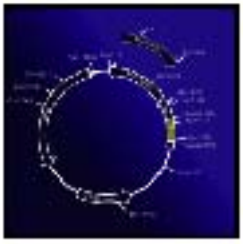


Plasmid DNA Purification

User manual

NucleoBond® PC 20

NucleoBond® PC 100

NucleoBond® PC 500

NucleoBond® BAC 100

NucleoBond® PC 2000

NucleoBond® PC 10000

March 2005/Rev. 03

MACHEREY-NAGEL

Protocol at a glance (Rev. 03)

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

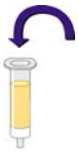
	step	Mini (AX 20)	Midi (AX 100)	Maxi (AX 500)	Mega (AX 2000)	Giga (AX 10000)	
1	**Cultivate and harvest bacterial cells**	4,500-6,000 x g 15 min at 4°C	4,500-6,000 x g 15 min at 4°C	4,500-6,000 x g 15 min at 4°C	4,500-6,000 x g 15 min at 4°C	4,500-6,000 x g 15 min at 4°C	
2	**Cell lysis**			high copy / 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	4 ml / 8 ml	12 ml / 24 ml	45 ml / 90 ml	120 ml / -	
	Buffer S3	0.4 ml / 0.8 ml	4 ml / 8 ml	12 ml / 24 ml	45 ml / 90 ml	120 ml / -	
3	**Equilibration of the column**	Buffer N2 1 ml	Buffer N2 2.5 ml	Buffer N2 6.0 ml	Buffer N2 20 ml	Buffer N2 100 ml	
4	**Clarification of the lysate**	Centrifugation 15 min 12,000 x g	Folded filter or centrifugation 25 min 12,000 x g	Folded filter or centrifugation 40 min 12,000 x g	Folded filter or centrifugation 50 min 12,000 x g	Folded filter or centrifugation 60 min 12,000 x g	
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 x 1.5 ml low copy 2 x 2 ml	Buffer N3 high copy 10 ml low copy 12 ml	Buffer N3 high copy 32 ml low copy 2 x 18 ml	Buffer N3 high copy 2 x 35 ml low copy 2 x 50 ml	Buffer N3 high copy 2 x 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 ≥ 15,000 x g 30 min at 4°C	Isopropanol 3.5 ml ≥ 15,000 x g 30 min at 4°C	Isopropanol 11 ml ≥ 15,000 x g 30 min at 4°C	Isopropanol 18 ml ≥ 15,000 x g 30 min at 4°C	Isopropanol 70 ml ≥ 15,000 x g 30 min at 4°C	
9	**Wash and dry DNA pellet**	70% ethanol 500 µl ≥ 15,000 x g 10 min at RT 5-10 min	70% ethanol 2 ml ≥ 15,000 x g 10 min at RT 5-10 min	70% ethanol 5 ml ≥ 15,000 x g 10 min at RT 10-20 min	70% ethanol 7 ml ≥ 15,000 x g 10 min at RT 30-60 min	70% ethanol 10 ml ≥ 15,000 x g 10 min at RT 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	

Table of contents

1 Kit contents

	NucleoBond® PC 20	
	20 preps	**100 preps**
Cat. No.	**740571**	**740571.100**
Buffer S1	20 ml	2 x 35 ml
Buffer S2	20 ml	2 x 35 ml
Buffer S3	20 ml	2 x 35 ml
Buffer N2	25 ml	125 ml
Buffer N3	2 x 35 ml	3 x 125 ml
Buffer N5	35 ml	125 ml
RNase A* (lyophilized)	2 mg	2 x 4 mg
NucleoBond® AX 20 columns	20	100
NucleoBond® folded filters	-	-
Plastic washer	10	10
Protocol	1	1

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

1 Kit contents *continued*

	NucleoBond® PC 100	
	20 preps	**100 preps**
Cat. No.	**740573**	**740573.100**
Buffer S1	120 ml	2 x 250 ml
Buffer S2	120 ml	2 x 250 ml
Buffer S3	120 ml	2 x 250 ml
Buffer N2	70 ml	2 x 150 ml
Buffer N3	240 ml	3 x 400 ml
Buffer N5	120 ml	3 x 200 ml
RNase A* (lyophilized)	12 mg	2 x 25 mg
NucleoBond® AX 100 columns	20	100
NucleoBond® folded filters	20	100
Plastic washer	10	10
Protocol	1	1

