doubled volumes of Buffers W1, W3, and R0.

10 – 100 µL	40 – 400 µL

Add **Buffer W1** and proceed with step 2.

400 µL	1.6 mL

B For solid samples (e.g., RNA pellets):

Add **Buffer W5**. Dissolve the pellet very carefully, if necessary, by incubation at 65 °C for 1 – 3 min.

1.2 mL	7.5 mL

Add **Buffer W6**, mix, and proceed with the centrifugation of step 2.

400 µL	2.5 mL

C For low-salt RNA solutions (e.g., "run-off" transcripts, pre-purified RNA):

Add **1/5 volume Buffer R3** and proceed with step 3.

2 Adjustment of binding conditions

Add **Buffer W3**, mix the sample and incubate for **5 min** at **room temperature**.

50 µL	200 µL

Add **Buffer R0** to the supernatant and mix carefully.

5 mL	18 mL

If necessary, centrifuge the solution (10 min, 12,000 x *g*, 4 °C) in order to remove insoluble particles and to avoid clogging of the column, and collect the clear supernatant.

3 Equilibration

Equilibrate a NucleoBond® AXR Column with **Buffer R1**.

	AXR 80	AXR 400
	1 mL	3 mL

4 Binding

Transfer the clear supernatant to the column.

5 Wash

Wash the NucleoBond® AXR Column with:

Buffer R1 for purification of tRNA or tRNA / mRNA / rRNA

or **Buffer R1 / 2 (1:1)** for purification of rRNA/mRNA

or **Buffer R2** for purification of rRNA or viral RNA (stringent wash)

AXR 80	AXR 400
6 mL	12 mL

6 Elution

Elute the RNA with **Buffer R3** preheated to 50 °C.

AXR 80	AXR 400
3 mL	6 mL

7 Precipitation

Add **isopropanol** to the eluate, mix, incubate **on ice for 15 min**, and centrifuge for **25 min** at **10,000 x *g*** and **4 °C**.

AXR 80	AXR 400
2.5 mL	5 mL

The RNA pellet is washed with 85 % ethanol, dried for 5 – 10 min and dissolved in an appropriate buffer for further use.

AXR 80	AXR 400
1 mL	1 mL

If complete removal of DNA is necessary for subsequent reactions, an additional enzymatic treatment with DNase is recommended (see ordering information for rDNase Set).

7 Protocols for NucleoBond® CB 20 / 100 / 500

7.1 Isolation of genomic DNA from blood and cell cultures

Before starting the preparation:

- Check if Buffer G1, G2, and Proteinase K were prepared according to section 4.
- Before starting the procedure chill 20 mL ddH_2O on ice.

AXG 20	AXG 100	AXG 500

1 Cell disruption

Cell culture: After washing the cells twice with PBS and centrifugation resuspend the cells in PBS to a final concentration of 10^7 cells/mL.

Add 1 volume of **Buffer G1** (ice-cold) and 3 volumes **ddH_2O** (ice-cold) to 1 volume whole blood or cell suspension. Example: For 1 mL cell suspension (~10^7 cells) or 1 mL blood add 1 mL of Buffer G1 and 3 mL ddH_2O.

1 vol G1 3 vol ddH_2O	1 vol G1 3 vol ddH_2O	1 vol G1 3 vol ddH_2O

Mix the suspension by inverting the tube 6 – 8 times and incubate the mixture for **10 min on ice**.

Centrifuge the mixture at **4 °C (important)** for **15 min** at **1,300 – 1,500 x *g*** (around 3,500 rpm). Discard the supernatant. A small red pellet is visible.

Add **Buffer G1** (ice-cold) and **ddH_2O** (ice-cold) and resuspend the pellet by vortexing (~5 – 10 s). Centrifuge the mixture at **4 °C (important)** for **15 min** at **1,300 – 1,500 x *g*** (around 3,500 rpm). Discard the supernatant. The pellet should be almost white.

