Bioanalysis

MACHEREY-NAGEL

User manual

Endotoxin-free plasmid DNA purification

- NucleoBond® PC 500 EF
- NucleoBond® PC 2000 EF
- NucleoBond® PC 10000 EF
- NucleoBond® PC Prep 100

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MACHEREY-NAGEL
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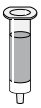
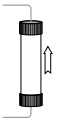
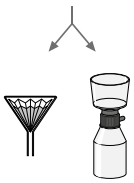
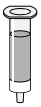
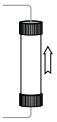
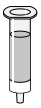
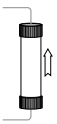
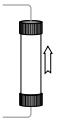
Endotoxin-free plasmid DNA purification (Maxi, Mega, Giga, Preparative scale)

Protocol at a glance (Rev. 19)

	Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)		Preparative scale (AX Prep 100)	
1 Cultivation and harvest of bacterial cells	4,500 – 6,000 x *g* 15 min at 4 °C	4,500 – 6,000 x *g* 15 min at 4 °C	4,500 – 6,000 x *g* 15 min at 4 °C		4,500 – 6,000 x *g* 15 min at 4 °C	
2 Cells lysis						
Buffer S1-EF	12 mL	40 mL	120 mL		1000 mL	
Buffer S2-EF	12 mL < 5 min at RT	40 mL < 5 min at RT	120 mL < 5 min at RT		1000 mL < 3 min at RT	
Buffer S3-EF	12 mL 5 min at 0 °C	40 mL /	120 mL /		1000 mL 25 min at 0 °C	
	Mix thoroughly – invert 10 – 15 times					
3 Equilibration of the column	Buffer N2-EF 5 mL	Buffer N2-EF 25 mL	Buffer N2-EF 100 mL		Buffer N2-EF 900 mL	
4 Clarification of the lysate	Folded Filter 20 min	Bottle Top Filter Type 1 5 min	Bottle Top Filter Type 2 5 min		1 st Sieving Fabric 2nd Folded Filters	
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	
6 Washing	Buffer N3-EF 2 × 24 mL Buffer N4-EF 2 × 12 mL	Buffer N3-EF 1 × 60 mL 2 × 40 mL Buffer N4-EF 60 mL	Buffer N3-EF 4 × 150 mL Buffer N4-EF 3 × 130 mL		Buffer N3-EF 900 mL Buffer N4-EF 1900 mL	
7 Elution	Buffer N5-EF 15 mL	Buffer N5-EF 25 mL	Buffer N5-EF 100 mL		Buffer N5-EF 470-600 mL	
8 Precipitation	Isopropanol 11 mL	Isopropanol 18 mL	Isopropanol 70 mL		Isopropanol 0.7 vol	
	4,5 – 15,000 x *g* 30 min at 12 °C	4,5 – 15,000 x *g* 30 min at 12 °C	4,5 – 15,000 x *g* 30 min at 12 °C		4,5 – 15,000 x *g* 60 min at 12 °C	
9 Washing and drying	70 % ethanol 5 mL	70 % ethanol 7 mL	70 % ethanol 10 mL		70 % ethanol 25 mL	
	4,5 – 15,000 x *g* 10 min at RT	4,5 – 15,000 x *g* 10 min at RT	4,5 – 15,000 x *g* 10 min at RT		4,5 – 15,000 x *g* 20 min at RT	
	10 – 20 min	30 – 60 min	30 – 60 min		60 min	
10 Reconstitution	Appropriate volume of TE-EF or H_2O-EF	Appropriate volume of TE-EF or H_2O-EF	Appropriate volume of TE-EF or H_2O-EF		Appropriate volume of TE-EF or H_2O-EF	

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1 Components

1.1 Kit contents

	NucleoBond® PC 500 EF	NucleoBond® PC 2000 EF	NucleoBond® PC 10000 EF
REF	**10 preps 740550**	**5 preps 740549**	**5 preps 740548**
Resuspension Buffer S1-EF	150 mL	250 mL	750 mL
Lysis Buffer S2-EF	150 mL	250 mL	750 mL
Neutralization Buffer S3-EF	150 mL	250 mL	750 mL
Equilibration Buffer N2-EF	100 mL	150 mL	600 mL
Wash Buffer N3-EF	800 mL	800 mL	4 × 800 mL
Wash Buffer N4-EF	350 mL	350 mL	2 × 1000 mL
Elution Buffer N5-EF	200 mL	200 mL	600 mL
Redissolving Buffer TE-EF	30 mL	30 mL	30 mL
70 % EtOH (Concentrate)*	35 mL	35 mL	35 mL
H_2O-EF	30 mL	30 mL	30 mL
RNase A (lyophilized)*	15 mg	25 mg	75 mg
NucleoBond® AX 500 / 2000/10000 Columns	10	5	5
NucleoBond® Folded Filters	10	-	-
NucleoBond® Bottle Top Filters (Type 1 or 2)	-	5 Type 1	5 Type 2
Plastic Washers (reusable)	5	5	-
User manual	1	1	1

* For preparation of working solutions and storage conditions see section 6.