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

1 Kit contents *continued*

	NucleoBond® PC 500			
	10 preps	**25 preps**	**50 preps**	**100 preps**
Cat. No.	**740574**	**740574.25**	**740574.50**	**740574.100**
Buffer S1	150 ml	2 x 200 ml	2 x 400 ml	3 x 500 ml
Buffer S2	150 ml	400 ml	2 x 400 ml	3 x 500 ml
Buffer S3	150 ml	400 ml	2 x 400 ml	3 x 500 ml
Buffer N2	70 ml	200 ml	2 x 200 ml	4 x 200 ml
Buffer N3	2 x 250 ml	2 x 500 ml	2 x 1000 ml	3 x 1000 ml 500 ml
Buffer N5	200 ml	500 ml	2 x 500 ml	3 x 500 ml 200 ml
RNase A* (lyophilized)	15 mg	2 x 25 mg	2 x 40 mg	3 x 50 mg
NucleoBond® AX 500 columns	10	25	50	100
NucleoBond® folded filters	10	25	50	100
Plastic washer	5	10	10	10
Protocol	1	1	1	1

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

1 Kit contents *continued*

	NucleoBond® PC 2000	NucleoBond® PC 10000
	5 preps	**5 preps**
Cat. No.	**740576**	**740593**
Buffer S1	250 ml	750 ml
Buffer S2	250 ml	750 ml
Buffer S3	250 ml	750 ml
Buffer N2	140 ml	500 ml 120 ml
Buffer N3	2 x 250 ml	1000 ml 2 x 200 ml
Buffer N5	200 ml	500 ml 120 ml
RNase A* (lyophilized)	25 mg	80 mg
NucleoBond® AX 2000 columns	5	-
NucleoBond® AX 10000 columns	-	5
NucleoBond® folded filters	5	10
Plastic washer	5	-
Protocol	1	1

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

1 Kit contents *continued*

	NucleoBond® BAC 100
	10 preps
Cat. No.	**740579**
Buffer S1	2 x 150 ml
Buffer S2	2 x 150 ml
Buffer S3	2 x 150 ml
Buffer N2	70 ml
Buffer N3	2 x 200 ml
Buffer N5	150 ml
RNase A* (lyophilized)	2 x 15 mg
NucleoBond® BAC 100 columns	10
NucleoBond® folded filters	10
Plastic washer	5
Protocol	1

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

2 Introduction

2.1 Properties

NucleoBond® AX is a silica-based anion-exchange resin, developed by MACHEREY-NAGEL, for routine separation of different classes of nucleic acids. **NucleoBond® AX** resin, covered under European Patent EP 0496822, forms the basis for the entire line of nucleic acid purification products presented in this User Manual. **NucleoBond® AX** resin consists of hydrophilic, macro porous silica beads coupled to a methyl-ethylamine functional group. The functional group provides a high overall charge density that permits the negatively charged phosphate backbone of plasmid DNA to bind with high specificity to the resin. Due to a specialized manufacturing process that is rigorously controlled and monitored, the beads are uniform in diameter and contain particularly large pores. These special properties allow for optimum flow rates through the column and more efficient binding of nucleic acids to the matrix. Thus, using the matrix you can achieve sharp, well-defined elution profiles for individual nucleic acid species (see Figure 1). **NucleoBond® AX** can separate distinct nucleic acids from each other and from proteins, carbohydrates, and other unwanted cellular components. The purified nucleic acid products are suitable for use in the most demanding molecular biology applications, including transfection, *in vitro* transcription, automated or manual sequencing, cloning, hybridization, and PCR.

