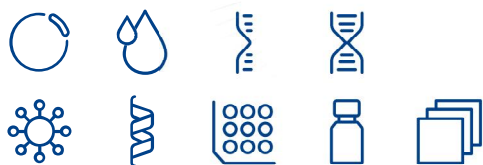


MACHEREY-NAGEL

User manual






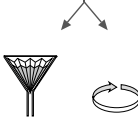
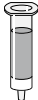
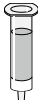




### Plasmid DNA purification

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

May 2019 / Rev. 12

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

## Protocol at a glance (Rev. 12)

	<b>Mini</b> (AX 20)	<b>Midi</b> (AX 100)	<b>Maxi</b> (AX 500)	<b>Mega</b> (AX 2000)	<b>Giga</b> (AX 10000)	
<b>1 Cultivate and harvest bacterial cells</b>	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	
<b>2 Cell lysis</b>	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	
<b>3 Equilibration of the column</b>	Buffer N2 1 mL	Buffer N2 2.5 mL	Buffer N2 6 mL	Buffer N2 20 mL	Buffer N2 100 mL	
<b>4 Clarification of the lysate</b>	Centrifugation  ≥ 5,000 x g 15 min	Folded Filter or centrifugation  ≥ 5,000 x g 25 min	Folded Filter or centrifugation  ≥ 5,000 x g 40 min	Folded Filter or centrifugation  ≥ 5,000 x g 50 min	Folded Filter or centrifugation  ≥ 5,000 x g 60 min	
<b>5 Binding</b>	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	
<b>6 Washing</b>	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	
<b>7 Elution</b>	Buffer N5 1 mL	Buffer N5 5 mL	Buffer N5 15 mL	Buffer N5 25 mL	Buffer N5 100 mL	
<b>8 Precipitation</b>	Isopropanol 0.75 mL  ≥ 5,000 x g 4 °C, 30 min	Isopropanol 3.5 mL  ≥ 5,000 x g 4 °C, 30 min	Isopropanol 11 mL  ≥ 5,000 x g 4 °C, 30 min	Isopropanol 18 mL  ≥ 5,000 x g 4 °C, 30 min	Isopropanol 70 mL  ≥ 5,000 x g 4 °C, 30 min	
<b>9 Wash and dry DNA pellet</b>	70 % ethanol 500 µL  ≥ 5,000 x g RT, 10 min  5–10 min	70 % ethanol 2 mL  ≥ 5,000 x g RT, 10 min  5–10 min	70 % ethanol 5 mL  ≥ 5,000 x g RT, 10 min  10–20 min	70 % ethanol 7 mL  ≥ 5,000 x g RT, 10 min  30–60 min	70 % ethanol 10 mL  ≥ 5,000 x g RT, 10 min  30–60 min	
<b>10 Reconstitute DNA</b>	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	

## Table of contents

1 Components	4
1.1 Kit contents	4
1.2 Reagents and equipment to be supplied by user	9
2 About this user manual	10
3 Product description	11
3.1 The basic principle	11
3.2 Kit specifications	11
3.3 High-/low-copy plasmid purification	12
3.4 Filtration of the lysate	12
3.5 Elution procedure	14
4 Storage conditions and preparation of working solutions	15
5 Safety instructions	16
6 Optimization of the plasmid isolation procedure	18
6.1 Estimation of optimal culture volume	18
6.2 SDS precipitation in Buffer S2	20
7 NucleoBond® plasmid purification	21
7.1 General procedure	21
7.2 High-copy plasmid purification (Mini, Midi, Maxi)	21
7.3 High-copy plasmid purification (Mega, Giga)	24
7.4 Low-copy plasmid purification (Mini, Midi)	27
7.5 Low-copy plasmid purification (Maxi/BAC, Mega)	30
8 Appendix	33
8.1 Determination of DNA yield and quality	33
8.2 Troubleshooting	33
8.3 Ordering information	39
8.4 References	40
8.5 Product use restriction / warranty	41

