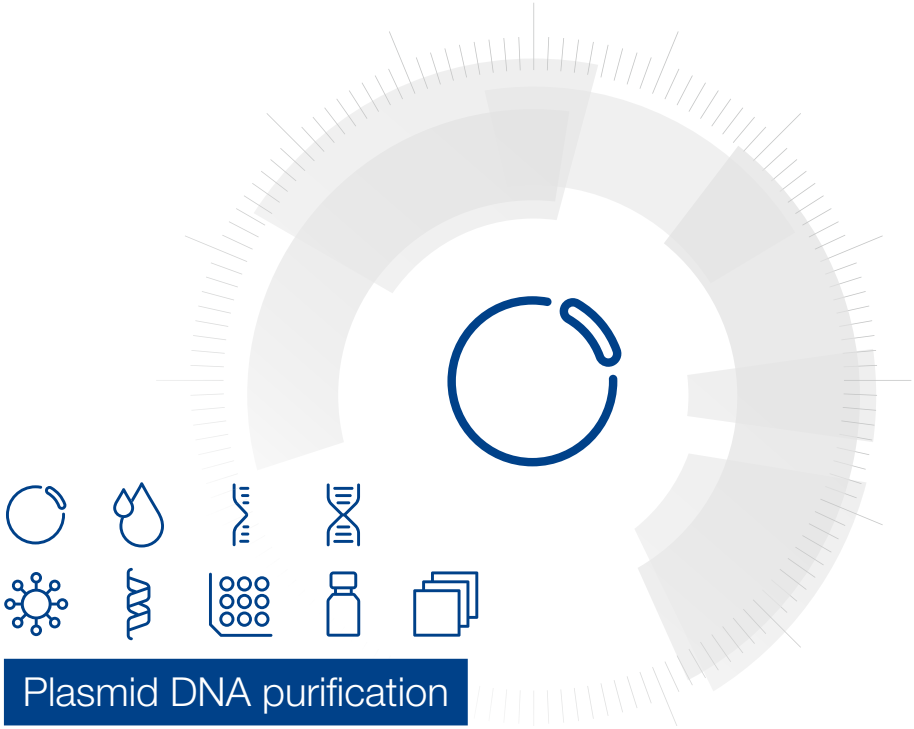
Bioanalysis

MACHEREY-NAGEL

User manual



Plasmid DNA purification

- NucleoBond® PC 20
- NucleoBond® PC 100
- NucleoBond® PC 500
- NucleoBond® BAC 100
- NucleoBond® PC 2000
- NucleoBond® PC 10000

September 2023 / Rev. 14

Plasmid DNA Purification (Mini, Midi, Maxi, Mega, Giga)

Protocol at a glance (Rev. 14)

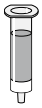
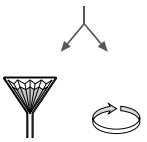
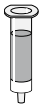
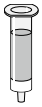
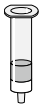
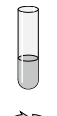
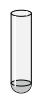
		Mini (AX 20)	Midi (AX 100)	Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)	
1	**Cultivate and harvest bacterial cells**	4,500 – 6,000 x *g* 4 °C, 15 min	4,500 – 6,000 x *g* 4 °C, 15 min	4,500 – 6,000 x *g* 4 °C, 15 min	4,500 – 6,000 x *g* 4 °C, 15 min	4,500 – 6,000 x *g* 4 °C, 15 min	
2	**Cell lysis**			High copy / low-copy			
	Buffer S1	0.4 mL / 0.8 mL	4 mL / 8 mL	12 mL / 24 mL	45 mL / 90 mL	120 mL / –	
	Buffer S2	0.4 mL / 0.8 mL RT, < 5 min	4 mL / 8 mL RT, < 5 min	12 mL / 24 mL RT, < 5 min	45 mL / 90 mL RT, < 5 min	120 mL / – RT, < 5 min	
	Buffer S3	0.4 mL / 0.8 mL 0 °C, 5 min	4 mL / 8 mL 0 °C, 5 min	12 mL / 24 mL 0 °C, 5 min	45 mL / 90 mL 0 °C, 5 min	120 mL / – 0 °C, 5 min	
3	**Equilibration of the column**	Buffer N2 1 mL	Buffer N2 2.5 mL	Buffer N2 6 mL	Buffer N2 20 mL	Buffer N2 100 mL	
4	**Clarification of the lysate**	Centrifugation ≥ 5,000 x *g* 15 min	Folded Filter or centrifugation ≥ 5,000 x *g* 25 min	Folded Filter or centrifugation ≥ 5,000 x *g* 40 min	Folded Filter or centrifugation ≥ 5,000 x *g* 50 min	Folded Filter or centrifugation ≥ 5,000 x *g* 60 min	
5	**Binding**	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column	
6	**Washing**	Buffer N3 High copy 2 × 1.5 mL Low copy 2 × 2 mL	Buffer N3 High copy 10 mL Low copy 12 mL	Buffer N3 High copy 32 mL Low copy 2 × 18 mL	Buffer N3 High copy 2 × 35 mL Low copy 2 × 50 mL	Buffer N3 High copy 2 × 100 mL	
7	**Elution**	Buffer N5 1 mL	Buffer N5 5 mL	Buffer N5 15 mL	Buffer N5 25 mL	Buffer N5 100 mL	
8	**Precipitation**	Isopropanol 0.75 mL ≥ 5,000 x *g* 4 °C, 30 min	Isopropanol 3.5 mL ≥ 5,000 x *g* 4 °C, 30 min	Isopropanol 11 mL ≥ 5,000 x *g* 4 °C, 30 min	Isopropanol 18 mL ≥ 5,000 x *g* 4 °C, 30 min	Isopropanol 70 mL ≥ 5,000 x *g* 4 °C, 30 min	
9	**Wash and dry DNA pellet**	70 % ethanol 500 µL ≥ 5,000 x *g* RT, 10 min 5 – 10 min	70 % ethanol 2 mL ≥ 5,000 x *g* RT, 10 min 5 – 10 min	70 % ethanol 5 mL ≥ 5,000 x *g* RT, 10 min 10 – 20 min	70 % ethanol 7 mL ≥ 5,000 x *g* RT, 10 min 30 – 60 min	70 % ethanol 10 mL ≥ 5,000 x *g* RT, 10 min 30 – 60 min	
10	**Reconstitute DNA**	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	