Kit contents continued

	NucleoBond® PC Prep 100
REF	**1 prep 740594**
Resuspension Buffer S1-EF	1000 mL
Lysis Buffer S2-EF	1000 mL
Neutralization Buffer S3-EF	1000 mL
Equilibration Buffer N2-EF	1000 mL
Wash Buffer N3-EF	1000 mL
Wash Buffer N4-EF	2 × 1000 mL
Elution Buffer N5-EF	600 mL
Redissolving Buffer TE-EF	60 mL
70 % EtOH (Concentrate)*	2 × 35 mL
H_2O-EF	60 mL
RNase A (lyophilized)*	100 mg
NucleoBond® AX Prep 100 Column	1
NucleoBond® AX 100 Columns	3
Sieving Fabric	3
NucleoBond® Folded Filters (Type 1 and 2)	2 × 5
User manual	1

* For preparation of working solutions and storage conditions see section 6.

1.2 Reagents and equipment to be supplied by user

Reagents

- Isopropanol (room-temperatured)
- 96 – 100 % ethanol (room-temperatured)
- Ice

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)
- Refrigerated centrifuge capable of reaching > 4,500 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 (see ordering information) or equivalent holder
- Peristaltic pump (for NucleoBond® PC Prep 100 only)

2 Kit specifications

- The kits are suitable for purifying endotoxin-free plasmids with < 0.1 EU/µg.
- **NucleoBond® 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 1).

Table 1: Kit specifications at a glance

	PC 500 EF	PC 2000 EF	PC 10000 EF	PC Prep 100
Use	For research use only			
Handling	Gravity flow column			
Sample material	Bacteria			
Target	Plasmid DNA			
Recomm. medium	LB			
Culture volume	30 – 150 mL	150 – 500 mL	500 mL–2 L	5 – 20 L
Max. pellet wet weight	0.75 g	2.5 g	10 g	90 g
Binding capacity	500 µg	2 mg	10 mg	100 mg
Endotoxin level	< 0.1 EU/µg			
Applications	Transfection studies, gene therapy			
Time/prep	100 min/ 2 preps	150 min/ 2 preps	180 min/ 2 preps	20 h/ prep

- **NucleoBond® PC EF/Prep 100** kits allow the purification of DNA that fulfills the following criteria:

Table 2: Criteria

Parameter	Method	Criterion
Structural integrity	Agarose gel Photo documentation	> 90 % ccc
RNA +ssDNA	HPLC Agarose gel	< 50 µg/mg
Chrom. DNA	HPLC Southern Blot/PCR	< 50 µg/mg
Endotoxin	LAL test	< 0.1 EU/µg
Protein	Bradford	< 10 µg/mg
Purity	A_{260}/A_{280} UV spectrum 220 – 320 nm	1.80 – 1.95 normal

- 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.

3 About this user manual

Experienced users who are performing the purification of high-copy plasmids using a **NucleoBond® PC EF** purification kit may refer to the Protocol at a glance instead of this user manual. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First time users are strongly advised to read this user manual.

Each procedural step in the protocols of this manual is arranged like the following example:

Maxi	Mega	Giga
(AX 500)	(AX 2000)	(AX 10000)

1 Carefully resuspend the pellet of bacterial cells in **Buffer S1-EF +RNase A**. Please see section 8.3 regarding difficult-to-lyse strains.

12 mL	40 mL	120 mL

For NucleoBond® PC 500 EF preparations refer to the buffer volumes and incubation times given in the white boxes. For Mega and Giga preparations refer to the grey and black boxes, respectively. The name of the buffer is highlighted in bold type.

For example in a **NucleoBond® PC 500 EF** preparation you are requested to resuspend the pelleted cells in **12 mL** of **Buffer S1-EF**.

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

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

4 Endotoxins

4.1 Localization, molecular structure, and function of endotoxins

In contrast to Gram positive bacteria which have only one lipid bilayer membrane surrounded by a thick cell wall, Gram negative bacteria have a second membrane enclosing the inner membrane and only a thin cell wall. The outer layer of this second membrane consists of amphiphilic lipopolysaccharides (LPS), also called endotoxins.

The structure of endotoxins can be divided into three domains:

1. The hydrophobic **Lipid A moiety** is anchoring the LPS inside the membrane and confers the toxicity to endotoxins. Its structure is highly conserved throughout all Gram negative bacteria.

2. The hydrophilic inner core of the polysaccharide part of LPS, the **R-antigen**, is a short sugar chain with a highly conserved sequence. It is harboring a lot of negative charges and is thought to function as the main barrier against hydrophobic substances like antibiotics and detergents.

3. The hydrophilic and extremely variable outer polysaccharide, the **O-antigen**, is involved, for example in cell adherence and interactions with the immune system of the host, i.e., it is responsible for the immunological properties and virulence of the bacteria.

4.2 Quantification of endotoxins

Endotoxins can be measured in highly sensitive photometric tests (“Pyrochrome”, Associates of Cape Cod, Inc.) and are expressed in endotoxin units (EU). For plasmid preparations the endotoxin level is given in EU per µg plasmid. A concentration of 0.1 EU/µg is usually considered endotoxin-free.

4.3 Removal of endotoxins

Endotoxins are released from cells in small amounts during cell growth and in very large quantities upon cell death and lysis and thus also during plasmid purification. Like intact cells the free LPS molecules induce inflammatory reactions of the mammalian immune system. Therefore they have to be removed quantitatively from plasmid preparations to guarantee high transfection rates and high viability of transfected cells.