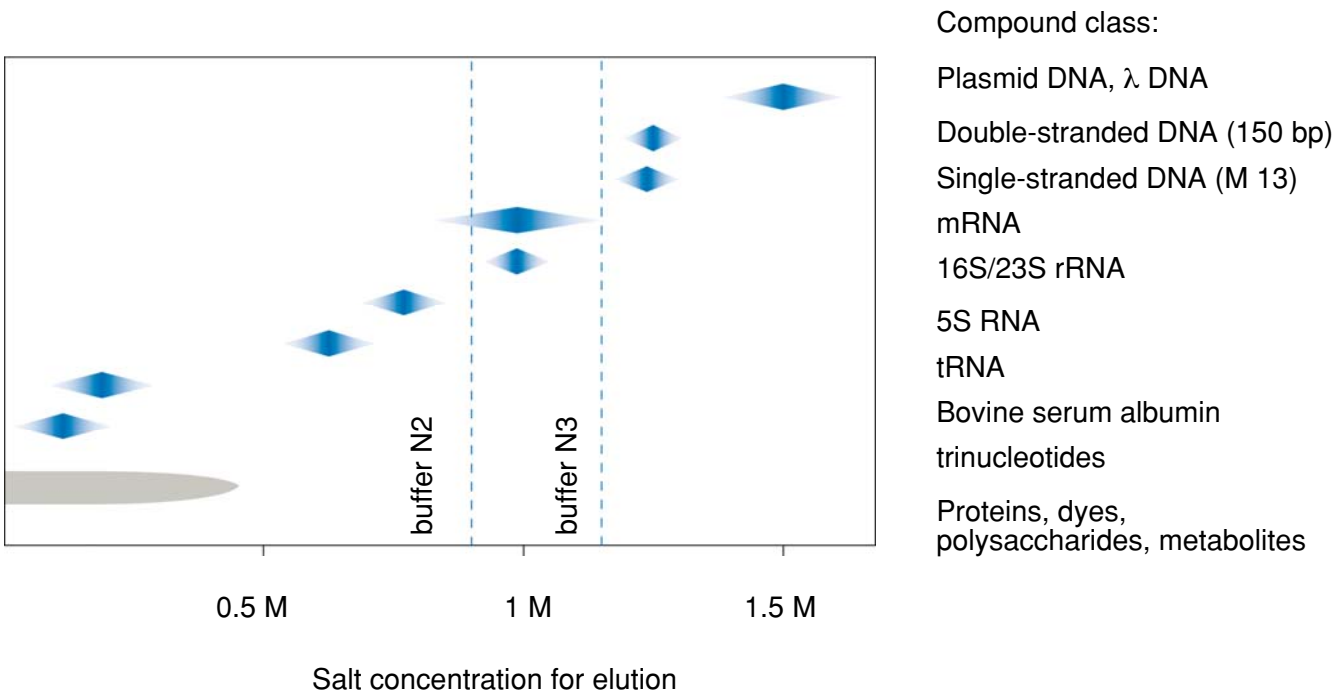


Figure 1. Elution profiles for distinct nucleic acid species using NucleoBond® AX columns. Nucleic acids can be eluted over a range of 0.5 M KCl to 1.5 M KCl; profiles for each nucleic acid are sharp and virtually non-overlapping.

2.2 About this user manual

Experienced users who are performing the purification of high-copy plasmids using a **NucleoBond® Plasmid** 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.

The protocols in this manual are organized as follows:

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

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

1 Carefully resuspend the pellet of bacterial cells in **buffer S1 + RNase A**. Please see section 6.3 regarding difficult-to-lyse strains.

0.4 ml	**4 ml**	**12 ml**

For example, if you are performing a Mini Prep to purify plasmid DNA using an AX 20 column you are requested to refer to the white boxes. In these boxes there are noted the volumes of buffers to be used.

The name of the buffer, the indicated volume is referring to, is highlighted in **bold type** within the instruction.

Referring to the a.m. example 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.

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, 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. 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**, appropriate 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 10,000).

- The protocols are suitable for purifying most plasmids ranging from 3->10 kb, cosmids from 10–50 kb, and very large constructs (P1 constructs, BACs, PACs) up to 300 kb.

- **NucleoBond® columns** are polypropylene columns containing **NucleoBond® AX silica resin** packed between two inert filter elements. **NucleoBond® columns** are used to purify plasmids, cosmids, P1 constructs, BACs, PACs, and phage λ DNA (not described in this user manual). The columns are available in several sizes to accommodate a wide range of purification needs (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	500 µg
AX 2000	2 mg
AX 10000	10 mg

- 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.