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Table of contents

1 Components

1.1 Kit contents

	NucleoBond® PC 20	
	20 preps	**100 preps**
REF	**740571**	**740571.100**
Resuspension Buffer S1	25 mL	100 mL
Lysis Buffer S2	25 mL	100 mL
Neutralization Buffer S3	25 mL	100 mL
Equilibration Buffer N2	25 mL	125 mL
Wash Buffer N3	125 mL	3 × 125 mL
Elution Buffer N5	32 mL	125 mL
RNase A (lyophilized)*	2.5 mg	10 mg
NucleoBond® AX 20 Columns	20	100
Plastic Washers	10	10
User Manual	1	1

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

1.1 Kit contents *continued*

	NucleoBond® PC 100	
	20 preps	**100 preps**
REF	**740573**	**740573.100**
Resuspension Buffer S1	100 mL	500 mL
Lysis Buffer S2	100 mL	500 mL
Neutralization Buffer S3	100 mL	500 mL
Equilibration Buffer N2	70 mL	500 mL
Wash Buffer N3	2 × 125 mL	1000 mL 125 mL
Elution Buffer N5	125 mL	1000 mL
RNase A (lyophilized)*	10 mg	50 mg
NucleoBond® AX 100 Columns	20	100
NucleoBond® Folded Filters	20	100
Plastic Washers	10	10
User Manual	1	1

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

1.1 Kit contents *continued*

	NucleoBond® PC 500			
	10 preps	**25 preps**	**50 preps**	**100 preps**
REF	**740574**	**740574.25**	**740574.50**	**740574.100**
Resuspension Buffer S1	150 mL	500 mL	750 mL	2 × 750 mL
Lysis Buffer S2	150 mL	500 mL	750 mL	2 × 750 mL
Neutralization Buffer S3	150 mL	500 mL	750 mL	2 × 750 mL
Equilibration Buffer N2	70 mL	200 mL	500 mL	1000 mL
Wash Buffer N3	2 × 250 mL	1000 mL	2 × 1000 mL	3 × 1000 mL 500 mL
Elution Buffer N5	200 mL	500 mL	1000 mL	2 × 1000 mL
RNase A (lyophilized)*	15 mg	50 mg	75 mg	2 × 75 mg
NucleoBond® AX 500 Columns	10	25	50	100
NucleoBond® Folded Filters XL	10	25	50	100
Plastic Washers	5	10	10	10
User Manual	1	1	1	1

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

1.1 Kit contents *continued*

	NucleoBond® PC 2000	NucleoBond® PC 10000
	5 preps	**5 preps**
REF	**740576**	**740593**
Resuspension Buffer S1	250 mL	750 mL
Lysis Buffer S2	250 mL	750 mL
Neutralization Buffer S3	250 mL	750 mL
Equilibration Buffer N2	125 mL	500 mL 125 mL
Wash Buffer N3	2 × 250 mL	1000 mL 125 mL
Elution Buffer N5	200 mL	500 mL 125 mL
RNase A (lyophilized)*	25 mg	75 mg
NucleoBond® AX 2000 Columns	5	–
NucleoBond® AX 10000 Columns	–	5
NucleoBond® Folded Filters XL	5	–
NucleoBond® Folded Filters Type 1	–	10
NucleoBond® Folded Filters Type 2	–	10
Plastic Washers	5	–
User Manual	1	1

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

1.1 Kit contents *continued*

	NucleoBond® BAC 100
	10 preps
REF	740579
Resuspension Buffer S1	250 mL
Lysis Buffer S2	250 mL
Neutralization Buffer S3	250 mL
Equilibration Buffer N2	70 mL
Wash Buffer N3	2 × 250 mL
Elution Buffer N5	200 mL
RNase A (lyophilized)*	25 mg
NucleoBond® BAC 100 Columns	10
NucleoBond® Folded Filters XL	10
Plastic Washers	5
User Manual	1

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

1.2 Reagents and equipment to be supplied by user

Reagents

- Isopropanol (room temperatured)
- 70 % ethanol (room temperatured)
- Ice
- Buffer for reconstitution of DNA (e.g., TE buffer or sterile H_2O)

Equipment

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37 °C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- Funnels to hold the NucleoBond® Folded Filters for lysate filtration (NucleoBond® PC 100, 500, 2000, 10000, BAC 100)
- NucleoBond® Rack (see ordering information) or equivalent holder
- Refrigerated centrifuge capable of reaching 5000 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

2 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoBond® Plasmid** kit is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

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

The protocols in this manual are organized as follows:

The volumes of the respective buffers used for a particular column size are high-lighted. Each procedural step is arranged like the following example (taken from section 7.2 High-copy plasmid purification):

	Mini	Midi	Maxi
	(AX 20)	**(AX 100)**	**(AX 500)**
1	**Cell lysis** Carefully resuspend the pellet of bacterial cells in **Buffer S1 + RNase A**.		
	0.4 mL	**4 mL**	**12 mL**

For example, if you are performing a Mini prep to purify plasmid DNA you will find volumes or incubation times in the white boxes.

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 pellet of the bacterial cells has to be resuspended in **0.4 mL** of **Buffer S1** when performing a Mini prep using **NucleoBond® AX 20 Columns**, in **4 mL** of **Buffer S1** when performing a Midi prep using **NucleoBond® AX 100 Columns**, and in **12 mL** of **Buffer S1** when performing a Maxi prep using **NucleoBond® AX 500 Columns**.

3 Product description

3.1 The basic principle

NucleoBond® PC/BAC kits employ a modified alkaline/SDS lysis procedure to prepare the bacterial cell pellet for plasmid purification. Both chromosomal and plasmid DNA are denatured under these alkaline conditions. Potassium acetate is then added to the denatured lysate, which causes the formation of a precipitate containing chromosomal DNA and other cellular compounds. The potassium acetate buffer also neutralizes the lysate. Plasmid DNA can revert to its native supercoiled structure and remains in solution. After equilibrating the appropriate **NucleoBond® Column** with equilibration buffer, plasmid DNA is bound to the anion-exchange resin and finally eluted after efficient washing of the column. After precipitation of the eluted DNA it can easily be dissolved in TE buffer for further use.