Due to their amphiphilic nature and their negative charge endotoxins behave like DNA and are co-purified with most common plasmid purification systems. Regular silica membrane kits with a purification procedure based on chaotropic salt lead to plasmid DNA with an endotoxin level of > 1000 EU/µg. Anion exchange kits like **NucleoBond® PC** reduce endotoxins to a level of > 1 EU/µg. However, since this may be still too high for successful transfection of very sensitive cells like primary or neuronal cells, **NucleoBond® PC EF** was developed to reduce the endotoxin level to < 0.1 EU/µg plasmid DNA using a patented procedure.

5 NuceoBond® PC EF purification system

5.1 The basic principle

NucleoBond® EF 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, which remains in solution, can revert to its native supercoiled structure. 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. Endotoxins are removed by Buffer N3-EF. After precipitation of the eluted DNA it can easily be dissolved in Buffer TE-EF or H_2O-EF for further use.

5.2 Convenient stopping points

Cell pellets can easily be stored for several months at -20 °C.

Cleared lysates can be kept on ice or at 4 °C for several days.

For optimal performance the column purification should not be interrupted. However, the columns can be left unattended for several hours since the columns do not run dry. This might cause only small losses in DNA yield.

The eluate can be stored for several days at 4 °C. Note that the eluate should be warmed up room temperature (15 – 25 °C) before precipitating the DNA to avoid co-precipitation of salt.

5.3 Filtration of the lysate

After alkaline lysis, the solution has to be clarified from, for example, the cell debris through the supplied **NucleoBond® Folded Filters** or **NucleoBond® Bottle Top Filters** in order to prevent clogging of the column.

- For the NucleoBond® AX 2000 (Mega) and AX 10000 (Giga) Column, use the supplied vacuum operated **NucleoBond® Bottle Top Filters** for filtration of the lysate. The bottle top filters (Figure 1) make the separation of the bacterial lysate and SDS precipitate easy, quick, and convenient. Adjust the bottle top filter to a suitable flask (e.g., Schott), load the bacterial lysate, and apply the vacuum. After 5 min the solution will have passed through. Load the resulting clear lysate onto the corresponding **NucleoBond® AX Column** and discard the bottle top filter.

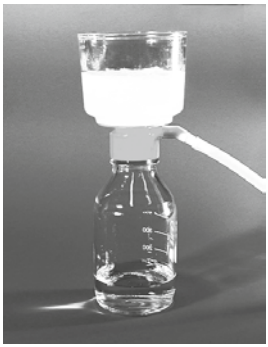


Figure 1 Correct use of the NucleoBond® Bottle Top Filter

- For the NucleoBond® AX 500 Column use the supplied **NucleoBond® Folded Filters** for filtration of the lysate (Figure 2). Folded filters are designed to eliminate the centrifugation step after alkaline lysis for plasmid isolation. The filters completely remove SDS and precipitate cellular debris from plasmid samples. For correct use please follow the instructions given in step 4 of the corresponding protocol.

Figure 2 Correct use of the NucleoBond® Folded Filter, NucleoBond® Column placed in a Plastic Washer (reusable)

5.4 Analytical check (refers only to PC Prep 100)

Before starting the **NucleoBond® PC Prep 100** purification, we recommend checking the fermented cell material by purifying the plasmid DNA from 20 mL and 40 mL of culture on a **NucleoBond® AX 100 Column** (supplied). Follow the instructions of the attached protocol.

From the yield resulting from 20 mL and 40 mL fermentation broth using a **NucleoBond® AX 100 Column**, the total yield of the **NucleoBond® Prep 100** procedure can be estimated. If necessary, the culture volume can be adapted accordingly with respect to a maximum pellet weight of 90 g and a maximum binding capacity of 100 mg (also see section 8.1).

In the case that a 20 mL fermentation broth (high copy plasmid) already results in 60 – 100 µg of plasmid DNA, yields obtained from a 40 mL fermentation broth will be invalid because the NucleoBond® AX 100 Column (Midi prep) has been overloaded. Take the yields obtained from the 20 mL fermentation broth as a basis for the calculation of how much fermentation broth can be used for the NucleoBond® AX Prep 100 Column (preparative scale).

	Midi
	(AX 100)
1	**Harvest of bacteria**
	Harvest bacteria from 20 and 40 mL fermentation broth 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-EF +RNase A**. Please see section 8.3 regarding difficult-to-lyse strains.
	4 mL
	Add **Buffer S2-EF** 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.
	4 mL
	Add precooled **Buffer S3-EF (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.
	4 mL
3	**Equilibration of the column**
	Equilibrate a NucleoBond® AX 100 (Midi) Column with **Buffer N2-EF**. Allow the column to empty by gravity flow. Discard flowthrough.
	2.5 mL

Midi

(AX 100)

4 Clarification of the lysate

Clear the bacterial lysate by following EITHER **option 1** or **option 2** as described below. This step is extremely important; excess precipitate left in suspension may clog the NucleoBond® Column in later steps.