3.3 Buffer compositions

Buffer S1:

- 50 mM Tris-HCl, 10 mM EDTA, 100 µg/ml RNase A, pH 8.0

Buffer S2:

- 200 mM NaOH, 1% SDS

Buffer S3:

- 2.8 M KAc, pH 5.1

Buffer N2:

- 100 mM Tris, 15% ethanol, 900 mM KCl, 0.15% Triton X-100, adjusted to pH 6.3 with H_3PO_4

Buffer N3:

- 100 mM Tris, 15% ethanol, 1.15 M KCl, adjusted to pH 6.3 with H_3PO_4

Buffer N5:

- 100 mM Tris, 15% ethanol, 1 M KCl, adjusted to pH 8.5 with H_3PO_4

Note: Keep all buffers tightly capped.

The concentration of KCl required for eluting the desired nucleic acid is highly dependent on the pH value of the eluent. For this reason, pH values must be carefully controlled if the buffers have been prepared by the customer. A deviation of more than 0.1 pH unit from the given values may affect yields. If you are consistently experiencing reduced product yields, check the pH of all buffers before continuing. Buffers should be adjusted with H_3PO_4 or KOH.

3.4 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. If you are purifying low-copy plasmids, you will need to supplement the **NucleoBond® PC** kits with additional buffers. We recommend the **NucleoBond® Buffer Set I** (Cat. No. 740601) for routine purification of low-copy plasmids.

The **NucleoBond® Buffer Set I** can be used in connection with **NucleoBond® PC kits** for the isolation of **low-copy plasmids**. In this combination it is sufficient for

- **NucleoBond® PC 500 kit** (Cat. No. 740574), 10 preparations low-copy plasmid purification
- **NucleoBond® PC 100 kit** (Cat. No. 740573), 20 preparations low-copy plasmid purification
- **NucleoBond® PC 20 kit** (Cat. No. 740571.100), 100 preparations low-copy plasmid purification.

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® AX 500 columns** (Cat. No. 740531) 5 preparations high-copy plasmid purification
- **NucleoBond® AX 100 columns** (Cat. No. 740521) 10 preparations high-copy plasmid purification
- **NucleoBond® AX 20 columns** (Cat. No. 740511) 50 preparations high-copy plasmid purification.

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 (also see section 6 for further information regarding the growing of bacterial cultures).

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

3.5 Filtration of the lysate

After alkaline lysis, the solution has to be clarified from e.g. the cell debris through the supplied **NucleoBond® folded filters** or **NucleoBond® bottle top filters** in order to prevent clogging of the column.

- Use the provided **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 cellular debris from plasmid samples. Furthermore, Folded Filters avoid shearing of large DNA constructs, such as PACs or BACs. Folded Filters should not be used with AX 20 (Mini) columns for plasmid preparation due to the small culture volumes which allow an easy and quick clearing of the lysate in a microcentrifuge. Due to the large culture volume two folded fiters (Type 1 and Type 2) are included in the **NucleoBond® PC 10000 kit** in order to guarantee an optimal removal of SDS and cellular debris from plasmid sample. For correct use please follow the instructions given in step 4 of the corresponding protocol.

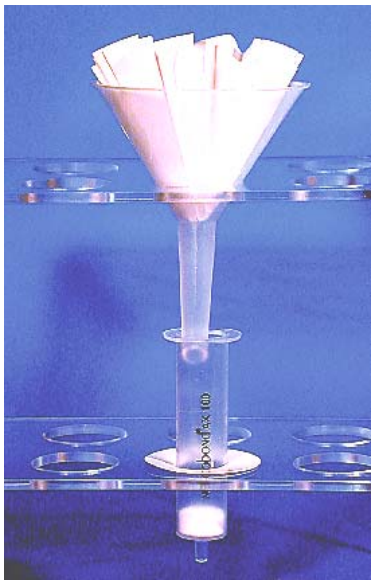


Figure 2: Correct use of the folded filters

- Alternatively:
  Centrifuge the solution with the given acceleration forces and times as written in step 4 of the corresponding protocols. Load the cleared lysate onto the column.