# 1 Components

## 1.1 Kit contents

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

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

1.1 Kit contents *continued*

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

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

**1.1 Kit contents *continued***

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

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

**1.1 Kit contents *continued***

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

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

**1.1 Kit contents *continued***

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

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



## 1.2 Reagents and equipment to be supplied by user

### Reagents

- Isopropanol (room temperature)
- 70 % ethanol (room temperature)
- Ice
- Buffer for reconstitution of DNA (e.g., TE buffer or sterile H<sub>2</sub>O)

### Equipment

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

## 2 About this user manual

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

All technical literature is available on the internet at [www.mn-net.com](http://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):

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

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

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

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

### 3 Product description

#### 3.1 The basic principle

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

#### 3.2 Kit specifications

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

**Table 1: NucleoBond® Column binding capacities**

<b>NucleoBond® Column</b>	<b>Binding capacity</b>
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.

### 3.3 High-/low-copy plasmid purification

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

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

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

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

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

### 3.4 Filtration of the lysate

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

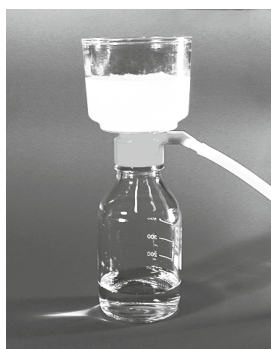
1) Use the **NucleoBond® Folded Filters** provided with the kits. The gentle filtration prevents shearing of plasmids and large constructs, such as cosmid, PAC, or BAC DNA.

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



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

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



**Figure 2** Correct use of the NucleoBond® Bottle Top Filter

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

### 3.5 Elution procedure

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

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

Before you start any **NucleoBond**<sup>®</sup> 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. Check buffer for precipitated SDS immediately before each use by carefully inverting the bottle a few times and inspecting the solution from above. The buffer must be absolutely clear. Precipitated SDS will result in dramatically reduced plasmid yield!

## 5 Safety instructions

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

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

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g. *Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	P-Sätze
N2, N3, N5	Ethanol 5–20 % <i>Ethanol 5–20 %</i> CAS 64-17-5d	 WARNING ACHTUNG	226	210
RNase A	RNase 90–100 % <i>(Enzym) RNase 90–100 %</i> CAS 9001-99-4	 DANGER GEFAHR	334	261sh, 342+311
S2	Sodium hydroxide solution 0.5–1.0 % <i>Natriumhydroxid-Lösung 0,5–1,0 %</i> CAS 1310-73-2	 DANGER GEFAHR	314	260sh, 280sh, 303+361+353 305+351+338, 310

### Hazard phrases

- H 226 Flammable liquid and vapor.  
*Flüssigkeit und Dampf entzündbar.*
- H 314 Causes severe skin burns and eye damage.  
*Verursacht schwere Verätzungen der Haut und schwere Augenschäden.*
- H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.  
*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*

### Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
- P 260sh Do not breathe dust/vapors.  
*Staub/Dampf nicht einatmen.*
- P 261sh Avoid breathing dust/vapors.  
*Einatmen von Staub/Dampf vermeiden.*
- P 280sh Wear protective gloves/eye protection.  
*Schutzhandschuhe/Augenschutz tragen..*



- P 303+361+353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].  
*BEI BERÜHRUNG MIT DER HAUT (oder dem Haar): Alle kontaminierten Kleidungsstücke sofort ausziehen. Haut mit Wasser abwaschen [oder duschen].*
- P 305+351+338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.  
*BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
- P 310 Immediately call a POISON CENTER/doctor.  
*Sofort GIFTINFORMATIONSZENTRUM/Arzt anrufen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER / doctor / ...  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM/Arzt/... anrufen.*

For further information please see Material Safety Data Sheets ([www.mn-net.com](http://www.mn-net.com)).  
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ([www.mn-net.com](http://www.mn-net.com)).*



