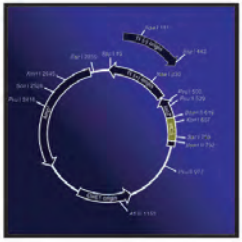

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Plasmid DNA Purification

User Manual

NucleoBond® PC 20

NucleoBond® PC 100

NucleoBond® PC 500

NucleoBond® BAC 100

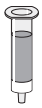
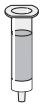
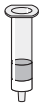
NucleoBond® PC 2000

NucleoBond® PC 10000

January 2011 / Rev. 08

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

Protocol-at-a-glance (Rev. 08)

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

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1 Components

1.1 Kit contents

	NucleoBond® PC 20	
	20 preps	**100 preps**
REF	**740571**	**740571.100**
Resuspension Buffer S1	25 mL	2 x 25 mL
Lysis Buffer S2	25 mL	2 x 25 mL
Neutralization Buffer S3	25 mL	2 x 25 mL
Equilibration Buffer N2	25 mL	125 mL
Wash Buffer N3	3 x 30 mL	3 x 125 mL
Elution Buffer N5	32 mL	120 mL
RNase A (lyophilized)*	2.5 mg	2 x 2.5 mg
NucleoBond® AX 20 Columns	20	100
Plastic Washers	10	10
User Manual	1	1

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

1.1 Kit contents *continued*

	NucleoBond® PC 100	
	20 preps	**100 preps**
REF	**740573**	**740573.100**
Resuspension Buffer S1	100 mL	2 x 250 mL
Lysis Buffer S2	4 x 25 mL	2 x 250 mL
Neutralization Buffer S3	100 mL	2 x 250 mL
Equilibration Buffer N2	70 mL	2 x 150 mL
Wash Buffer N3	250 mL	3 x 400 mL
Elution Buffer N5	120 mL	3 x 200 mL
RNase A (lyophilized)*	10 mg	2 x 25 mg
NucleoBond® AX 100 Columns	20	100
NucleoBond® Folded Filters	20	100
Plastic Washers	10	10
User Manual	1	1

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

1.1 Kit contents *continued*

	NucleoBond® PC 500			
	10 preps	**25 preps**	**50 preps**	**100 preps**
REF	**740574**	**740574.25**	**740574.50**	**740574.100**
Resuspension Buffer S1	150 mL	2 x 200 mL	2 x 400 mL	3 x 500 mL
Lysis Buffer S2	150 mL	400 mL	2 x 400 mL	3 x 500 mL
Neutralization Buffer S3	150 mL	400 mL	2 x 400 mL	3 x 500 mL
Equilibration Buffer N2	70 mL	200 mL	2 x 200 mL	4 x 200 mL
Wash Buffer N3	2 x 250 mL	2 x 500 mL	2 x 1000 mL	3 x 1000 mL 500 mL
Elution 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 XL	10	25	50	100
Plastic Washers	5	10	10	10
User Manual	1	1	1	1

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

1.1 Kit contents *continued*

	NucleoBond® PC 2000	NucleoBond® PC 10000
	5 preps	**5 preps**
REF	**740576**	**740593**
Resuspension Buffer S1	250 mL	750 mL
Lysis Buffer S2	250 mL	750 mL
Neutralization Buffer S3	250 mL	750 mL
Equilibration Buffer N2	150 mL	500 mL 120 mL
Wash Buffer N3	2 x 250 mL	1000 mL 2 x 200 mL
Elution 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 XL	5	–
NucleoBond® Folded Filters Type 1	–	10
NucleoBond® Folded Filters Type 2	–	10
Plastic Washers	5	–
User Manual	1	1

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

1.1 Kit contents *continued*

	NucleoBond® BAC 100
	10 preps
REF	**740579**
Resuspension Buffer S1	2 x 150 mL
Lysis Buffer S2	2 x 150 mL
Neutralization Buffer S3	2 x 150 mL
Equilibration Buffer N2	70 mL
Wash Buffer N3	2 x 200 mL
Elution Buffer N5	150 mL
RNase A (lyophilized)*	2 x 15 mg
NucleoBond® BAC 100 Columns	10
NucleoBond® Folded Filters XL	10
Plastic Washers	5
User Manual	1