- For the AX 2000 (Mega) column and AX 10000 (Giga) column also the vacuum operated NucleoBond® bottle top filters can be used for filtration of the lysate. The **NucleoBond® bottle top filters** (Figure 3) make the separation of the bacterial lysate and SDS precipitate easily, quickly, and conveniently. When using a **NucleoBond® bottle top filter** it is not necessary to centrifuge the solution first, as described in step 4, option 1 and 2 of the corresponding protocol. Adjust the bottle top filter to a suitable flask (e.g. Schott), load the bacterial lysate and apply the vacuum. After 3-5 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 3: Correct use of the NucleoBond® bottle top filter

3.6 Elution procedures

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 (20-25°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 (20-25°C)** 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!

- All kit components can be stored at room temperature (20-25°C) and are stable 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 of 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 up to 6 months.

- Buffer S2 should be stored at room-temperature (20-25°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.

5 Safety instructions - risk and safety phrases

The following components of the NucleoBond® PC kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
RNase A	RNase A, lyophilized	Xn*	May cause sensitization by inhalation and skin contact	R 42/43	S 7-16-22
S2	sodium hydroxide < 2 %	Xi**	Irritating to eyes and skin	R 36/38	S 22-24-26-36/37

Risk Phrases

R 36/38	Irritating to eyes and skin
R 42/43	May cause sensitization by inhalation and skin contact

Safety Phrases

S 7	Keep container tightly closed
S 16	Keep away from sources of ignition - No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 36/37	Wear suitable protective clothing and gloves

* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

** Label not necessary, if quantity below 25 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

6 Growing of bacterial cultures

6.1 General considerations

Yield and quality of plasmid DNA depends on e.g. 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 2. Overnight cultures in flasks usually reach, under vigorous shaking, an OD_{600} of 3-6, while fermentation cultures reach 10 and more. Therefore, please refer not only to the culture volume, but also check OD_{600} and pellet wet weight, too in particular if richer culture media like 2xYT or TB are used. If too much bacterial material is used, lysis and precipitation steps are inefficient and finally **NucleoBond® columns** are overloaded causing 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.

Table 2: Recommended culture volume

Copy plasmids	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
	1-100 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

For AX 20 and AX 100 it is not necessary to measure the wet weight but depending on the media used, OD_{600} should be determined.

For a low copy protocol using AX 10000 (Giga) columns please call our Technical Service Center.

6.2 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 2xYT (Yeast/Tryptone) or TB (Terrific Broth) can be used. By using 2xYT 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.

For Mini and Midi preps cultivation in flasks is recommended. At least for Mega and Giga preps the use of an appropriate fermentation system is recommended in order to optimize cultivation conditions.

6.3 Difficult-to-lyse strains

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

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) with selecting antibiotics added to the medium.

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

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**. Please see section 6.3 regarding difficult-to-lyse strains.

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 (20-25°C) for 2–3 min (max 5 min). Do not vortex, as this will release contaminating chromosomal DNA from the 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

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

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 flow-through.

1.0 ml	2.5 ml	6.0 ml

4 Clarification of the lysate

Clear the bacterial lysate by following EITHER **option 1 (Midi, Maxi)** or **option 2 (Mini, Midi, Maxi)**, described below. This step is extremely important; excess precipitate left in suspension may clog the NucleoBond® column in later steps.

Option 1. Filter the suspension. *Place a NucleoBond® folded filter in a small funnel for support, and prewet the filter with a few drops of buffer N2 or sterile deionized H_2O. Load the bacterial lysate onto the wet filter and collect the flow-through.*

Note*: Do not use NucleoBond® folded filters with AX 20 columns (Mini preps).*

Alternatively:

Option 2. *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,* ***either*** *repeat this step* ***or*** *proceed with option 1.*

15 min	25 min	40 min

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 flow-through for analysis.

6 Washing

Wash the column with **buffer N3.** Repeat as indicated. Discard flow-through.

2 x 1.5 ml	10 ml	32 ml

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

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 some hours. In this case the eluate should be prewarmed 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 **≥ 15,000 x g for 30 min at 4°C**. Carefully discard the supernatant.

0.75 ml	3.5 ml	11.0 ml

9 Wash and dry DNA pellet

Add room-temperature **70% ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 15,000 x g for 10 min at room temperature (20-25°C).**

500 µl	2 ml	5 ml

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (20-25°C)**, no less than the indicated time.

Drying for longer periods will not harm the quality of the plasmid DNA.