The symbol shown on labels refers to further safety information in this section.  
*Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.*

## 6 Optimization of the plasmid isolation procedure

### 6.1 Estimation of optimal culture volume

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

**Table 2: Culture volumes**

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

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

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

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

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

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

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

*For example: 30 mL of a culture grown to a dilution factor corrected OD<sub>600</sub> of 3.0 would result in pellets of  $3.0 \times 30 = \text{ODV } 90$ .*

The **NucleoBond® PC** kits use lysis buffer volumes which are much larger than typically needed and ensure complete plasmid release. Maximum OD<sub>600</sub> values for the recommended culture volumes are given in the table below.

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

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

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

## 6.2 SDS precipitation in Buffer S2

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

## 7 NucleoBond® plasmid purification

### 7.1 General procedure

Prepare an overnight culture:

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

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

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

#### 1 Cultivate and harvest bacterial cells

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

#### 2 Cell lysis

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

0.4 mL	4 mL	12 mL
--------	------	-------

Add **Buffer S2** to the suspension. Mix gently by inverting the tube 6–8 times. Incubate the mixture at room temperature (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
--------	------	-------

#### 3 Equilibration of the column

**Equilibrate** a NucleoBond® AX 20 (Mini), AX 100 (Midi) or AX 500 (Maxi) Column with Buffer N2. Allow the column to empty by gravity flow. Discard flowthrough.

1.0 mL	2.5 mL	6.0 mL
--------	--------	--------

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

#### 4 Clarification of the lysate

##### NucleoBond® PC 20

Centrifuge the lysate at  $\geq 5,000 \times g$  for **5–10 minutes** at room temperature or better 4°C.

##### NucleoBond® PC 100, 500

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

#### 5 Binding

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

#### 6 Washing

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

**2 x 1.5 mL**

**10 mL**

**32 mL**

#### 7 Elution

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

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

**1 mL**

**5 mL**

**15 mL**

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

#### 8 Precipitation

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

**0.75 mL**

**3.5 mL**

**11.0 mL**

---

<b>Mini</b> (AX 20)	<b>Midi</b> (AX 100)	<b>Maxi</b> (AX 500)
------------------------	-------------------------	-------------------------

---

**9 Wash and dry DNA pellet**

Add **room temperature 70 % ethanol** to the pellet. Vortex briefly and centrifuge at  $\geq 5,000 \times g$  for **10 min** at **room temperature (18–25 °C)**.

<b>500 <math>\mu</math>L</b>	<b>2 mL</b>	<b>5 mL</b>
------------------------------	-------------	-------------

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.*

<b>5–10 min</b>	<b>5–10 min</b>	<b>10–20 min</b>
-----------------	-----------------	------------------

---

**10 Reconstitute DNA**

**Dissolve pellet** in an appropriate volume of buffer TE or sterile deionized H<sub>2</sub>O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10–60 min (3D-shaker).

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

---

### 7.3 High-copy plasmid purification (Mega, Giga)

Mega	Giga
(AX 2000)	(AX 10000)

#### 1 Cultivate and harvest bacterial cells

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

#### 2 Cell lysis

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

45 mL	120 mL
-------	--------

Add **Buffer S2** to the suspension. Mix gently by inverting the tube 6–8 times. Incubate the mixture at room temperature (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.

45 mL	120 mL
-------	--------

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

45 mL	120 mL
-------	--------

#### 3 Equilibration of the column

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

20 mL	100 mL
-------	--------

#### 4 Clarification of the lysate

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

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

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

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



**Mega****(AX 2000)****Giga****(AX 10000)****5 Binding**

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

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

**6 Washing**

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

**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).

**8 Precipitation**

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

**18 mL****70 mL****9 Wash and dry DNA pellet**

Add **room temperature 70% ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 5,000 x g** for **10 min** at **room temperature (18–25 °C)**.