200 µL G1 750 µL ddH_2O	1 mL G1 3 mL ddH_2O	2 mL G1 6 mL ddH_2O

Small red spots on the pellet are not critical for the procedure. If the whole pellet is slightly red, repeat this washing step.

Add **Buffer G2** and completely resuspend the pellet by vortexing for 15 – 30 s

1 mL	5 mL	10 mL

Add **Proteinase K** (20 mg/mL) and incubate the mixture for **60 min** at **50 °C**.

50 µL	100 µL	200 µL

AXG 20	AXG 100	AXG 500

2 Equilibration

Equilibrate the NucloBond® AXG Column with **Buffer N2**

1 mL	2 mL	5 mL

3 Binding

Add **Buffer N2** (room temperature) to the sample. Vortex the mixture for 15 s at maximum speed. Load the sample onto the column. Allow it to enter the resin by gravity flow.

1 mL	5 mL	10 mL

4 Wash

Wash the column with **Buffer N3**.

3 × 1 mL	3 × 4 mL	3 × 8 mL

5 Elution

Elute the genomic DNA with **Buffer N5**. A second elution step with the same volume of elution buffer will increase the yield slightly (15 – 20 %).

1 mL	5 mL	8 mL

6 Precipitation

Add 0.7 volume of **isopropanol** (room temperature), mix, incubate **30 – 60 min** at **room temperature** and centrifuge at **4 °C** (~15,000 rpm) for **25 min**.

700 µL	3.5 mL	5.6 mL

If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g., TE, pH 8) overnight on a shaker or at 55 °C for 1– 2 hours. If a white pellet is obtained, additionally wash it with 70 % ethanol and redissolve it as described above.

8 Protocols for NucleoBond® AXG Columns and NucleoBond® Buffer Set III / IV

8.1 Isolation of genomic DNA from bacteria

For the isolation of genomic DNA from bacteria MACHEREY-NAGEL does not offer ready-to-use kits. The columns as well as the buffer solutions can be ordered separately. The NucleoBond® Buffer Set III (REF 740603) contains all necessary buffers (Buffers G3, G4, N2, N3, and N5), Proteinase K, as well as RNase A. Lysozyme is not included in this buffer set.

Please note:

Gram-positive bacteria are more difficult to lyse. Reagents like lysozyme, lysostaphin, etc. are recommended and compatible with this method. When using clinical samples, tissue, or other inhomogenous material for DNA isolation, additional homogenisation techniques (Ultra-Turrax, Dounce homogenisator, etc.) in combination with an enzymatic digest (lyticase, lysozyme, lysostaphin) may be necessary. In general follow our standard protocol for the isolation of genomic DNA from bacteria.

In order to obtain pure DNA as well as a good flow rate of the column an overloading of the column must be avoided!

For the first time it is better to start using a low cell number (AXG 20: 4×10^9, AXG 100: 2×10^{10}, AXG 500: 1×10^{11}, cells can be increased stepwise).

If bacteria are used that contain plasmid DNA and genomic DNA start with half of the culture volume recommended for non plasmid containing bacteria.

Before starting the preparation:

- Check if Buffer G3 and Proteinase K were prepared according to section 4.
- If lysozyme is required, redissolve it in sterile or ddH_2O (100 mg/mL).The solution should be divided in aliquots and stored at -20 °C.

AXG 20	AXG 100	AXG 500

1 Cell disruption

Pellet the bacterial cells from an appropriate volume of culture by centrifugation at **3,000 – 5,000 x *g*** for **10 min**. Discard the supernatant.

Resuspend the bacterial pellet in **Buffer G3** by vortexing.

1 mL	5 mL	8 mL

Add the ***lysozyme (optional)*** and the **Proteinase K** stock solution.

20 µL lysozyme 25 µL Proteinase K	20 µL lysozyme 25 µL Proteinase K	20 µL lysozyme 25 µL Proteinase K

Incubate the mixture at **37 °C**.

20 min	40 min	60 min

Add **Buffer G4** and mix by vortexing.