5-10 min	5-10 min	10-20 min

10 Reconstitute DNA

Redissolve the DNA pellet in an appropriate volume of buffer TE or sterile deionized H_2O. Depending on the type of centrifugation-tube, redissolve 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**. Please see section 6.3 regarding difficult-to-lyse strains.

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 (20-25°C) for 2–3 min (max 5 min). Do not vortex as this will release contaminating chromosomal DNA from the 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 flow-through.

20 ml	100 ml

Mega	Giga
(AX 2000)	(AX 10000)

4 Clarification of the lysate

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

Option 1. Filter the suspension. *Place a NucleoBond® folded filter in a large funnel for support. Prewet the filter with a few drops of buffer N2 or sterile deionized H_2O and load lysate.*

Note: *NucleoBond® PC 10000 (Giga) kits contain two types of folded filters (Type 1 and Type 2) in order to guarantee an optimal and fast filtration. Put folded filter Type 2 into folded filter Type 1 and prewet the filters with a few drops of buffer N2 or sterile deionized H_2O before loading lysate.*

For Giga Columns, we recommend dividing the samples in half to clear the lysate: use two NucleoBond® folded filters and two funnels simultaneously. Then combine the flow-through before proceeding with step 5.

For AX 2000 (Mega) and AX 10000 (Giga) columns alternatively the vacuum operated NucleoBond® bottle top filters (not included) can be used for filtration of the lysate. For correct use of the NucleoBond® bottle top filters see section 3.5.

Alternative:

Option 2*. 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,* ***either*** *repeat this step* ***or*** *proceed with option 1.*

50 min	60 min

5 Binding

Load the cleared lysate from step 4 onto the NucleoBond® column. Allow the column to empty by gravity flow. You may want to save all or part of the flow-through for analysis.

6 Washing

Wash the column with **buffer N3.** Repeat as indicated. Discard flow-through.

2 x 35 ml	2 x 100 ml

Mega	Giga
(AX 2000)	(AX 10000)

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 some hours. In this case the eluate should be prewarmed to room temperature before the plasmid DNA is precipitated.

25 ml	100 ml

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

8 Precipitation

Add room-temperature **isopropanol** to precipitate the eluted plasmid DNA. Centrifuge at **≥ 15,000 x g for 30 min at 4°C**. Carefully discard the supernatant.

18 ml	70 ml

9 Wash and dry DNA pellet

Add room-temperature **70% ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 15,000 x g for 10 min at room temperature (20-25°C)**.

7 ml	10 ml

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (20-25°C)**.

Drying for longer periods will not harm the quality of the plasmid DNA.

30 - 60 min	30 - 60 min

10 Reconstitute DNA

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

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

7.4 Low-copy plasmid purification (Mini-Midi)

Mini	Midi
(AX 20)	(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**. Please see section 6.3 regarding difficult-to-lyse strains.

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 (20-25°C) for 2–3 min (max 5 min). Do not vortex, as this will release contaminating chromosomal DNA from the cellular debris into the suspension.

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.

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 flow-through.

1 ml	2.5 ml

Mini	Midi
(AX 20)	(AX 100)

4 Clarification of the lysate

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

Option 1. Filter the suspension. *Place a NucleoBond® folded filter in a small funnel for support, and prewet the filter with a few drops of buffer N2 or sterile deionized H_2O. Load the lysate onto the wet filter and collect the flow-through.*

Note*: Do not use NucleoBond® folded filters with AX 20 columns (Mini preps).*

Alternative:

Option 2*. 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,* ***either*** *repeat this step* ***or*** *proceed with option 1.*

15 min	25 min

5 Binding

Load the cleared lysate from step 4 onto the NucleoBond® column. Allow the column to empty by gravity flow. You may want to save all or part of the flow-through for analysis.

6 Washing

Wash the column with **buffer N3.** Repeat as indicated. Discard flow-through.

2 x 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 some hours. In this case the eluate should be prewarmed to room temperature before the plasmid DNA is precipitated.

1 ml	5 ml

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

Mini	Midi
(AX 20)	(AX 100)

8 Precipitation

Add room-temperature **isopropanol** to precipitate the eluted plasmid DNA. Centrifuge at **≥ 15,000 x g for 30 min at 4°C**. Carefully discard the supernatant.