3.2 Kit specifications

- **NucleoBond® Plasmid** purification kits contain **NucleoBond® Columns**, buffers, and RNase A. Kits are available for each column size: Mini (PC 20), Midi (PC 100), Maxi (PC 500, BAC 100), Mega (PC 2000), and Giga (PC 10000).
- The protocols are suitable for purifying most plasmids and cosmids ranging from 3 – 50 kbp, and very large constructs (P1 constructs, BACs, PACs) up to 300 kbp.
- **NucleoBond® Columns** are available in several sizes to allow tailored purification of plasmid DNA from different culture volumes. (see Table 1).

Table 1: NucleoBond® Column binding capacities

NucleoBond® Column	Binding capacity
AX 20	20 µg
AX 100	100 µg
AX 500	500 µg
BAC 100	100 µg
AX 2000	2 mg
AX 10000	10 mg
Use	For research use only

- 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.

3.3 High-/low-copy plasmid purification

NucleoBond® PC kits are recommended for the isolation of high-copy plasmids (> 20 copies/cell), however, low-copy plasmids (< 20 copies/cell) can be isolated as well. Purifying low-copy plasmids requires additional buffers S1, S2 and S3 (see ordering information).

In connection with **NucleoBond® AX Columns** the **NucleoBond® Buffer Set I** can be used for the isolation of high-copy plasmids. In this combination it is sufficient for

- **NucleoBond® PC 500 Columns** (REF 740531) 5 high-copy plasmid preparations
- **NucleoBond® PC 100 Columns** (REF 740521) 10 high-copy plasmid preparations
- **NucleoBond® PC 20 Columns** (REF 740511) 50 high-copy plasmid preparations

The **NucleoBond® BAC 100** kit is recommended for the isolation of **low-copy plasmids** and contains sufficient buffer to perform 10 maxi preps. The kit contains BAC 100 columns, which can bind up to 500 µg of plasmid DNA. Typically yields are 10 – 100 µg from 500 mL fermentation broth depending on copy number and size of constructs.

The protocol for the isolation of low-copy plasmids using the NucleoBond® BAC 100 kit can be found in section 7.5.

3.4 Filtration of the lysate

After alkaline lysis, the solution has to be cleared from precipitated protein and cell debris in order to prevent clogging of the NucleoBond® Columns. Three options for lysate clarification are suitable:

1) Use the **NucleoBond® Folded Filters** provided with the kits. The gentle filtration prevents shearing of plasmids and large constructs, such as cosmid, PAC, or BAC DNA.

For NucleoBond® PC 100, 500, 2000, BAC 100 place one filter in a funnel of appropriate size. For NucleoBond® PC 10000 put a folded filter of type 1 into a folded filter of type 2 and place the combination in a funnel. The two types of filters differ in pore size to allow a fast and complete removal of large amounts of precipitate. Wet the filter(s) with a few drops of Equilibration Buffer N2 and load the bacterial lysate onto the wet filter(s). Either collect the flowthrough in a separate vessel or position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one time saving step (see Figure 2).

Figure 1 Correct use of the NucleoBond® Folded Filter, NucleoBond® Column placed in a Plastic Washer

2) Use **NucleoBond® Bottle Top Filters** for a very fast and complete, vacuum assisted filtration. Two different types of filters can be ordered separately (see ordering information) for NucleoBond® PC 2000 (Type 1) or NucleoBond® PC 10000 (Type 2). Attach a bottle top filter to a suitable flask (e.g., Schott), load the bacterial lysate and apply the vacuum (see Figure 2). The lysate is cleared and sterile filtered within 3 – 5 minutes and can then be loaded onto the NucleoBond® Column.

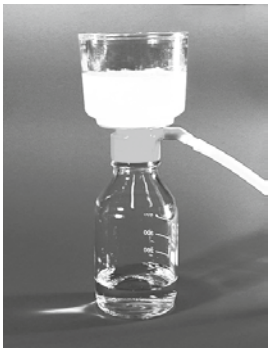


Figure 2 Correct use of the NucleoBond® Bottle Top Filter

3) Clearing the lysate by **centrifugation** is recommended for either very small sample volumes (NucleoBond® PC 20) or very large volumes (low-copy protocol). Centrifuge the lysate for 5 – 30 minutes at > 12.000 x *g* at room temperature or preferably 4 °C. Apply the cleared lysate directly to the NucleoBond® Column or pass the lysate through a wet folded filter to remove remaining particles before loading the column.

3.5 Elution procedure

Elution is carried out into a fresh tube with the volume of elution buffer indicated in the corresponding protocol. The plasmid DNA is precipitated by the addition of **room temperature (15 – 25 °C) isopropanol**. Do not let the plasmid DNA solution drop into a vial with isopropanol, because this leads to spontaneous co-precipitation of salt.

Only use **room temperature** isopropanol to prevent spontaneous co-precipitation of salt.

4 Storage conditions and preparation of working solutions

Attention: Buffer S2 contains sodium dodecylsulfate and sodium hydroxide. Wear gloves and goggles!

Storage conitions:

- All kit components can be stored at **15 – 25 °C** and are stable for up to two years.

Before you start any **NucleoBond®** plasmid DNA purification prepare the following:

- Dissolve the **lyophilized RNase A** by the addition of 1 mL **Resuspension Buffer S1**. Wearing gloves is recommended. Pipette up and down until the RNase A is dissolved completely. Transfer the RNase A solution back to the bottle containing Buffer S1 and shake well. Indicate date of RNase A addition. The final concentration of RNase A is 100 µg/mL Buffer S1. Store Buffer S1 with RNase A at 4 °C. The solution will be stable at this temperature for at least 6 months.
- **Lysis Buffer S2** should be stored at **15 – 25 °C** since the containing SDS may precipitate at temperatures below 20 °C. If precipitation occurs, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is redissolved. Check buffer for precipitated SDS immediately before each use by carefully inverting the bottle a few times and inspecting the solution from above. The buffer must be absolutely clear. Precipitated SDS will result in dramatically reduced plasmid yield!

5 Safety instructions

When working with the Nucleobond® PC kits, 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)***.

The waste generated with the Nucleobond® PC kits 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 RNase A treatment, but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according to 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 Optimization of the plasmid isolation procedure

6.1 Estimation of optimal culture volume

NucleoBond® PC kits are available in different formats ranging from mini to giga scale. See the table below for an overview of cell input and the corresponding expected yield.