Option 1: Centrifuge the suspension. *Centrifuge at > 12,000 x g for the minimum time indicated below at 4 °C. If the suspension contains residual precipitate after the first centrifugation, repeat this step.*

25 min

Option 2. Filter the suspension. *Place a NucleoBond® Folded Filter (not provided) in a small funnel for support, and prewet the filter with a few drops of Buffer N2-EF or sterile deionized H_2O. Load the lysate onto the wet filter and collect the flowthrough*

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-EF**. Discard flowthrough.

10 mL

Wash the column with **Buffer N4-EF**. Discard flowthrough.

5 mL

7 Elution

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

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 prewarmed to room temperature before the plasmid DNA is precipitated.

5 mL

8 Precipitation

Add **room-temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **≥ 15,000 x *g*** for **30 min** at **12 °C**. Carefully discard the supernatant.

3.5 mL

	Midi (AX 100)
9	**Washing and drying**
	Add **room-temperature endotoxin-free 70 % EtOH** to the pellet. Vortex briefly and centrifuge at **4,5 – 15,000 x *g*** for **10 min** at **room temperature.**
	For preparation of endotoxin-free 70 % EtOH refer to section 6.
	2 mL
	Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature** at least for the indicated time.
	Drying for longer periods will not harm the quality of the plasmid DNA but over-drying may render the DNA less soluble.
	5 – 10 min
10	**Reconstitution**
	Dissolve pellet in an appropriate volume of **Buffer TE-EF** or **H_2O-EF**. 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.

5.5 Elution procedure

Elution is carried out into a new tube with the volume of elution buffer indicated in the corresponding protocol. The plasmid DNA is precipitated by the addition of **room-temperature 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.

5.6 Disposal of column resin (PC Prep 100 only)

Rinse the **NucleoBond® AX Prep 100 Column** with **350 mL** of **0.2 M HCl** to deactivate residual plasmid DNA and other potential biohazards.

Column resin or the entire column can then be disposed of according to local regulations.

6 Storage conditions and preparation of working solutions

- All kit components can be stored at 15 – 25 °C and are stable until see package label.
- Storage of **Buffer S2-EF** below 20 °C may cause precipitation of SDS. If salt precipitate is observed, incubate buffer at 30 – 40 °C for several minutes and mix well until all precipitate is redissolved completely. Cool down to room temperature before use.

Before the first use of **NucleoBond® PC EF**, prepare the following:

- Dissolve the **lyophilized RNase A** by adding 1 mL of Resuspension Buffer S1-EF. Pipette up and down until the RNase A is completely dissolved. Transfer the RNase A solution back to the bottle containing Buffer S1-EF and shake well. Note the date of RNase A addition. The final concentration of RNase A is 100 µg/mL Buffer S1-EF. Store Buffer S1-EF with RNase A at 4 °C. The solution will be stable at this temperature for at least 12 months.
- Precool **Neutralization Buffer S3-EF** to 4 °C.
- The **NucleoBond® Bottle Top Filters** are designed to be used with a 1 Liter, 45 mm-neck vacuum-resistant glass bottle (bottles are not included in the kit). Use any vacuum source, for example, a vacuum pump or house vacuum that generates reduced pressure between -0.2 and -0.8 bar. Do not use scratched bottles and wear goggles when working with the **NucleoBond® Bottle Top Filter** system under vacuum conditions.
- Add indicated volume of 96 – 100 % ethanol to endotoxin-free water in bottles labeled “70% EtOH”.

	NucleoBond® PC 500 EF	NucleoBond® PC 2000 EF	NucleoBond® PC 10000 EF
REF	**10 preps 740550**	**5 preps 740549**	**5 preps 740548**
70 % EtOH (Concentrate)	35 mL Add 80 mL ethanol	35 mL Add 80 mL ethanol	35 mL Add 80 mL ethanol

	NucleoBond® PC Prep 100
REF	**1 prep 740594**
70 % EtOH (Concentrate)	2 × 35 mL Add 80 mL ethanol to each bottle

7 Safety instructions

When working with the **NucleoBond® PC EF** and **NucleoBond® PC Prep 100** 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 at http://***www.mn-net.com/msds***).

The waste generated with the **NucleoBond® PC EF** kit and **NucleoBond® PC Prep 100** 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 treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

7.1 Disposal

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

8 Growing of bacterial cultures

8.1 General considerations

Yield and quality of plasmid DNA depend on, for example, the type of growing media and antibiotics, the bacterial host, plasmid type, size, or copy number. Therefore, these factors should be taken into consideration. For cultivation of bacterial cells, we recommend LB medium. The suggested bacterial culture volumes for each column size as well as expected plasmid yields are listed in Table 3, section 8.1. Overnight-cultures in flasks usually reach, under vigorous shaking, an OD600 of 3 – 6, while fermentation cultures reach 10 and more. Therefore, please refer not only to the culture volume, but also check OD600 and pellet wet weight, particularly if richer culture media like 2xYT or TB are used. If too much bacterial material is used, lysis and precipitation steps are inefficient and cause decreased yield and plasmid quality.

As a general rule, 1 Liter *E.coli* culture grown in LB medium yields a pellet of about 3 – 20 g wet weight. The expected yield for a high-copy-number plasmid is 1 – 3 mg per gram wet weight.