400 µL	1.2 mL	4 mL

Incubate the mixture at **50 °C** for **30 min**. If the lysate is not clear after incubation with Proteinase K the incubation time should be prolonged. If any insoluble cell components are observed, the sample should be clarified by a short centrifugation (5,000 x *g*, 5 min).

Note: It is very important to obtain a clear lysate in order to avoid clogging of the column.

2 Equilibration

Equilibrate the column with **Buffer N2.**

1 mL	2 mL	5 mL

3 Binding

Add **Buffer N2** (room temperature) to the sample. Vortex the mixture for 15 s at maximum speed. Load the sample onto the column. Allow it to enter the resin by gravity flow.

1 mL	5 mL	10 mL

AXG 20	AXG 100	AXG 500

4 Wash

Wash the column with **Buffer N3**.

3 × 1 mL	3 × 4 mL	3 × 8 mL

5 Elution

Elute the genomic DNA with **Buffer N5**. A second elution step with the same volume will increase the yield slightly (15 – 20 %).

1 mL	4 mL	8 mL

6 Precipitation

Add 0.7 volume of **isopropanol** (room temperature), mix, incubate **30 – 60 min** at **room temperature** and centrifuge at **4 °C** (~15,000 rpm) for 25 min.

700 µL	3.5 mL	5.6 mL

If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g., TE, pH 8) overnight on a shaker or at 55 °C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70 % ethanol and redissolve it as described above.

8.2 Isolation of genomic DNA from yeast

For the isolation of genomic DNA from yeast MACHEREY-NAGEL does not offer ready-to-use kits. The columns and the most important buffers can be ordered separately. Our NucleoBond® Buffer Set III (REF 740603) contains all necessary buffer solutions (Buffers G3, G4, N2, N3, and N5), Proteinase K, and RNase A. Sorbitol buffer as well as lyticase or zymolase stock solution have to be prepared fresh. These two enzymes are not included in the buffer set.

Before starting the preparation:

- Check if Buffer R4 was prepared according to section 4.
- Prepare lyticase / zymolase solution for total DNA from yeast (1 M sorbitol, 100 mM EDTA, 14 mM ß-mercaptoethanol, 50 – 100 U/mL lyticase or zymolase pH 7.4).
- Check that isopropanol and 70 % ethanol is available.

	AXG 20	AXG 100	AXG 500
1	**Cell disruption**		
	Resuspend the yeast cell pellet in **lyticase / zymolase solution** and incubate at **30 °C** for **30 min**.		
	600 µL lyticase / zymolase	**3 mL lyticase / zymolase**	**15 mL lyticase / zymolase**
	Centrifuge the mixture for **10 min** at **5,000 x *g*** to pellet the spheroblasts. Remove the supernatant and resuspend the cell pellet in **Buffer G3** by vortexing.		
	1 mL	**4 mL**	**12 mL**
	Add **Proteinase K** stock solution.		
	25 µL	**100 µL**	**450 µL**
	Incubate the mixture at **37 °C**.		
	20 min	**40 min**	**60 min**
	Add **Buffer G4** and mix by vortexing.		
	400 µL	**1.2 mL**	**4 mL**

AXG 20	AXG 100	AXG 500

Incubate the mixture at **50 °C** for **30 min**. If the lysate is not clear after incubation with Proteinase K the incubation time should be prolonged. If any insoluble cell components are observed, the sample should be clarified by a short centrifugation (5,000 x *g*, 5 min).

Note: It is very important to obtain a clear lysate in order to avoid clogging of the column.

2 Equilibration

Equilibrate the column with **Buffer N2**.

1 mL	2 mL	5 mL

3 Binding

Add **Buffer N2** (room temperature) to the sample. Vortex the mixture for 15 s at maximum speed. Load the sample onto the column. Allow it to enter the resin by gravity flow.

1 mL	5 mL	10 mL

4 Wash

Wash the column with **Buffer N3**.

3 × 1 mL	3 × 4 mL	3 × 8 mL

5 Elution

Elute the genomic DNA with **Buffer N5**. A second elution step with the same volume will increase the yield slightly (10 – 15 %).