Table 2: Culture volumes

Plasmid type	LB culture volume	Wet weight of pellet	Recommended column size	Average yield
High copy	1 – 5 mL	–	AX 20 (Mini)	3 – 20 µg
	5 – 30 mL	–	AX 100 (Midi)	20 – 100 µg
	30 – 150 mL	0.75 g	AX 500 (Maxi)	100 – 500 µg
	150 – 500 mL	2.5 g	AX 2000 (Mega)	500 µg–2 mg
	500 – 2,000 mL	10 g	AX 10000 (Giga)	2 mg–10 mg
Low copy	3 – 10 mL	–	AX 20 (Mini)	3 – 20 µg
	10 – 100 mL	–	AX 100 (Midi)	20 – 100 µg
	100 – 500 mL	1.5 – 2.2 g	AX 500 (Maxi)	100 – 500 µg
	100 – 500 mL	1.5 – 2.2 g	BAC 100 (Maxi)	100 µg
	500 – 2,000 mL	5 – 7.5 g	AX 2000 (Mega)	500 µg–2 mg

Please keep in mind that these are average values. The actual yield is depending on many factors during cultivation like copy number, propagation and size of plasmid, accumulation of toxic metabolic products, selective markers, choice of culture medium, incubation time and temperature and other factors.

These factors are independent of the plasmid purification procedure but can result in a low plasmid yield.

The capacity of the lysis buffer system is one important factor for all kinds of plasmid preparation procedures that follow a modified alkaline lysis. The modified alkaline lysis uses the detergent sodium dodecyl sulfate to disrupt bacterial cells. An alkaline pH, which is generated by the concentration of sodium hydroxide, is also important for effective lysis.

As a consequence, dodecyl sulfate and hydroxide ions are consumed during cell lysis. The more bacterial cells are resuspended in a certain amount of lysis buffer, the less efficient the lysis will be. Therefore, cell input and plasmid yield will always follow a linear increase until a maximum cell input is reached from where on lysis efficiency and thereby plasmid yield rapidly decrease.

Using more than the recommended culture volumes or using rich culture media, designed for reaching high cell densities, might result in suboptimal lysis and decreased yield.

To avoid excess cell input it is recommended to measure the optical density of the culture at 600 nm. Within the linear range of the photometer, usually from 0.1 to 1.0, the OD600 is proportional to the amount of cells. The dilution factor corrected OD600 gives an impression of the cell titer. Multiplication with the pelleted volume in mL is shown as ODV, which describes the amount of cells in a pellet.

For example: 30 mL of a culture grown to a dilution factor corrected OD600 of 3.0 would result in pellets of 3.0 × 30 = ODV 90.

The **NucleoBond® PC** kits use lysis buffer volumes which are much larger than typically needed and ensure complete plasmid release. Maximum OD600 values for the recommended culture volumes are given in the table below.

	Format	**Recommended culture volume**	**Maximum ODV** according to lysis buffer capacity	**Maximum OD_{600} at maximum recommended culture volume**
High copy	Mini (PC 20)	1 – 5 mL	20	4.0
	Midi (PC 100)	5 – 30 mL	200	6.7
	Maxi (PC 500)	30 – 150 mL	600	4.0
	Mega (PC 2000)	150 – 500 mL	2,250	4.5
	Giga (PC 10000)	500 – 2,000 mL	6,000	3.0
Low copy	Mini (PC 20)	3 – 10 mL	40	4.0
	Midi (PC 100)	10 – 100 mL	400	4.0
	Maxi (PC 500)	100 – 500 mL	1,200	2.4
	Maxi (BAC 100)	100 – 500 mL	1,200	2.4
	Mega (PC 2000)	500 – 2,000 mL	12,000	6.0

Maximum ODV values in the table above correspond to empiric data and should not be exceeded. If possible, decrease pelleted culture volume or increase volumes of Lysis Buffers S1, S2 and S3 equally to increase lysis capacity. An increase of 1 mL S1, S2 and S3 each gains another ODV 50 in lysis capacity.

For example: Using 15 mL of buffer S1, S2 and S3 each instead of 12 mL (additional 3 mL each), the ODV of a PC 500 buffer system can be increased to a maximum ODV of 750.

6.2 SDS precipitation in Buffer S2

SDS in buffer S2 is the main component for cell disruption but tends to precipitate at temperatures slightly below room temperature. During transportation and during storage in ware houses, ambient temperatures might be below the critical precipitation temperature. Upon SDS precipitation, buffer S2 loses its lysis activity resulting in no or extremely low plasmid yields. Precipitated SDS can easily be resolubilized by a short incubation at elevated temperatures (e. g., 37 °C for 5 minutes, mix carefully afterwards). Be aware that precipitated SDS might form a firm layer at the bottom of the bottle which can be difficult to see. Invert bottle with Lysis Buffer S2 carefully (avoid excessive foaming) and inspect the buffer for the presence of precipitates. **Always store buffer S2 at room temperature!**

7 NucleoBond® plasmid purification

7.1 General procedure

Prepare an overnight culture:

- Set up an overnight bacterial culture by inoculating the appropriate volume of LB medium (plus antibiotic) with a single colony picked from a freshly streaked plate. Shake the culture overnight (12 – 16 h) under selective conditions.

7.2 High-copy plasmid purification (Mini, Midi, Maxi)

	Mini	Midi	Maxi
	(AX 20)	(AX 100)	(AX 500)
1	**Cultivate and harvest bacterial cells**		
	Harvest bacteria from an LB culture by centrifugation at **4,500 – 6,000 x *g*** for **15 min** at **4 °C**.		
2	**Cell lysis**		
	Carefully resuspend the pellet of bacterial cells in **Buffer S1 + RNase A**.		
	0.4 mL	**4 mL**	**12 mL**
	Add **Buffer S2** to the suspension. Mix gently by inverting the tube 6 – 8 times. Incubate the mixture at **room temperature** for **2– 3 min** (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.		
	0.4 mL	**4 mL**	**12 mL**
	Add pre-cooled **Buffer S3 (4 °C)** to the suspension. Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. **Incubate** the suspension on ice for **5 min**.		
	0.4 mL	**4 mL**	**12 mL**
3	**Equilibration of the column**		
	Equilibrate a NucleoBond® AX 20 (Mini), AX 100 (Midi) or AX 500 (Maxi) Column with Buffer N2. Allow the column to empty by gravity flow. Discard flowthrough.		
	1.0 mL	**2.5 mL**	**6.0 mL**

	Mini	Midi	Maxi
	(AX 20)	(AX 100)	(AX 500)

4 Clarification of the lysate

NucleoBond® PC 20

Centrifuge the lysate at **≥ 5,000 x *g*** for **5 – 10 minutes** at room temperature or better 4 °C.