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

Table 3: Culture volumes

Plasmid type	LB culture volume	Wet weight of pellet	Recommended column size	Average yield
High copy	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
	5 – 20 L	90 g	AX Prep 100 (preparative scale)	80 – 100 mg
Low copy	100 – 500 mL	1.5 – 2.2 g	AX 500 (Maxi)	100 – 500 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 EF** 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.

Table 4: Estimation of optimal culture volume

	Format	Recommended culture volume	Maximum ODV according to lysis buffer capacity	Maximum OD_{600} at maximum recommended culture volume
High copy	Maxi (PC 500 EF)	30 – 150 mL	600	4.0
	Mega (PC 2000 EF)	150 – 500 mL	2,250	4.5
	Giga (PC 10000 EF)	500 – 2,000 mL	6,000	3.0
	Preparative scale (PC Prep 100)	up to 90 g cell pellet		
Low copy	Maxi (PC 500 EF)	100 – 500 mL	1,200	2.4
	Mega (PC 2000 EF)	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-EF, S2-EF and S3-EF equally to increase lysis capacity. An increase of 1 mL S1-EF, S2-EF and S3-EF each gains another ODV 50 in lysis capacity.

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

8.2 Selection of culture media

The cultivation of cells is recommended at 37 °C in LB (Luria-Bertani) medium at constant shaking (200 – 250 rpm). Alternatively, rich media like 2 x YT (Yeast/Tryptone) or TB (Terrific Broth) can be used. By using 2 x YT or TB, bacteria grow faster and reach the stationary phase much earlier than in LB medium (≤ 12 h). This may lead to a higher percentage of dead or starving cells when starting the preparation. The resulting plasmid DNA from overgrown cultures may be partially degraded or contaminated with chromosomal DNA.

At least for Mega, Giga and preparative scale (PC Prep 100) preps the use of an ap-propriate fermentation system is recommended in order to optimize cultivation conditions.

8.3 Difficult to lyse strains

Isolate plasmid DNA from difficult-to-lyse strains by first resuspending the pellet in Buffer S1-EF containing lysozyme (2 mg/mL final concentration). Incubate at 37 °C for 30 minutes, then continue with the addition of Buffer S2-EF, and proceed with the appropriate NucleoBond® protocol.

8.4 Chloramphenicol amplification of low-copy plasmids

To dramatically increase the low copy number of pMB1/colE1 derived plasmids grow the cell culture to mid or late log phase (OD600 ≈ 0.6 – 2.0) under selective conditions with an appropriate antibiotic. Then add 170 µg/mL chloramphenicol and continue incubation for a further 8 – 12 hours. Chloramphenicol inhibits host protein synthesis and thus prevents replication of the host chromosome. Plasmid replication, however, is independent of newly synthesized proteins and continues for several hours until up to 2000 – 3000 copies per cell are accumulated*.

Alternatively, the cell culture can be grown with only partial inhibition of protein synthesis under low chloramphenicol concentrations (10 – 20 µg/mL) resulting in a 5 – 10-fold greater yield of plasmid DNA**.

Both methods show the positive side effect of much less genomic DNA per plasmid, but they obviously work only with plasmids that do not carry the chloramphenicol resistance gene. Furthermore, the method is only effective with low copy number plasmids under stringent control (e.g., pBR322). All modern high copy number plasmids (e.g., pUC) are already under relaxed control due to mutations in the plasmid copy number control genes and show no significant additional increase in their copy number.

9 NucleoBond® PC EF plasmid DNA purification

9.1 Endotoxin-free plasmid DNA purification (Maxi, Mega, Giga)

	Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)

1 Cultivation and harvest of bacterial cells

Set up an overnight bacterial culture by diluting an appropriate volume of starter culture into the respective volume of **LB medium** with selecting antibiotics. Shake the culture overnight (12 – 16 h).

Centrifuge the culture at **4,500 – 6,000 x *g*** for **15 min** at **4 °C**. Carefully discard the supernatant.

2 Cell lysis

Carefully resuspend the pellet of bacterial cells in **Buffer S1-EF +RNase A**. Please see section 8.3 regarding difficult-to-lyse strains.

12 mL	40 mL	120 mL

Add **Buffer S2-EF** 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.

12 mL	40 mL	120 mL

Add precooled **Buffer S3-EF** (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.

12 mL	40 mL	120 mL

Maxi	Mega	Giga
(AX 500)	(AX 2000)	(AX 10000)

AX 500 (Maxi):

Incubate the suspension for 5 min on ice before continuing with step 4, "Clarification of the lysate".

AX 2000/10000 (Mega, Giga):

Pour the lysate immediately into the NucleoBond® Bottle Top Filter Type 1 (AX 2000) or Type 2 (AX 10000) and continue as described in step 4 of this protocol.

To save time, the equilibration of the NucleoBond® Columns (see step 3) can be started during the clarification of the lysate as described in step 4.

3 Equilibration of the column

Equilibrate a NucleoBond® Column with Buffer N2-EF. Allow the column to empty by gravity flow. Discard flowthrough.