**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.

**30–60 min****30–60 min**

**Mega**

**(AX 2000)**

**Giga**

**(AX 10000)**

---

**10 Reconstitute DNA**

**Dissolve pellet** in an appropriate volume of buffer TE or sterile deionized H<sub>2</sub>O. 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	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**.

<b>0.8 mL</b>	<b>8.0 mL</b>
---------------	---------------

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.

<b>0.8 mL</b>	<b>8.0 mL</b>
---------------	---------------

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**.

<b>0.8 mL</b>	<b>8.0 mL</b>
---------------	---------------

### 3 Equilibration of the column

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

<b>1 mL</b>	<b>2.5 mL</b>
-------------	---------------

### 4 Clarification of the lysate

#### NucleoBond® PC 20

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

#### NucleoBond® PC 100, 500

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

**Mini**  
**(AX 20)****Midi**  
**(AX 100)****5 Binding**

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

**6 Washing**

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

**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 several hours. In this case the eluate should be preheated 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).*

**8 Precipitation**

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

**0.75 mL****3.5 mL****9 Wash and dry DNA pellet**

Add **room temperature 70% ethanol** to the pellet. Vortex briefly and centrifuge at **≥ 5,000 x g** for **10 min** at **room temperature (18–25 °C)**.

**0.5 mL****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**

**Mini**

**(AX 20)**

**Midi**

**(AX 100)**

---

**10 Reconstitute DNA**

**Dissolve pellet** in an appropriate volume of buffer TE or sterile deionized H<sub>2</sub>O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10–60 min (3D-shaker).

---

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

---

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

	Maxi (AX 500 / BAC 100)	Mega (AX 2000)
<b>1 Cultivate and harvest bacterial cells</b>		
Harvest bacteria from an LB culture by centrifugation at <b>4,500–6,000 x g</b> for <b>15 min</b> at <b>4 °C</b> .		
<b>2 Cell lysis</b>		
Carefully resuspend the pellet of bacterial cells in <b>Buffer S1 + RNase A</b> .		
	24 mL	90 mL
Add <b>Buffer S2</b> to the suspension. Mix gently by inverting the tube 6–8 times. Incubate the mixture at room temperature (18–25 °C) for <b>2–3 min</b> (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 <b>Buffer S3 (4 °C)</b> 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. <b>Incubate</b> the suspension on ice for <b>5 min</b> .		
	24 mL	90 mL
<b>3 Equilibration of the column</b>		
<b>Equilibrate</b> a NucleoBond® AX 500 (Maxi), BAC 100 (Maxi), or AX 2000 (Mega) Column with <b>Buffer N2</b> . Allow the column to empty by gravity flow. Discard flowthrough.		
	6 mL	20 mL
<b>4 Clarification of the lysate</b>		
Place a <b>NucleoBond® Folded Filter</b> in a funnel of appropriate size.		
Wet the filter with a few drops of Buffer N2 and load the bacterial lysate onto the wet filter. Either collect the flowthrough in a separate vessel and proceed with step 5 <b>OR</b> 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 <b>3.4</b> for more information on how to set up the funnel and filter and for alternative clarification methods like centrifugation.		

	Maxi (AX 500 / BAC 100)	Mega (AX 2000)
<b>5 Binding</b>		
	Load the cleared <b>lysate</b> from step 4 onto the NucleoBond® Column. Allow the column to empty by gravity flow.	
<b>6 Washing</b>		
	Wash the column with <b>Buffer N3</b> . Repeat as indicated. Discard flowthrough.	
	2 x 18 mL	2 x 50 mL
<b>7 Elution</b>		
	Elute the plasmid DNA with <b>Buffer N5</b> . 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
	<i>Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 10).</i>	
<b>8 Precipitation</b>		
	Add <b>room temperature isopropanol</b> to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at <b>≥ 5,000 x g</b> for <b>30 min</b> . Carefully discard the supernatant.	
	11 mL	18 mL
<b>9 Wash and dry DNA pellet</b>		
	Add <b>room temperature 70 % ethanol</b> to the pellet. Vortex briefly and centrifuge at <b>≥ 5,000 x g</b> for <b>10 min</b> at <b>room temperature (18–25 °C)</b> .	
	5 mL	7 mL
	Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at <b>room temperature (18–25 °C)</b> no less than the indicated time.	
	<i>Drying for longer periods of time will not harm the quality of plasmid DNA but over-drying may render the DNA less soluble.</i>	