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 **≥ 15,000 x g for 10 min at room temperature (20-25°C)**.

500 µl	2 ml

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (20-25°C).**

Drying for longer periods will not harm the quality of the plasmid DNA.

5-10 min	5-10 min

10 Reconstitute DNA

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

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

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

Maxi	Mega
(AX 500 / BAC 100)	(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**. Please see section 6.3 regarding difficult-to-lyse strains.

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 (20-25°C) for 2–3 min (max 5 min). Do not vortex, as this will release contaminating chromosomal DNA from the 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 flow-through.

6 ml	20 ml

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

4 Clarification of the lysate

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

Note: for purification of BAC DNA it is recommended to follow option 1.

Option 1. Filter the suspension. *Place a NucleoBond® folded filter in a large funnel for support. Prewet the filter with a few drops of buffer N2 or sterile deionized H_2O.* ***Shortly*** *spin down the lysate at low g-force in order to let the cellullar debris settle before loading on the NucleoBond® folded filter When the centrifuge has stopped, carefully decant the partially cleared lysate onto the wet filter and collect the flow-through.*

For the AX 2000 (Mega) column alternatively the vacuum operated NucleoBond® bottle top filters (not included) can be used for filtration of the lysate.

Alternative:
Option 2. 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,* ***either*** *repeat this step* ***or*** *proceed with option 1.*

40 min	50 min

5 Binding

Load the cleared lysate from step 4 onto the NucleoBond® column. Allow the column to empty by gravity flow. You may want to save all or part of the flow-through for analysis.

6 Washing

Wash the column with **buffer N3.** Repeat as indicated. Discard flow-through.

2 x 18 ml	2 x 50 ml

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

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 some hours. In this case the eluate should be prewarmed 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 **≥ 15,000 x g for 30 min at 4°C**. Carefully discard the supernatant.

11 ml	18 ml

9 Wash and dry DNA pellet

Add room-temperature **70% ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 15,000 x g for 10 min at room temperature (20-25°C)**.

5 ml	7 ml

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (20-25°C).**

Drying for longer periods will not harm the quality of the plasmid DNA.

10-20 min	30-60 min

10 Reconstitute DNA

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

Determine plasmid yields 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 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 percentage of ccc form / structural integrity of isolated plasmid DNA.

- Plasmid quality is checked by UV spectroscopy (quotient 260 nm/ 280 nm). 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.

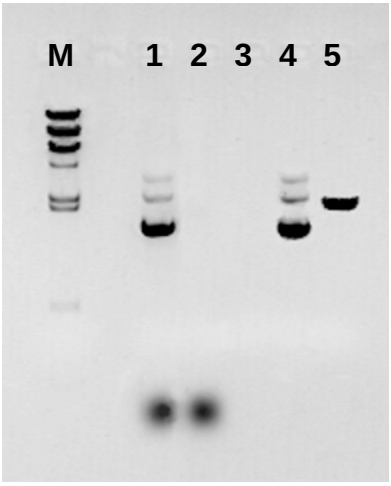
8.2 Troubleshooting

If you experience problems with reduced yield or purity, it is recommended to check at which purification step of the procedure the problem occured. Firstly, the bacterial culture has to be checked for sufficient growth (OD_{600}) in the presence of an appropriate selective antibiotic (see Table 4). Secondly, aliquots of the cleared lysate, the flow-through, the combined washing steps (buffer N3), 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. The volumes outlined in Table 3 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. The gel picture (see Figure 4) will help you to address the specific questions outlined in this section more quickly and efficiently.

Table 3: NucleoBond® PC volumes required for analytical check					
Sample	**Purification step**	**Volume required [µl]**			
		PC 100	**PC 500**	**PC 2000**	**PC 10000**
I	**Cleared lysate, after protocol step 4**	600	400	300	200
II	**Flow-through, after protocol step 5**	600	400	300	200
III	**Washing flow-through, after protocol step 6**	500	300	200	100
IV	**Eluate, after protocol step 7**	300	200	100	100

Figure 4 Analytical check of NucleoBond® PC 500 purification samples
Plasmid: pUC18, bacterial strain: *E.coli* DH5α. 20 µl of each sample has been analyzed on a 1% 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%.