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

1.2 Reagents and equipment to be supplied by user

Reagents

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

Equipment

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37 °C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- Funnels to hold the NucleoBond® Folded Filters for lysate filtration (NucleoBond® PC 100, 500, 2000, 10000, BAC 100)
- NucleoBond® Rack (see ordering information) or equivalent holder
- Refrigerated centrifuge capable of reaching ≥ 15,000 x *g* with rotor for the appropriate centrifuge tubes or bottles
- Centrifugation tubes or vessels with suitable capacity for the volumes specified in the respective protocol

2 Introduction

2.1 Properties

NucleoBond® AX is a patented silica-based anion-exchange resin, developed by MACHEREY-NAGEL, for routine separation of different classes of nucleic acids. **NucleoBond® AX Resin** forms the basis for the entire line of nucleic acid purification products presented in this User Manual. **NucleoBond® AX Resin** consists of hydrophilic, macro porous silica beads coupled to a methyl-ethylamine functional group. The functional group provides a high overall charge density that permits the negatively charged phosphate backbone of 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.

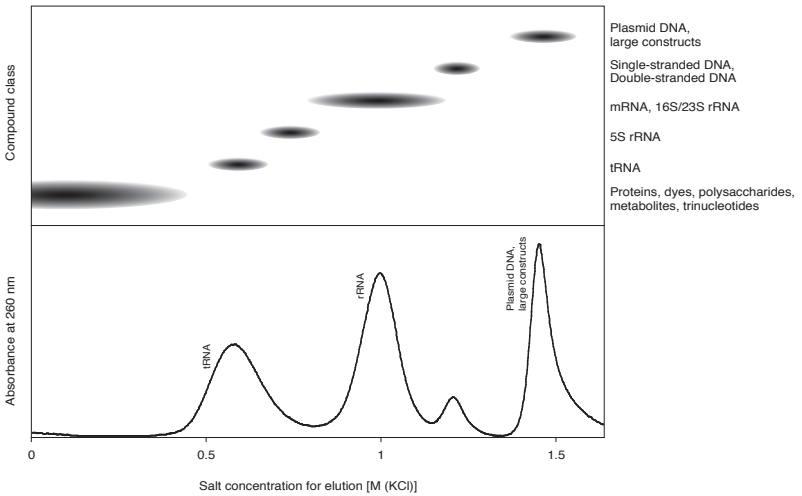

Figure 1: Elution profile of NucleoBond® AX Resin at pH 7.0

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

2.2 About this User Manual

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

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

The protocols in this manual are organized as follows:

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

	Mini	Midi	Maxi
	(AX 20)	**(AX 100)**	**(AX 500)**
1	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 you will find volumes or incubation times in the white boxes.

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

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

3 Product description

3.1 The basic principle

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

3.2 Kit specifications

- **NucleoBond® Plasmid** purification kits contain **NucleoBond® Columns**, 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 10000).
- The protocols are suitable for purifying most plasmids ranging from 3–10 kbp, cosmids from 10–50 kbp, and very large constructs (P1 constructs, BACs, PACs) up to 300 kbp.
- **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: NucleoBond® Column binding capacities

NucleoBond® Column	Binding capacity
AX 20	20 µg
AX 100	100 µg
AX 500	500 µg
BAC 100	100 µg
AX 2000	2 mg
AX 10000	10 mg

- 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

Resuspension Buffer S1:

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

Lysis Buffer S2:

- 200 mM NaOH, 1 % SDS

Neutralization Buffer S3:

- 2.8 M KAc, pH 5.1

Equilibration Buffer N2:

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

Wash Buffer N3:

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

Elution 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 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** (REF 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 (REF 740574), 10 preparations low-copy plasmid purification
- **NucleoBond® PC 100** kit (REF 740573), 20 preparations low-copy plasmid purification
- **NucleoBond® PC 20** kit (REF 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® PC 500 Columns** (REF 740531) 5 preparations high-copy plasmid purification
- **NucleoBond® PC 100 Columns** (REF 740521) 10 preparations high-copy plasmid purification
- **NucleoBond® PC 20 Columns** (REF 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 cleared from precipitated protein and cell debris in order to prevent clogging of the NucleoBond® Columns. There are the following three options:

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

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

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

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

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

Figure 3: Correct use of the NucleoBond® Bottle Top Filter

3.6 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 (18–25 °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 (18–25 °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!

Storage conitions:

- All kit components can be stored at room temperature (18–25 °C) and are stable for up to one year.