5 mL	25 mL	100 mL

4 Clarification of the lysate

Clear the bacterial lysate by using NucleoBond® Folded Filters (PC 500 EF) or NucleoBond® Bottle Top Filters (PC 2000/10000 EF). This step is extremely important; excess precipitate left in suspension may clog the NucleoBond® Column in later steps.

AX 500 (Maxi): Place the NucleoBond® Folded Filter in a small funnel for support, and prewet the filter with a few drops of Buffer N2-EF or sterile deionized H_2O. Load the bacterial lysate onto the wet filter and collect the flowthrough.

AX 2000/10000 (Mega, Giga): Pour the lysate immediately into the NucleoBond® Bottle Top Filter Type 1 (AX 2000) or Type 2 (AX 10000) and incubate at room temperature for 10 min. Switch on the vacuum source (optimal -0.4 to -0.6 bar) in order to filtrate the lysate through the NucleoBond® Bottle Top Filter. After all liquid has passed the filter (3 – 5 min) switch off the vacuum source.

AX 10000 (Giga): *It is possible to stir the precipitate with a spatula gently onto the filter. In order to recover residual liquid, switch on vacuum again for another minute.*

20 min	5 min	5 min

Alternatively: Centrifuge the crude lysate at high speed (> 4,5 – 12,000 x g) at 4 °C for 40 min (Maxi), 50 min (Mega) and 60 min (Giga). Subsequently after centrifugation, carefully remove the supernatant from the white precipitate and load it onto the equilibrated NucleoBond® Column.

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.

	Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)

6 Washing

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

Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
2 × 24 mL	1 × 60 mL 2 × 40 mL	4 × 150 mL

Wash the column with **Buffer N4-EF**. Repeat as indicated. Discard flowthrough.

Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
2 × 12 mL	1 × 60 mL	3 × 130 mL

7 Elution

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

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.

Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
15 mL	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 **4,5 – 15,000 x *g*** for **30 min** at **12 °C**. Carefully discard the supernatant.

Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
11 mL	18 mL	70 mL

9 Washing and drying

Add **room-temperature endotoxin-free 70 % EtOH** to the pellet. Vortex briefly and centrifuge at **4,5 – 15,000 x *g*** for **10 min** at **room temperature.**

For preparation of endotoxin-free 70 % EtOH refer to section 6.

Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
5 mL	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 overdrying may render the DNA less soluble.

Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
5 – 10 min	30 – 60 min	30 – 60 min

	Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)
10	**Reconstitution** Dissolve pellet in an appropriate volume of **Buffer TE-EF** or **H_2O-EF**. Depending on the type of centrifugationtube, 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.		

9.2 Plasmid DNA purification (preparative scale)

The following **NucleoBond® PC Prep 100** protocol is based on the application of 10 Liters of LB culture, corresponding to up to 90 g of bacterial cells.

The **NucleoBond® AX Prep 100 Column** is for single use only. Pressure within the column should not exceed 7 kg/cm^2 (100 PSI) during usage.

Use only oven-baked glassware or new disposable plasticware for handling the purified plasmid DNA to prevent contamination with endotoxins. This is important for the elution, precipitation, and reconstitution step.

	Preparative scale (AX Prep 100)
1	**Cultivation and harvest of bacterial cells** Set up an overnight bacterial culture by diluting an appropriate volume of starter culture into the respective volume of **LB medium** with selecting antibiotics. Shake the culture overnight (12 – 16 h). Centrifuge the culture at **4,500 – 6,000 x *g*** for **15 min** at **4 °C**. Carefully discard the supernatant.
2	**Cell lysis** Carefully resuspend the pellet of bacterial cells (up to 90 g) in **Buffer S1-EF +RNase A** in a 4 – 5 L wide-mouth bottle with screw closure by shaking the suspension for **5 – 10 minutes**. *Alternatively, stirring at a low speed (about 30 rpm) for one hour at RT can be performed. Check that the bacterial pellet is resuspended completely and no aggregates are left.*
	1000 mL
	Add **Buffer S2-EF** (equilibrated to RT > 20 °C to avoid SDS precipitation) to the suspension. Immediately mix the suspension by gently inverting the flask **6 – 10 times**, resulting in a **clear and very viscous solution**. **Do not stir the resulting lysate**, since this may release contaminating chromosomal DNA from debris into the suspension. Incubate the mixture at room-temperature for up to **3 min**.

Preparative scale

(AX Prep 100)

1000 mL

Add precooled **Buffer S3-EF (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 at least **25 min**.

1000 mL

3 Equilibration of the column

Fix the column **upright** at a lab frame. Equilibrate the NucleoBond® AX Prep 100 Column in an **upward direction** with Buffer N2-EF at a flow rate of 15 – 20 mL/min at room temperature.

First, cut the tube at the column in the middle. The resulting inlet tube of the column is connected to the silicon tube of a peristaltic or piston pump for loading. Check the tightness of all fitting connections and use cable binders to prevent leakage where necessary. If the column runs dry during use, rehydrate column bed by pushing new buffer through the column upwards via a peristaltic pump.

900 mL

4 Clarification of the lysate

Step I:

Put the **Sieving Fabric (3 pieces of 4 Layers each)** into a funnel on a 3 L Erlenmeyer flask or a 3 L glass bottle and load the lysate. **Collect** the flowthrough on ice.