NucleoBond® PC 100, 500

Place a NucleoBond® Folded Filter in a funnel of appropriate size. Wet the filter with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter. Either collect the flowthrough in a separate vessel and proceed with step 5 **OR** position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one time saving step (skip step 5). See section 3.4 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

5 Binding

Load the cleared **lysate** from step 4 onto the NucleoBond® Column. Allow the column to empty by gravity flow.

6 Washing

Wash the column with **Buffer N3**. Repeat as indicated. Discard flowthrough.

2 × 1.5 mL	10 mL	32 mL

7 Elution

Elute the plasmid DNA with **Buffer N5**.

We recommend precipitating the eluate as soon as possible (step 8). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.

1 mL	5 mL	15 mL

Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 10).

8 Precipitation

Add **room temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **≥ 5,000 x *g*** for **30 min**. Carefully discard the supernatant.

0.75 mL	3.5 mL	11.0 mL

Mini	Midi	Maxi
(AX 20)	(AX 100)	(AX 500)

9 Wash and dry DNA pellet

Add **room temperature 70 % ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 5,000 x *g*** for **10 min** at **room temperature**.

500 µL	2 mL	5 mL

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature** no less than the indicated time.

Drying for longer periods of time will not harm the quality of plasmid DNA but over-drying may render the DNA less soluble.

5 – 10 min	5 – 10 min	10 – 20 min

10 Reconstitute DNA

Dissolve pellet in an appropriate volume of buffer TE or sterile deionized H_2O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).

Determine plasmid yield by UV spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

7.3 High-copy plasmid purification (Mega, Giga)

	Mega	Giga
	(AX 2000)	**(AX 10000)**

1 Cultivate and harvest bacterial cells

Harvest bacteria from an LB culture by centrifugation at **4,500 – 6,000 x *g*** for **15 min** at **4 °C**.

2 Cell lysis

Carefully resuspend the pellet of bacterial cells in **Buffer S1 + RNase A**.

45 mL	**120 mL**

Add **Buffer S2** to the suspension. Mix gently by inverting the tube 6 – 8 times. Incubate the mixture at **room temperature** for **2 – 3 min** (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

45 mL	**120 mL**

Add pre-cooled **Buffer S3 (4 °C)** to the suspension. Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. **Incubate** the suspension on ice for **5 min**.

45 mL	**120 mL**

3 Equilibration of the column

Equilibrate a NucleoBond® AX 2000 (Mega), AX 10000 (Giga) Column with **Buffer N2**. Allow the column to empty by gravity flow. Discard flowthrough.

20 mL	**100 mL**

4 Clarification of the lysate

For **NucleoBond® PC 2000** place a **NucleoBond® Folded Filter XL** in a funnel of appropriate size.

For **NucleoBond® PC 10000** put a **NucleoBond® Folded Filter of type 1** into a folded filter of **type 2** and place the combination in a funnel.

Wet the filter(s) with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter(s). Either collect the flowthrough in a separate vessel and proceed with step 5 **OR** position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one time saving step (skip step 5).

See section 3.4 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

	Mega (AX 2000)	Giga (AX 10000)

5 Binding

Load the cleared **lysate** from step 4 onto the NucleoBond® Column. Allow the column to empty by gravity flow.

Optional: You may want to save all or part of the flowthrough for analysis.

6 Washing

Wash the column with **Buffer N3**. Repeat as indicated. Discard flowthrough.

Mega	Giga
2 × 35 mL	**2 × 100 mL**

7 Elution

Elute the plasmid DNA with **Buffer N5**.

We recommend precipitating the eluate as soon as possible (step 8). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.

Mega	Giga
25 mL	**100 mL**

Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 10).

8 Precipitation

Add **room temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **≥ 5,000 x *g*** for **30 min**. Carefully discard the supernatant.

Mega	Giga
18 mL	**70 mL**

9 Wash and dry DNA pellet

Add **room temperature 70 % ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 5,000 x *g*** for **10 min** at **room temperature**.

Mega	Giga
7 mL	**10 mL**

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature** no less than the indicated time.

Drying for longer periods of time will not harm the quality of plasmid DNA but over-drying may render the DNA less soluble.

Mega	Giga
30 – 60 min	**30 – 60 min**

	Mega	Giga
	(AX 2000)	(AX 10000)

10 Reconstitute DNA

Dissolve pellet in an appropriate volume of buffer TE or sterile deionized H_2O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).

Determine plasmid yield by UV spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

7.4 Low-copy plasmid purification (Mini, Midi)

	Mini (AX 20)	Midi (AX 100)

1 Cultivate and harvest bacterial cells

Harvest bacteria from an LB culture by centrifugation at **4,500 – 6,000 x *g*** for **15 min** at **4 °C**.

2 Cell lysis

Carefully resuspend the pellet of bacterial cells in **Buffer S1 + RNase A**.

Mini	Midi
0.8 mL	**8.0 mL**

Add **Buffer S2** to the suspension. Mix gently by inverting the tube 6 – 8 times. Incubate the mixture at **room temperature** for **2 – 3 min** (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

Mini	Midi
0.8 mL	**8.0 mL**

Add pre-cooled **Buffer S3 (4 °C)** to the suspension. Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. **Incubate** the suspension on ice for **5 min**.

Mini	Midi
0.8 mL	**8.0 mL**

3 Equilibration of the column

Equilibrate a NucleoBond® AX 20 (Mini), AX 100 (Midi) Column with **Buffer N2**. Allow the column to empty by gravity flow. Discard flowthrough.

Mini	Midi
1 mL	**2.5 mL**

4 Clarification of the lysate

NucleoBond® PC 20

Centrifuge the lysate at **≥ 5,000 x *g*** for **5 – 10 minutes** at room temperature or better 4 °C.