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

- Dissolve the **lyophilized RNase A** by the addition of 1 mL **Resuspension Buffer S1**. Wearing gloves is recommended. Pipette up and down until the RNase A is dissolved completely. Transfer the RNase A solution back to the bottle containing Buffer S1 and shake well. Indicate date of RNase A addition. The final concentration of RNase A is 100 µg/mL Buffer S1. Store Buffer S1 with RNase A at 4 °C. The solution will be stable at this temperature for at least 6 months.

- **Lysis Buffer S2** should be stored at room-temperature (18–25 °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 22-24
S2	Sodium hydroxide <2 %	✖ Xi*	Irritating to eyes and skin	R 36/38	S 26-37/39-45

Risk phrases

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

Safety phrases

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 37/39	Wear suitable gloves and eye/face protection
S 45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

* Hazard labeling not necessary if quantity per bottle below 25 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

6 Growing of bacterial cultures

6.1 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 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, particularly if richer culture media like 2 x YT 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
	10–100 mL	–	AX 100 (Midi)	20–100 µg
	100–500 mL	1.5–2.2 g	AX 500 (Maxi)	100–500 µg
	100–500 mL	1.5–2.2 g	BAC 100 (Maxi)	100 µg
	500–2,000 mL	5–7.5 g	AX 2000 (Mega)	500 µg–2 mg

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 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 (≤ 2 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

For plasmid purification of (e.g., Gram-positive bacteria or strains with a more resistant cell wall) it might be advantageous to start the preparation with a lysozyme treatment. Therefore, resuspend the cell pellet in Buffer S1 containing **2 mg/mL lysozyme** and incubate at **37 °C** for **30 minutes**. Proceed then with the lysis procedure according to the **NucleoBond®** standard protocol.

6.4 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 (OD_{600} ≈ 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.

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

** Frenkel L, Bremer H: *Increased amplification of plasmids pBR322 and pBR327 by low concentrations of chloramphenicol*, DNA (5), 539–544, 1986.

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 (18–25 °C) for 2–3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

0.4 mL	4 mL	12 mL

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

0.4 mL	4 mL	12 mL

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

Note: Complete removal of precipitated protein and cell debris is essential to avoid clogging of the NucleoBond® Column.

NucleoBond® PC 20

Centrifuge the lysate at >1 2,000 x *g* for 5–10 minutes at room temperature or better 4°C.

NucleoBond® PC 100, 500

Place a NucleoBond® Folded Filter in a funnel of appropriate size. Wet the filter with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter. Either collect the flow-through in a separate vessel and proceed with step 5 **OR** position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one time-saving step (skip step 5). See section 3.5 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

5 Binding

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

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 m	32 mL

7 Elution

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

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

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

1 mL	5 mL	15 mL

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

8 Precipitation

Add **room-temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at **≥ 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 (18–25 °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 (18–25 °C)** no less than the indicated time.

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

5–10 min	5–10 min	10–20 min

10 Reconstitute DNA

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

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

7.3 High-copy plasmid purification (Mega, Giga)

	Mega (AX 2000)	Giga (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.

Mega	Giga
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 (18–25°C) for 2–3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

Mega	Giga
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.

Mega	Giga
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.

Mega	Giga
20 mL	100 mL

	Mega (AX 2000)	Giga (AX 10000)

4 Clarification of the lysate

Note: Complete removal of precipitated protein and cell debris is essential to avoid clogging of the NucleoBond® Column.

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

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

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

See section 3.5 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

5 Binding

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

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 35 mL	2 x 100 mL

7 Elution

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

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

25 mL	100 mL

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

	Mega	Giga
	(AX 2000)	(AX 10000)

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.

Mega	Giga
18 mL	70 mL

9 Wash and dry DNA pellet

Add **room-temperature 70 % ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 15,000 x *g*** for **10 min** at **room temperature (18–25 °C)**.

Mega	Giga
7 mL	10 mL

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (18–25 °C)** no less than the indicated time.

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

Mega	Giga
30–60 min	30–60 min

10 Reconstitute DNA

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

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

7.4 Low-copy plasmid purification (Mini, Midi)

	Mini (AX 20)	Midi (AX 100)
1	**Cultivate and harvest bacterial cells**	
	Harvest bacteria from an LB culture by centrifugation at **4,500–6,000 x *g*** for **15 min** at **4 °C**.	
2	**Cell lysis**	
	Carefully resuspend the pellet of bacterial cells in **Buffer S1 + RNase A**. 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 (18–25 °C) for 2–3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from 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

Note: Complete removal of precipitated protein and cell debris is essential to avoid clogging of the NucleoBond® Column.