**Maxi**

(AX 500 / BAC 100)

**Mega**

(AX 2000)

**10–20 min**

**30–60 min**

---

**10 Reconstitute DNA**

**Dissolve pellet** in an appropriate volume of buffer TE or sterile deionized H<sub>2</sub>O. Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10–60 min (3D-shaker).

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

---



## 8 Appendix

### 8.1 Determination of DNA yield and quality

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

### 8.2 Troubleshooting

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

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

**Table 3: Information about antibiotics according to Maniatis\***

Antibiotic	Stock solution (concentration)		Storage	Working concentration	
Ampicillin	50 mg/mL	in H <sub>2</sub> O	- 20 °C	20–50	µg/mL
Carbenicillin	50 mg/mL	in H <sub>2</sub> O	- 20 °C	20–60	µg/mL
Chloramphenicol	34 mg/mL	in EtOH	- 20 °C	25–170	µg/mL
Kanamycin	10 mg/mL	in H <sub>2</sub> O	- 20 °C	10–50	µg/mL
Streptomycin	10 mg/mL	in H <sub>2</sub> O	- 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: Volume recommendations for purification analyses**

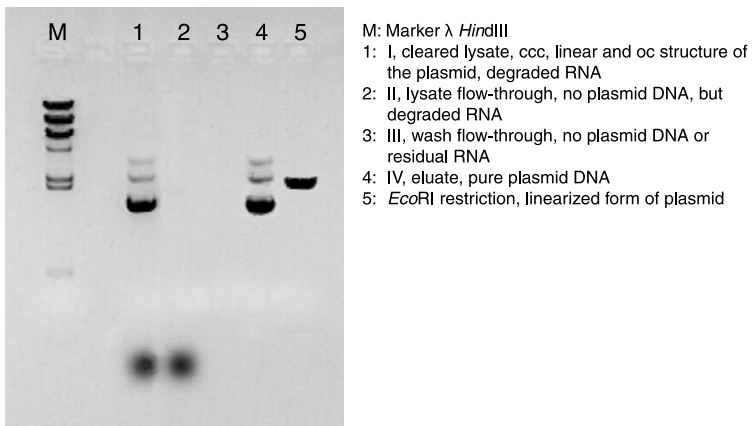
Sample	Purification step	Volume required [µL]			
		PC 100	PC 500	PC 2000	PC 10000
I	Cleared lysate after protocol step 4	600	400	300	200
II	Column flowthrough after protocol step 5	600	400	300	200
III	Wash flowthrough after protocol step 6	500	300	200	100
IV	Eluate after protocol step 7	300	200	100	100

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

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

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

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



**Figure 3 Exemplary analytical check of NucleoBond® PC 500 purification samples**

Plasmid: pUC18, bacterial strain: *E. coli* DH5 $\alpha$ ®. 20  $\mu$ L 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	<i>SDS- or other precipitates are present in the sample</i> <ul style="list-style-type: none"><li>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.</li></ul>
	<i>Sample/lysate is too viscous</i> <ul style="list-style-type: none"><li>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.</li></ul>
	<i>Plasmid did not propagate</i> <ul style="list-style-type: none"><li>Check plasmid content in the cleared lysate by precipitation of an aliquot. Use colonies from fresh plates for inoculation and add appropriate amounts of antibiotics to the culture media.</li></ul>
	<i>Alkaline lysis was inefficient</i> <ul style="list-style-type: none"><li>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.</li></ul>
	<i>Lysate prepared incorrectly</i> <ul style="list-style-type: none"><li>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.</li></ul>