Alternatively: Clarification can also be achieved by centrifuging the crude lysate at 3,000 – 5,000 x g for 1 h at 4 °C. Subsequently after centrifugation, remove the supernatant from the white precipitate carefully.

Step II:

Moisten the NucleoBond® Folded Filter **Type 1** (fixed in a funnel) carefully with deionized water, apply the flowthrough from step I and collect the filtrate. Apply the filtrate onto a prewet NucleoBond® Folded Filter **Type 2**. Collect the clear flowthrough and estimate the volume for step 4. Store the cleared lysate on ice.

Cleared lysate can be stored on ice for hours. If further precipitate appears, filter the lysate again before loading it onto the NucleoBond® AX Prep 100 Column (additional filters not included, see ordering information).

We recommend using a 0.2 – 0.4 µm CA-filter for filtration (e.g., NucleoBond® Bottle Top Filters, see ordering information).

1 h

Preparative scale

(AX Prep 100)

5 Binding

Load the cleared lysate from step 4 onto the NucleoBond® AX Prep 100 Column (equilibrated with Buffer N2-EF) at a flow rate of up to 4 mL/min. Check the real flow rate of the pump.

Depending on the volume of the cleared lysate, the flow rate can be reduced to get a reasonable loading time. It is convenient to keep the cleared lysate stored in an icebox during the loading of the column overnight at 3 mL/min flow rate. Do not cool the column itself.

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

6 Washing

Wash the column with **Buffer N3-EF** at a flow rate of 15 – 20 mL/min at room temperature.

900 mL

Wash the column with **Buffer N4-EF** at a flow rate of 15 – 20 mL/min at room temperature.

1900 mL

7 Elution

Elute the plasmid DNA with **Buffer N5-EF** at a maximal flow rate of 4 mL/min at room temperature into oven-baked glassware or endotoxin-free plastic vials.

Discard the first 200 mL of the eluate as this is the dead volume of the column. The following **270 – 400 mL of eluate** will contain the purified plasmid DNA.

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 prewarmed to room temperature before the plasmid DNA is precipitated.

If possible, check plasmid concentration at A_{260} during elution continuously to get maximal yield in minimal elution volume.

Store the eluate on ice during elution, if possible.

470 – 600 mL

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

Preparative scale

(AX Prep 100)

8 Precipitation

Add **room-temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **4,5 – 15,000 x *g*** for **30 min** at **12 °C**. Carefully discard the supernatant.

Make sure the temperature in the plasmid suspension will not exceed 25 °C to prevent reduced yield.

In order to prevent salt precipitation, temperatures below 5 °C should be avoided.

0.7 vol

9 Washing and drying

Add **room-temperature endotoxin-free 70 % EtOH** to the pellet. Vortex briefly and centrifuge at **4,5 – 15,000 x** *g* for **20 min** at **room temperature.**

Repeat this step once.

For preparation of endotoxin-free 70 % EtOH refer to section 6.

25 mL

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at room temperature for at least the indicated time.

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

60 min

10 Reconstitution

Dissolve pellet in an appropriate volume of **Buffer TE-EF** or **H_2O-EF**. Depending on the type of centrifugationtube, 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.

10 Appendix

10.1 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.
- Endotoxins can be measured in highly sensitive photometric tests (e.g, "Limolus Amebocyte Lysate (LAL') Pyrochrome", Lonza Cambrex BioWhittaker) and are expressed in endotoxin units (EU). **NucleoBond® PC EF/PC Prep 100** purified plasmid DNA typically contains less than 0.1 EU/µg.
- Depending on further use of the purified plasmid, more sophisticated analytical methods may have to be applied for quantification of byproducts.

10.2 Troubleshooting

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

First, the bacterial culture has to be checked for sufficient growth (OD600) in the presence of an appropriate selective antibiotic (see Table 4). **Second**, aliquots of the cleared lysate, the flowthrough, the combined washing steps (Buffer N3-EF and N4-EF), and the eluate should be kept for further analysis by agarose gel electrophoresis.

Refer to Table 3 to choose a fraction volume yielding approximately 5 µg of plasmid DNA assuming 500 µg, 2000 µg, 10000 µg, and 100 mg were loaded onto the **NucleoBond® AX 500, AX 2000, AX 10000, and AX Prep 100 Column**, respectively. 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 5: NucleoBond® PC EF volumes required for an analytical check

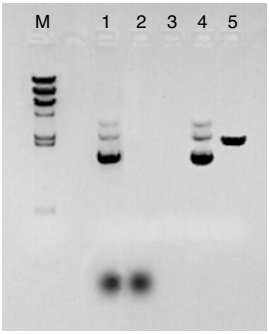
Sample	Purification step	Volume required (µL)			
		PC 500 EF	PC 2000 EF	PC 10000 EF	PC Prep 100 AX 100 check
I	Cleared lysate, after protocol step 4	400	300	200	600
II	Column flowthrough, after protocol step 5	400	300	200	600
III	Wash flowthrough, after protocol step 6	600	400	500	500
IV	Eluate, after protocol step 7	200	100	100	300

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, for example, the dominant plasmid bands which should only be present in the eluate and in the lysate before loading to proof plasmid production in your cell culture (lane 1). Plasmid DNA found in the wash fractions, however, narrows down the problem to wrong or bad wash buffers (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, for example, too harsh lysis conditions.