NucleoBond® PC 20

Centrifuge the lysate at >1 2,000 x *g* for 5–10 minutes at room temperature or better 4°C.

NucleoBond® PC 100, 500

Place a NucleoBond® Folded Filter in a funnel of appropriate size. Wet the filter with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter. Either collect the flow-through in a separate vessel and proceed with step 5 **OR** position funnel and filter directly on top of the NucleoBond® Column to clear and load the lysate in one time-saving step (skip step 5). See section 3.5 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

5 Binding

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

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.

Mini	Midi
2 x 2 mL	12 mL

7 Elution

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

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

Mini	Midi
1 mL	5 mL

Mini	Midi
(AX 20)	(AX 100)

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

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 (18–25 °C)**.

500 µL	2 mL

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (18–25 °C)**.

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

5–10 min	5–10 min

10 Reconstitute DNA

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

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

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

	Maxi	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 (18–25 °C) for 2–3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

24 mL	90 mL

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

24 mL	90 mL

3 Equilibration of the column

Equilibrate a NucleoBond® AX 500 (Maxi), BAC 100 (Maxi), or AX 2000 (Mega) Column with **Buffer N2**. Allow the column to empty by gravity flow. Discard flow-through.

6 mL	20 mL

4 Clarification of the lysate

Clear the bacterial 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.

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

Note: Complete removal of precipitated protein and cell debris is essential to avoid clogging of the NucleoBond® Column.

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

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

See section 3.5 for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.

5 Binding

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

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 18 mL	2 x 50 mL

7 Elution

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

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

15 mL	25 mL

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

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

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 (18–25 °C)**.

5 mL	7 mL

Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (18–25 °C)** no less than the indicated time.

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

10–20 min	30–60 min

10 Reconstitute DNA

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

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

8 Appendix

8.1 Determination of DNA yield and quality

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

8.2 Troubleshooting

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

First, the bacterial culture has to be checked for sufficient growth (OD_{600}) in the presence of an appropriate selective antibiotic (see Table 3). **Second**, 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.

Table 3: Information about antibiotics according to Maniatis*

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

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

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

Table 4: NucleoBond® PC volumes required for an analytical check

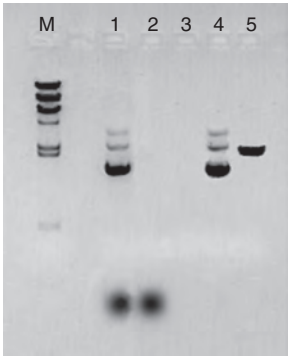
Sample	Purification step	Volume required [µL]			
		PC 100	PC 500	PC 2000	PC 10000
I	Cleared lysate after protocol step 4	600	400	300	200
II	Column flow-through after protocol step 5	600	400	300	200
III	Wash flow-through after protocol step 6	500	300	200	100
IV	Eluate after protocol step 7	300	200	100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

The exemplary gel picture (see Figure 4) 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 flow-through samples (lane 1 and 2). It might also occur in the wash fraction but must be absent in the eluate.

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



M: Marker λ *Hin*dIII

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

2: II, lysate flow-through, no plasmid DNA, but degraded RNA

3: III, wash flow-through, no plasmid DNA or residual RNA

4: IV, eluate, pure plasmid DNA

5: *Eco*RI restriction, linearized form of plasmid

Figure 4: Exemplary analytical check of NucleoBond® PC 500 purification samples

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

Problem	Possible cause and suggestions
No or low plasmid DNA yield	*SDS- or other precipitates are present in the sample* • Load the S1 / S2 / S3 lysate sample onto the NucleoBond® Column immediately after finishing the initial lysis steps. SDS and cell debris are removed by filtration with NucleoBond® Folded Filters / NucleoBond® Bottle Top Filters but if the cleared lysate is stored on ice for a longer period, new precipitate may appear. If precipitate is visible, it is recommended to filter the lysate again immediately before loading it onto the NucleoBond® Column. *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 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 2, section 6.1. *Lysate incorrectly prepared* • After storage below 20 °C, SDS in Buffer S2 may precipitate causing inefficient lysis. Check Buffer S2 for precipitates before use and preheat the bottle to 30–40 °C if necessary in order to redissolve SDS.