NucleoBond® PC 100, 500

Place a NucleoBond® Folded Filter in a funnel of appropriate size. Wet the filter with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter. Either collect the flowthrough in a separate vessel and proceed with step 5 **OR** position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one timesaving step (skip step 5). See section 3.4 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

	Mini	Midi
	(AX 20)	(AX 100)

5 Binding

Load the cleared **lysate** from step 4 onto the NucleoBond® Column. Allow the column to empty by gravity flow.

6 Washing

Wash the column with Buffer N3. Repeat as indicated. Discard flowthrough.

Mini	Midi
2 × 2 mL	12 mL

7 Elution

Elute the plasmid DNA with **Buffer N5**. Preheating Buffer N5 to 50 °C prior to elution may improve yields for high-molecular weight constructs such as BACs.

We recommend precipitating the eluate as soon as possible (step 8). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.

Mini	Midi
1 mL	5 mL

Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 10).

8 Precipitation

Add **room temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **≥ 5,000 x *g*** for **30 min**. Carefully discard the supernatant.

Mini	Midi
0.75 mL	3.5 mL

9 Wash and dry DNA pellet

Add **room temperature 70 % ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 5,000 x *g*** for **10 min at room temperature**.

Mini	Midi
0.5 mL	2 mL

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature**.

Drying for longer periods of time will not harm the quality of plasmid DNA but over-drying may render the DNA less soluble.

Mini	Midi
5 – 10 min	5 – 10 min

	Mini (AX 20)	Midi (AX 100)
10	**Reconstitute DNA**	
	Dissolve pellet in an appropriate volume of buffer TE or sterile deionized H_2O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).	
	Determine plasmid yield by UV spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.	

7.5 Low-copy plasmid purification (Maxi/BAC, Mega)

	Maxi (AX 500 / BAC 100)	Mega (AX 2000)
1	**Cultivate and harvest bacterial cells**	
	Harvest bacteria from an LB culture by centrifugation at **4,500 – 6,000 x *g*** for **15 min** at **4 °C**.	
2	**Cell lysis**	
	Carefully resuspend the pellet of bacterial cells in **Buffer S1 + RNase A**.	
	24 mL	**90 mL**
	Add **Buffer S2** to the suspension. Mix gently by inverting the tube 6 – 8 times. Incubate the mixture at **room temperature** for **2 – 3 min** (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.	
	24 mL	**90 mL**
	Add pre-cooled **Buffer S3 (4 °C)** to the suspension. Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. **Incubate** the suspension on ice for **5 min**.	
	24 mL	**90 mL**
3	**Equilibration of the column**	
	Equilibrate a NucleoBond® AX 500 (Maxi), BAC 100 (Maxi), or AX 2000 (Mega) Column with **Buffer N2**. Allow the column to empty by gravity flow. Discard flowthrough.	
	6 mL	**20 mL**

	Maxi (AX 500 / BAC 100)	Mega (AX 2000)

4 Clarification of the lysate

Place a **NucleoBond® Folded Filter** in a funnel of appropriate size.

Wet the filter with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter. Either collect the flowthrough in a separate vessel and proceed with step 5 **OR** position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one time saving step (skip step 5).

See section **3.4** for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

5 Binding

Load the cleared **lysate** from step 4 onto the NucleoBond® Column. Allow the column to empty by gravity flow.

6 Washing

Wash the column with **Buffer N3**. Repeat as indicated. Discard flowthrough.

	2 × 18 mL	2 × 50 mL

7 Elution

Elute the plasmid DNA with **Buffer N5**. Preheating Buffer N5 to 50 °C prior to elution may improve yields for high-molecular weight constructs such as BACs.

We recommend precipitating the eluate as soon as possible (step 8). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.

	15 mL	25 mL

Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 10).

8 Precipitation

Add **room temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **≥ 5,000 x *g*** for **30 min**. Carefully discard the supernatant.

	11 mL	18 mL

	Maxi	Mega
	(AX 500 / BAC 100)	**(AX 2000)**
9	**Wash and dry DNA pellet**	
	Add **room temperature 70 % ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 5,000 x *g*** for **10 min** at **room temperature**.	
	5 mL	**7 mL**
	Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature** no less than the indicated time.	
	Drying for longer periods of time will not harm the quality of plasmid DNA but over-drying may render the DNA less soluble.	
	10 – 20 min	**30 – 60 min**
10	**Reconstitute DNA**	
	Dissolve pellet in an appropriate volume of buffer TE or sterile deionized H_2O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).	
	Determine plasmid yield by UV spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.	

8 Appendix

8.1 Determination of DNA yield and quality

- Plasmid yield is measured by UV spectroscopy by using the following relationship: 1 OD at 260 nm (1 cm path length) is equivalent to 50 µg plasmid DNA/mL.
- Plasmid quality is checked initially by running a 1 % agarose gel. This will give information on the percentage of ccc form/structural integrity of isolated plasmid DNA.
- Plasmid quality is checked by UV spectroscopy (quotient A_{260}/A_{280}). A value of 1.80 – 1.90 is an indication for pure plasmid DNA.
- Depending on further use of the purified plasmid, more sophisticated analytical methods may have to be applied for quantification of byproducts.

8.2 Troubleshooting

If you experience problems with reduced yield or purity, it is recommended to check which purification step of the procedure is causing the difficulties.

First, the bacterial culture has to be checked for sufficient growth (OD600) in the presence of an appropriate selective antibiotic (see Table 3). Second, aliquots of the cleared lysate, the flowthrough, the combined washing steps (Buffer N3), and the eluate should be kept for further analysis by agarose gel electrophoresis.