1 mL	5 mL	8 mL

6 Precipitation

Add 0.7 volume of **isopropanol** (room temperature), mix, incubate **30 – 60 min** at **room temperature** and centrifuge at **4 °C** (~15,000 rpm) for 25 min.

700 µL	3.5 mL	5.6 mL

If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g., TE, pH 8) overnight on a shaker or at 55 °C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70 % ethanol and redissolve it as described above.

8.3 Isolation of genomic DNA from tissue

For the isolation of genomic DNA from tissue MACHEREY-NAGEL does not offer ready-to-use NucleoBond® kits. The columns as well as the buffer solutions can be ordered separately. Our NucleoBond® Buffer Set IV (REF 740604) contains all necessary buffer solutions (Buffers G2, N2, N3, and N5), Proteinase K, and RNase A.

Please note:

In order to achieve a high yield of DNA, the tissue samples should be kept in liquid nitrogen at all time before the preparation.

If the tissue sample is treated with 20 % glycerol or 20 % DMSO, centrifuge the sample. and discard the supernatant.

Before starting the preparation:

- Check if Buffer G2 and Proteinase K were prepared according to section 4.

AXG 20	AXG 100	AXG 500

1 Cell disruption

Thoroughly homogenize the tissue mechanically (Ultra Turrax) in **Buffer G2**. Alternatively, the tissue can be homogenized with a mortar and pestle under liquid nitrogen. The fine powder is dissolved in Buffer G2.

Note: Homogenize the tissue as good as possible. This step is very important for the lysis procedure as well as for a good flow rate of the NucleoBond® AXG Columns.

2 mL	5 mL	10 mL

Transfer the homogenate to a 15 or 50 mL screw cap tube. Add the **Proteinase K** stock solution (20 mg/mL) to the homogenate. Mix well by vortexing for 30 s.

25 µL	100 µL	450 µL

Incubate the sample at **50 °C** for **2 hours**. If the lysate is not clear after incubation with Proteinase K the incubation time should be prolonged. If any insoluble cell components are observed, the sample should be clarified by a short centrifugation (5,000 x *g*, 5 min).

Note: It is very important to obtain a clear lysate in order to avoid clogging of the column.

	AXG 20	AXG 100	AXG 500

2 Equilibration

Equilibrate the column with **Buffer N2.**

1 mL	2 mL	5 mL

3 Binding

Add **Buffer N2** (room temperature) to the sample. Vortex the mixture for 15 s at maximum speed. Load the sample onto the column. Allow it to enter the resin by gravity flow.

1 mL	5 mL	10 mL

4 Wash

Wash the column with **Buffer N3**.

3 × 1 mL	3 × 4 mL	3 × 8 mL

5 Elution

Elute the genomic DNA with **Buffer N5**. A second elution step with the same volume will increase the yield slightly (10 – 15 %).

1 mL	5 mL	8 mL

6 Precipitation

Add 0.7 volume of **isopropanol** (room temperature), mix, incubate **30 – 60 min** at **room temperature** and centrifuge at **4 °C** (~15,000 rpm) for **25 min**.

700 µL	3.5 mL	5.6 mL

If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g., TE, pH 8) overnight on a shaker or at 55 °C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70 % ethanol and redissolve it as described above.

9 Appendix

9.1 Troubleshooting

If any problems with the preparation arise proceed as follows: In order to get an idea of what has been the problem, please collect the flow-through, the wash and the eluate fraction. Precipitate the fractions and load them on an agarose gel. In combination with this troubleshooting guide this will help to solve your problem.