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

Table 4: Information about antibiotics according to Maniatis*			
Antibiotic	**Stock solution (concentration)**	**Storage**	**Working concentration**
Ampicillin	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, Fritsh EF, Sambrook J: *Molecular cloning. A laboratory manual,* Cold Spring Harbour, Cold Spring, New York 1982.

Problem	Possible cause and suggestions
	SDS- or other precipitates are present in the sample • Load the S1/2/3 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 or centrifugation but if the cleared lysate is stored on ice for a longer period, new precipitates may appear. If precipitate is visible, it is recommended to filter respectively centrifuge the lysate again immediately before loading it onto the NucleoBond® column.
	pH or salt concentrations of buffers are too high • Especially if the customer prepares additional buffer it is recommended to thoroughly check the pH of each buffer. Adjust pH or prepare new buffers if necessary.
	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 cartridge will be insufficient.
No or low plasmid DNA yield	*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 2, section 6.1.
	Lysate incorrectly prepared • After storage below 20°C, SDS in buffer S2 may precipitate. This will lead to a suboptimal SDS concentration in buffer S2 causing inefficient lysis. Check buffer S2 for precipitates before use and prewarm the bottle if necessary in order to redissolve SDS (30-40°C will be sufficient).

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 blocks the column but also significantly reduces yields. *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 or 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. *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 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 potential plasmid DNA 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 smeared over the wall 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 will not resuspend in buffer	*Pellet was over dried* • Try dissolving at temperatures for a longer period of time (e.g., 2 h at 37°C or overnight at RT), best under constant spinning (3D-shaker). *There is 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* • Check isopropanol purity, and perform precipitation at room-temperature (20-25°C) but centrifuge at 4°C. 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 contaminated with cellular debris or genomic DNA due to inefficient lysis* • Reduce the culture volume, or increase the amount of buffers S1, S2, and S3 used during the lysis steps. *DNA is degraded* • Make sure that all equipment (pipettes, 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 proceed for longer than 5 min.
NucleoBond® folded filters clog during filtration	*Culture volumes used are too large* • Reduce the culture volume or increase the amount of buffers 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	Cat. No.	Pack of
NucleoBond® PC 20	740571	20 preps
NucleoBond® PC 20	740571.100	100 preps
NucleoBond® AX 20	740511	20 columns
NucleoBond® PC 100	740573	20 preps
NucleoBond® PC 100	740573.100	100 preps
NucleoBond® AX 100	740521	20 columns
NucleoBond® AX 100 big pack	740521.100	100 columns
NucleoBond® PC 500	740574	10 preps
NucleoBond® PC 500	740574.25	25 preps
NucleoBond® PC 500	740574.50	50 preps
NucleoBond® PC 500	740574.100	100 preps
NucleoBond® AX 500	740531	10 columns
NucleoBond® AX 500 big pack	740531.50	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
NucleoBond® Finalizer (includes 20 NucleoBond® Finalizer, 2 syringes of 30 ml, 2 syringes of 1 ml)	740519.20	20 filters
NucleoBond® Finalizer (includes 20 NucleoBond® Finalizer, 20 syringes of 30 ml, 20 syringes of 1 ml)	740520.20	20 sets
NucleoBond® folded filters (for AX 100 columns)	740561	50

Product	Cat. No.	Pack of
NucleoBond® folded filters XL (for AX 500/2000, BAC 100 columns)	740577	50
NucleoBond® bottle top filters (for AX 2000/10000 columns)	on request	
NucleoBond® buffer set I	740601	1 set
NucleoBond® buffer S1	740516.1	500 ml
NucleoBond® buffer S2	740517.1	500 ml
NucleoBond® buffer S3	740518.1	500 ml
NucleoBond® buffer N2	740527.1	500 ml
NucleoBond® buffer N3	740528.1	1000 ml
NucleoBond® buffer N5	740529.1	500 ml
NucleoBond® rack small (for AX 20 columns)	740562	1
NucleoBond® rack large (for AX 100, AX 500, AX 2000, AX 10000 columns)	740563	1
RNase A	740505	100 mg
RNase A	740505.50	50 mg

8.4 References

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

8.5 Product use restriction / warranty

NucleoBond® PC/BAC kit components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoBond® PC/BAC** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole

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