Table 3: Information about antibiotics according to Maniatis*

Antibiotic	Stock solution (concentration)		Storage	Working concentration	
Ampicillin	50 mg/mL	in H_2O	- 20 °C	20 – 50	µg/mL
Carbenicillin	50 mg/mL	in H_2O	- 20 °C	20 – 60	µg/mL
Chloramphenicol	34 mg/mL	in EtOH	- 20 °C	25 – 170	µg/mL
Kanamycin	10 mg/mL	in H_2O	- 20 °C	10 – 50	µg/mL
Streptomycin	10 mg/mL	in H_2O	- 20 °C	10 – 50	µg/mL
Tetracycline	5 mg/mL	in EtOH	- 20 °C	10 – 50	µg/mL

Refer to Table 4 to choose a fraction volume yielding approximately 5 µg of plasmid DNA. The volumes outlined in Table 4 refer to maximum yield/binding capacity of each column size used for the preparation (please also see Tables 1 and 2). **Precipitate the nucleic acids by adding 0.7 volumes of isopropanol, centrifuge the sample, wash the pellet using 70 % ethanol, centrifuge again, air dry for 10 minutes, dissolve the DNA in 100 µL TE buffer, pH 8.0, and run 20 µL on a 1 % agarose gel.**

Table 4: Volume recommendations for purification analyses

		Volume required [µL]			
Sample	**Purification step**	**PC 100**	**PC 500**	**PC 2000**	**PC 10000**
I	**Cleared lysate after protocol step 4**	600	400	300	200
II	**Column flowthrough after protocol step 5**	600	400	300	200
III	**Wash flowthrough after protocol step 6**	500	300	200	100
IV	**Eluate after protocol step 7**	300	200	100	100

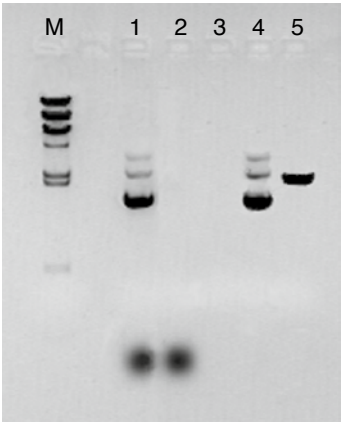
The exemplary gel picture (see Figure 3) will help you to address the specific questions outlined in the following section more quickly and efficiently.

It shows the dominant plasmid bands which should only be present in the eluate and in the lysate before loading to proof plasmid production in the bacterial culture (lane 1). Plasmid DNA found in the wash fractions, however, narrows down the problem to inappropriate washing conditions (e.g., wrong pH, buffer components precipitated, evaporation of liquid due to wrong storage).

RNA might be visible as a broad band at the bottom of the gel for the lysate and the lysate flowthrough samples (lane 1 and 2). It might also occur in the wash fraction but must be absent in the eluate.

Genomic DNA should not be visible at all but would show up in the gel slot or right below indicating e.g., too harsh lysis conditions.

* Maniatis T, Fritsch EF, Sambrook J: Molecular cloning. A laboratory manual, Cold Spring Harbor, Cold Spring, New York 1982.



M: Marker λ *Hin*dIII
1: I, cleared lysate, ccc, linear and oc structure of the plasmid, degraded RNA
2: II, lysate flow-through, no plasmid DNA, but degraded RNA
3: III, wash flow-through, no plasmid DNA or residual RNA
4: IV, eluate, pure plasmid DNA
5: *Eco*RI restriction, linearized form of plasmid

Figure 3 Exemplary analytical check of NucleoBond® PC 500 purification samples
Plasmid: pUC18, bacterial strain: *E. coli* DH5α®. 20 µL of each precipitated sample has been analyzed on a 1 % TAE agarose gel. Equal amounts of plasmid DNA before (lane 1) and after (lane 4) purification using NucleoBond® PC 500 are shown with a recovery of > 90 %.

Problem	Possible cause and suggestions
No or low plasmid DNA yield	*SDS- or other precipitates are present in the sample* • Load the S1 / S2 / S3 lysate sample onto the NucleoBond® Column immediately after finishing the initial lysis steps. SDS and cell debris are removed by filtration with NucleoBond® Folded Filters / NucleoBond® Bottle Top Filters but if the cleared lysate is stored on ice for a longer period, new precipitate may appear. If precipitate is visible, it is recommended to filter the lysate again immediately before loading it onto the NucleoBond® Column. *Sample/lysate is too viscous* • Watch maximal volumes and pellet wet weights given in the manual. Otherwise, filtration of the lysate and flow rate of the column will be insufficient. *Plasmid did not propagate* • Check plasmid content in the cleared lysate by precipitation of an aliquot. Use colonies from fresh plates for inoculation and add appropriate amounts of antibiotics to the culture media. *Alkaline lysis was inefficient* • If culture volume or pellet weight is too high, alkaline lysis becomes inefficient. Refer to the recommended culture volumes listed in Table 2, section 6.1. *Lysate prepared incorrectly* • After storage below 20 °C, SDS in Buffer S2 may precipitate causing inefficient lysis. Check Buffer S2 for precipitates before use and preheat the bottle to 30 – 40 °C if necessary in order to redissolve SDS.

Problem	Possible cause and suggestions
Column is blocked	*Sample is too viscous* • Do NOT attempt to purify lysate prepared from a culture volume larger than recommended for any given column size. Increasing culture volumes not only block the column, but can also reduce yields due to inefficient lysis. *Precipitates occur during storage* • Check cleared lysate for precipitates, especially if the lysate was stored for a longer time before loading. If necessary, clear the lysate again by filtration. *Lysate was not completely cleared* • Centrifuge at higher speed for a longer period of time, or use additional NucleoBond® Folded Filters to clear the lysate.
Cellular DNA or RNA contamination of plasmid DNA	*Lysis treatment was too harsh* • Be sure not to incubate the lysate in Buffer S2 for more than 5 min. *Excessive mixing during lysis allowed genomic DNA to shear off into the lysis buffer* • If the lysate is too viscous to mix properly or gently, reduce culture volumes. *RNase digestion was inefficient* • RNase was not added to Buffer S1 or stored too long. Add new RNase to Buffer S1. See ordering information, section 8.3.
No nucleic acid pellet formed after precipitation	*Pellet was lost* • Handle the precipitate with care. Decant solutions carefully. Measure DNA yield in Buffer N5 in order to calculate the expected plasmid DNA amount that should be recovered after precipitation. *Pellet did not resuspend in buffer* • Again, handle the pellet with care. Especially, if the DNA was precipitated in a > 15 mL tube the “pellet” may be spread at the inner side of the tube. Dissolve DNA with an appropriate volume of TE buffer by rolling the tube for at least 30 min. *Nucleic acid did not precipitate* • Check volumes of precipitating solvent, making sure to use at least 0.7 volumes of isopropanol and centrifuge for longer periods of time.