Problem	Possible cause and suggestions
Column is blocked	*Sample is too viscous* • Do NOT attempt to purify lysate prepared from a culture volume larger than recommended for any given column size. Increasing culture volumes not only block the column but can also reduce yields due to inefficient lysis. *Precipitates occur during storage* • Check cleared lysate for precipitates, especially if the lysate was stored for a longer time before loading. If necessary, clear the lysate again by filtration. *Lysate was not completely cleared* • Centrifuge at higher speed for a longer period of time, or use additional NucleoBond® Folded Filters to clear the lysate.
Cellular DNA or RNA contamination of plasmid DNA	*Lysis treatment was too harsh* • Be sure not to incubate the lysate in Buffer S2 for more than 5 min. *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.

Problem	Possible cause and suggestions
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.
Nucleic acid pellet does not resuspend in buffer	*Pellet was over dried* • Try dissolving at higher temperatures for a longer period of time (e.g., 2 h at 37 °C or overnight at RT), best under constant spinning (3D-shaker). *Residual salt or organic solvent in the pellet* • Wash the pellet with additional low-viscosity organic solvent (70 % ethanol), or increase the resuspension buffer volume.
Nucleic acid pellet is opaque or white instead of clear and glassy	*Salt has co-precipitated with the pellet* • Use room-temperature isopropanol and check isopropanol purity. Do not precipitate by allowing the eluate to drip directly from the column into a tube containing isopropanol. Add isopropanol only after eluate has been collected. • Try resuspending the pellet in Buffer N2, and reload onto the NucleoBond® Column. Be sure to wash the column several times with Buffer N2 before loading the redissolved pellet onto the column.

Problem	Possible cause and suggestions
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, S2, and S3 used during the lysis steps. *DNA is degraded* • Make sure that all equipment (pipettors, centrifuge tubes, etc.) are clean and nuclease-free. Make sure that the alkaline lysis step (i.e., the incubation of sample after addition of Buffer S2) does not 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 Buffer S1, S2, and S3 used during the lysis steps. *Incubation time too short* • Make sure that S1 / S2 / S3 lysate was incubated according to the protocol.

8.3 Ordering information

Product	REF	Pack of
NucleoBond® PC 20	740571	20 preps
	740571.100	100 preps
NucleoBond® AX 20	740511	20 columns
NucleoBond® PC 100	740573	20 preps
	740573.100	100 preps
NucleoBond® AX 100	740521	20 columns
	740521.100	100 columns
NucleoBond® PC 500	740574	10 preps
	740574.25	25 preps
	740574.50	50 preps
	740574.100	100 preps
NucleoBond® AX 500	740531	10 columns
	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 (for use with NucleoBond® PC 100, PC 500, PC 500 EF, NucleoBond® Xtra Midi, Midi EF)	740519.20	20 filters 2 syringe sets
NucleoBond® Finalizer Plus (for use with NucleoBond® PC 100, PC 500, PC 500 EF, NucleoBond® Xtra Midi, Midi EF)	740520.20	20 filters 20 syringe sets
NucleoBond® Finalizer Large (for use with NucleoBond® PC 2000, PC 2000 EF, NucleoBond® Xtra Maxi, Maxi EF)	740418.20	20 large filters 2 syringe sets
NucleoBond® Finalizer Large Plus (for use with NucleoBond® PC 2000, PC 2000 EF, NucleoBond® Xtra Maxi, Maxi EF)	740419.20	20 large filters 20 syringe sets
NucleoBond® Folded Filters (for NucleoBond® AX 100 Columns)	740561	5

Product	REF	Pack of
NucleoBond® Folded Filters XL (for NucleoBond® AX 500/2000, BAC 100 Columns)	740577	50
NucleoBond® Bottle Top Filters Type 1 (for NucleoBond® AX 2000 Columns)	740547.5	5
NucleoBond® Bottle Top Filters Type 2 (for NucleoBond® AX 10000 Columns)	740553.5	5
NucleoBond® Buffer Set I	740601	1 set
Buffer S1	740516.1	500 mL
Buffer S2	740517.1	500 mL
Buffer S3	740518.1	500 mL
Buffer N2	740527.1	500 mL
Buffer N3	740528.1	1000 mL
Buffer N5	740529.1	500 mL
NucleoBond® Rack Small (for NucleoBond® AX 20 Columns)	740562	1
NucleoBond® Rack Large (for NucleoBond® AX 100, 500, 2000, and 10000 Columns)	740563	1
RNase A (lyophilized)	740505.50 740505	50 mg 100 mg

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

8.4 References

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

8.5 Product use restriction/warranty

NucleoBond® PC / BAC kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

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