Problem	Possible cause and suggestions
No quantitative adsorption of nucleic acids	*Salt concentration of the sample is too high* • Dilute the sample or precipitate and redissolve it. *pH value of the sample is higher than pH 6.5* • Adjust the pH of the sample. *pH or salt concentrations of buffers are too high* • Adjust pH or prepare new buffers. *High viscosity sample* • Increase the volume of sample preparation buffers to reduce viscosity. *Column was overloaded with nucleic acid* • Use a bigger column or purify excess DNA on a new column. *No nucleic acid in the sample* • Check the pH of all buffers used and repeat the purification.
No elution of RNA, dsDNA or ssDNA	*No nucleic acid adsorbed* • See above *Salt concentration or pH of the washing buffer are too high* • Adjust pH or prepare a new buffer. *Salt concentration of the elution buffer or its pH are too low* • Adjust pH or prepare a new buffer.
No clear RNA separation	*Column overloaded* • Do not overload the column because this will result in decreased yield and purity of RNA preparations. If you are in doubt, use first a small amount of sample in order to find out the RNA content. Afterwards, use the appropriate amount of sample according to the limited lysing capacity of the lysis buffer and according to the capacity of the column as indicated in the protocol.

Column blocked	*Viscosity of the sample is too high* • Use larger buffer volumes for sample preparation. Use a prolonged centrifugation step to get a clear supernatant. Mix the sample with one volume of equilibration buffer.
RNA contamination in DNA fraction	*RNase A digestion was insufficient* • Add more RNase. Increase volume of wash buffer.
No nucleic acid after precipitation	*Nucleic acid pellet was lost* • Handle with care. *Nucleic acid was not resuspended* • Handle with care. *Nucleic acid was not precipitated* • Check organic solvent. Mix the suspension and use a longer centrifugation time.
Insufficient resuspension of purified nucleic acid	*Nucleic acid was overdried* • Dissolve for a longer time at somewhat higher temperature. *Residual salt or organic solvent in the pellet* • Wash the pellet with an organic solvent of low viscosity. • Increase buffer volume.
Strong white pellet after precipitation	*Coprecipitation of the salt* • Check the purity of the isopropanol. • Perform precipitation at room temperature (except centrifugation). • Do not let the eluate drop directly into a vial with isopropanol.

9.2 Ordering information

Product	REF	Pack of
NucleoBond® RNA/DNA 80	740650	25 preps
NucleoBond® RNA/DNA 400	740651	10 preps
NucleoBond® CB 20	740507	20 preps
NucleoBond® CB 100	740508	20 preps
NucleoBond® CB 500	740509	10 preps
NucleoBond® Buffer Set III	740603	1 set
NucleoBond® Buffer Set IV	740604	1 set
NucleoBond® AXG 20	740544	20 columns
NucleoBond® AXG 100	740545	20 columns
NucleoBond® AXG 500	740546	10 columns
NucleoBond® Xtra Combi Rack	740415	1
NucleoBond® Rack Large	740563	1
NucleoBond® Smart Rack	740413	1
rDNase Set	740963	1 set
RNase A	740505.50 740505	50 mg 100 mg

9.3 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-NAGELS 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.

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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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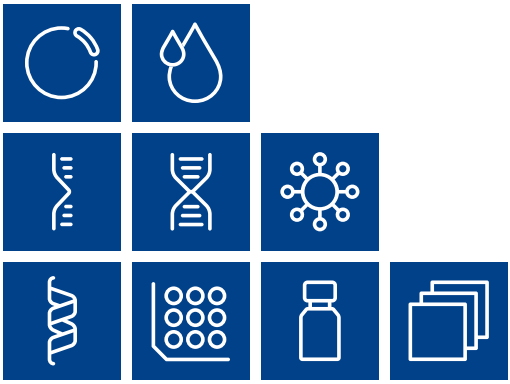
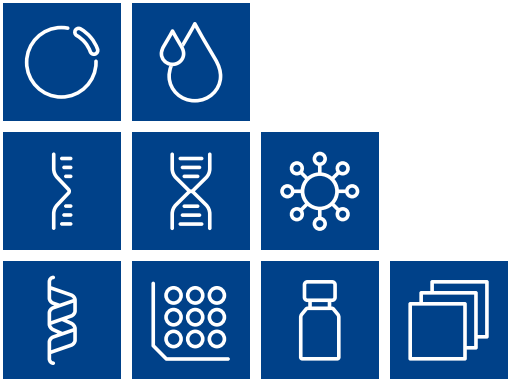


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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