M: Marker λ *Hin*dIII

1: I, cleared lysate, ccc, linear and oc structure of the plasmid, degraded RNA

2: II, lysate flowthrough, no plasmid DNA, but degraded RNA

3: III, wash flowthrough, 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 %.

Table 6: 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

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

Problem	Possible cause and suggestions
No or low plasmid DNA yield	*SDS-or other precipitates are present in the sample* • Load the S1-EF/S2-EF/S3-EF 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. *Column overloaded with nucleic acids* • Use a larger column or purify excess nucleic acids on a new column. Refer to the recommended culture volumes listed in the table at the beginning of each protocol. *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 antibiotic concentration to plates and 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 3, section 8.1. *Lysate incorrectly prepared* • After storage below 20 °C, SDS in Buffer S2-EF may precipitate causing inefficient lysis. Check Buffer S2-EF for precipitates before use and prewarm the bottle to 30 – 40 °C if necessary in order to redissolve SDS. *Flow rates too high (NucleoBond® PC Prep 100)* • Do not exceed recommended flow rates for loading and eluting the plasmid DNA.

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* • Use additional NucleoBond® Folded Filters to clear the lysate. *High back pressure during purification (NucleoBond® PC Prep Prep 100)* • Cleared lysate contains particulate matter. Make sure that there is no cell debris in the lysate. Particulate matter may clog the inlet frit or in-line filters. If necessary, repeat filtration or centrifugation steps. Be sure to equilibrate and use the column in the correct direction (see protocol and scheme).
Cellular DNA or RNA contamination of plasmid DNA	*Lysis treatment was too harsh* • Be sure not to incubate the lysate in Buffer S2-EF for more than 5 min. *Overzealous 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-EF or stored too long. Add new RNase to Buffer S1-EF. See ordering information, section 10.3.
No nucleic acid pellet formed after precipitation	*Pellet was lost* • Handle the precipitate with care. Decant solutions carefully. Measure DNA yield in Buffer N5-EF in order to calculate the potential plasmid DNA that should be recovered after precipitation. *Pellet was smeared over the tube wall* • Dissolve DNA with an appropriate volume of Buffer TE-EF 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 endotoxin-free 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. Centrifuge at 12 °C. • Try resuspending the pellet in Buffer N2-EF, and repeat the precipitation step.
Purified plasmid does not perform well in subsequent reactions	*DNA is contaminated with cellular debris or genomic DNA due to inefficient lysis* • Reduce the culture volume, or increase the amount of Buffer S1-EF, S2-EF, and S3-EF 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-EF) does not proceed for longer than 5 min. *Washing steps inefficient (NucleoBond® PC Prep 100)* • Wash the column extensively with Buffer N3-EF and N4-EF, respectively, until UV absorbance has reached the initial value of equilibration.
NucleoBond® Folded Filters clog during filtration	*Culture volumes used are too large* • Reduce the culture volume or increase the amount of Buffer S1-EF, S2-EF, and S3-EF used during the lysis steps. *Incubation time too short* • Make sure that S1-EF/S2-EF/S3-EF lysate was incubated according to the protocol.

10.3 Ordering information

Product	REF	Pack of
NucleoBond® PC 500 EF	740550	10 preps
NucleoBond® AX 500	740531	10 columns
NucleoBond® AX 500	740531.50	50 columns
NucleoBond® PC 2000 EF	740549	5 preps
NucleoBond® AX 2000	740525	10 columns
NucleoBond® PC 10000 EF	740548	5 preps
NucleoBond® AX 10000	740534	5 columns
NucleoBond® PC Prep 100	740594	1 prep
NucleoBond® Folded Filters XL (for NucleoBond® AX 500 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® 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.

10.4 References

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

10.5 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-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.

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.

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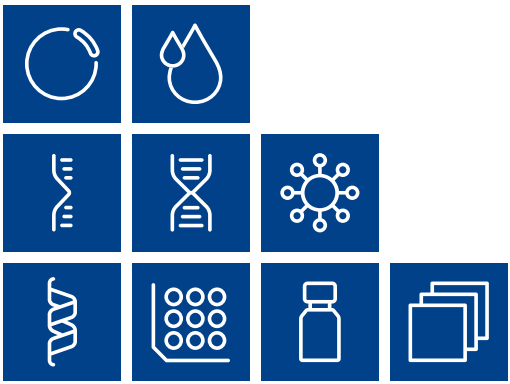

Plasmid DNA
Clean up
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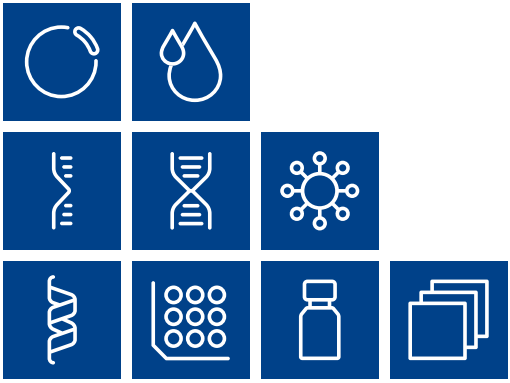
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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