Problem	Possible cause and suggestions
Nucleic acid pellet does not resuspend in buffer	*Pellet was over dried* • Try dissolving at higher temperatures for a longer period of time (e.g., 2 h at 37 °C or overnight at RT), best under constant spinning (3D-shaker). *Residual salt or organic solvent in the pellet* • Wash the pellet with additional low-viscosity organic solvent (70 % ethanol), or increase the resuspension buffer volume.
Nucleic acid pellet is opaque or white instead of clear and glassy	*Salt has co-precipitated with the pellet* • Use room temperature isopropanol and check isopropanol purity. Do not precipitate by allowing the eluate to drip directly from the column into a tube containing isopropanol. Add isopropanol only after eluate has been collected. • Try resuspending the pellet in Buffer N2, and reload onto the NucleoBond® Column. Be sure to wash the column several times with Buffer N2 before loading the redissolved pellet onto the column.
Purified plasmid does not perform well in subsequent reactions	*DNA is* con*taminated with cellular debris or genomic DNA due to inefficient ly*sis • Reduce the culture volume, or increase the amount of Buffer S1, S2, and S3 used during the lysis steps. *DNA is degraded* • Make sure that all equipment (pipettors, centrifuge tubes, etc.) are clean and nuclease-free. Make sure that the alkaline lysis step (i.e., the incubation of sample after addition of Buffer S2) does not exceed.
NucleoBond® Folded Filters clog during filtration	*Culture volumes used are too large* • Reduce the culture volume or increase the amount of Buffer S1, S2, and S3 used during the lysis steps. *Incubation time too short* • Make sure that S1 / S2 / S3 lysate was incubated according to the protocol.

8.3 Ordering information

Product	REF	Pack of
NucleoBond® PC 20	740571 740571. 100	20 preps 100 preps
NucleoBond® AX 20	740511	20 columns
NucleoBond® PC 100	740573 740573.100	20 preps 100 preps
NucleoBond® AX 100	740521 740521.100	20 columns 100 columns
NucleoBond® PC 500	740574 740574.25 740574.50 740574.100	10 preps 25 preps 50 preps 100 preps
NucleoBond® AX 500	740531 740531.50	10 columns 50 columns
NucleoBond® PC 2000	740576	5 preps
NucleoBond® AX 2000	740525	10 columns
NucleoBond® PC 10000	740593	5 preps
NucleoBond® AX 10000	740534	5 columns
NucleoSnap® Finisher Midi (for use with NucleoBond® Xtra Midi, NucleoBond® PC 100)	740434.10 740434.50	10 preps 50 preps
NucleoSnap® Finisher Maxi (for use with NucleoBond® Xtra Maxi, NucleoBond® PC 500)	740435.10 740435.50	10 preps 50 preps
NucleoSpin® Finisher Midi (for use with NucleoBond® Xtra Midi, NucleoBond® PC 100)	740439.10 740439.50	10 preps 50 preps
NucleoBond® Finalizer (for use with NucleoBond® PC 100, PC 500, PC 500 EF, NucleoBond® Xtra Midi, Midi EF)	740519.20	20 filters 2 syringe sets

Product	REF	Pack of
NucleoBond® Finalizer Plus (for use with NucleoBond® PC 100, PC 500, PC 500 EF, NucleoBond® Xtra Midi, Midi EF)	740520.20	20 filters 20 syringe sets
NucleoBond® Finalizer Large (for use with NucleoBond® PC 2000, PC 2000 EF, NucleoBond® Xtra Maxi, Maxi EF)	740418.20	20 large filters 2 syringe sets
NucleoBond® Finalizer Large Plus (for use with NucleoBond® PC 2000, PC 2000 EF, NucleoBond® Xtra Maxi, Maxi EF)	740419.20	20 large filters 20 syringe sets
NucleoBond® Folded Filters (for NucleoBond® AX 100 Columns)	740561	5
NucleoBond® Folded Filters XL (for NucleoBond® AX 500/2000, BAC 100 Columns)	740577	50
NucleoBond® Bottle Top Filters Type 1 (for NucleoBond® AX 2000 Columns)	740547.5	5
NucleoBond® Bottle Top Filters Type 2 (for NucleoBond® AX 10000 Columns)	740553.5	5
NucleoBond® Buffer Set I	740601	1 set
Buffer S1	740516.1	500 mL
Buffer S2	740517.1	500 mL
Buffer S3	740518.1	500 mL
Buffer N2	740527.1	500 mL
Buffer N3	740528.1	1000 mL
Buffer N5	740529.1	500 mL
NucleoBond® Rack Small (for NucleoBond® AX 20 Columns)	740562	1
NucleoBond® Rack Large (for NucleoBond® AX 100, 500, and 2000 columns)	740563	1
RNase A (lyophilized)	740505.50 740505	50 mg 100 mg

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

8.4 References

Birnboim, H. C. and Doly, J., (1979) Nucl. Acids Res. 7, 1513-1523

8.5 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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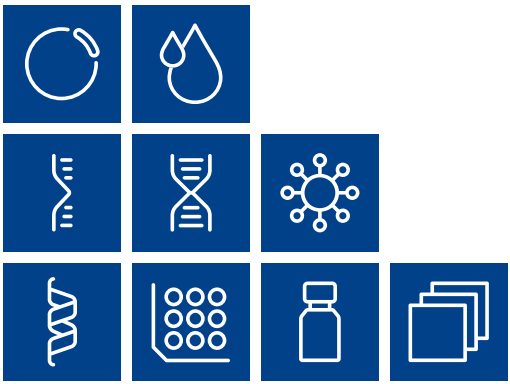
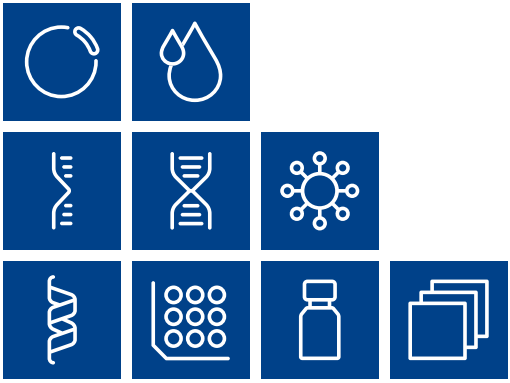


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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