<b>Problem</b>	<b>Possible cause and suggestions</b>
Column is blocked	<p><i>Sample is too viscous</i></p> <ul style="list-style-type: none"> <li>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.</li> </ul>
	<p><i>Precipitates occur during storage</i></p> <ul style="list-style-type: none"> <li>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.</li> </ul> <p><i>Lysate was not completely cleared</i></p> <ul style="list-style-type: none"> <li>Centrifuge at higher speed for a longer period of time, or use additional NucleoBond® Folded Filters to clear the lysate.</li> </ul>
Cellular DNA or RNA contamination of plasmid DNA	<p><i>Lysis treatment was too harsh</i></p> <ul style="list-style-type: none"> <li>Be sure not to incubate the lysate in Buffer S2 for more than 5 min.</li> </ul> <p><i>Excessive mixing during lysis allowed genomic DNA to shear off into the lysis buffer</i></p> <ul style="list-style-type: none"> <li>If the lysate is too viscous to mix properly or gently, reduce culture volumes.</li> </ul>
	<p><i>RNase digestion was inefficient</i></p> <ul style="list-style-type: none"> <li>RNase was not added to Buffer S1 or stored too long. Add new RNase to Buffer S1. See ordering information, section 8.3.</li> </ul>
No nucleic acid pellet formed after precipitation	<p><i>Pellet was lost</i></p> <ul style="list-style-type: none"> <li>Handle the precipitate with care. Decant solutions carefully. Measure DNA yield in Buffer N5 in order to calculate the expected plasmid DNA amount that should be recovered after precipitation.</li> </ul> <p><i>Pellet did not resuspend in buffer</i></p> <ul style="list-style-type: none"> <li>Again, handle the pellet with care. Especially, if the DNA was precipitated in a &gt; 15 mL tube the "pellet" may be spread at the inner side of the tube. Dissolve DNA with an appropriate volume of TE buffer by rolling the tube for at least 30 min.</li> </ul>
	<p><i>Nucleic acid did not precipitate</i></p> <ul style="list-style-type: none"> <li>Check volumes of precipitating solvent, making sure to use at least 0.7 volumes of isopropanol and centrifuge for longer periods of time.</li> </ul>

<b>Problem</b>	<b>Possible cause and suggestions</b>
Nucleic acid pellet does not resuspend in buffer	<i>Pellet was over dried</i>
	<ul style="list-style-type: none"> <li>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).</li> </ul>
Nucleic acid pellet is opaque or white instead of clear and glassy	<i>Residual salt or organic solvent in the pellet</i>
	<ul style="list-style-type: none"> <li>Wash the pellet with additional low-viscosity organic solvent (70 % ethanol), or increase the resuspension buffer volume.</li> </ul>
Purified plasmid does not perform well in subsequent reactions	<i>Salt has co-precipitated with the pellet</i>
	<ul style="list-style-type: none"> <li>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.</li> <li>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.</li> </ul>
NucleoBond® Folded Filters clog during filtration	<i>DNA is contaminated with cellular debris or genomic DNA due to inefficient lysis</i>
	<ul style="list-style-type: none"> <li>Reduce the culture volume, or increase the amount of Buffer S1, S2, and S3 used during the lysis steps.</li> </ul>
	<i>DNA is degraded</i>
	<ul style="list-style-type: none"> <li>Make sure that all equipment (pipettors, centrifuge tubes, etc.) are clean and nuclease-free. Make sure that the alkaline lysis step (i.e., the incubation of sample after addition of Buffer S2) does not exceed.</li> </ul>
	<i>Culture volumes used are too large</i>
	<ul style="list-style-type: none"> <li>Reduce the culture volume or increase the amount of Buffer S1, S2, and S3 used during the lysis steps.</li> </ul>
	<i>Incubation time too short</i>
	<ul style="list-style-type: none"> <li>Make sure that S1 / S2 / S3 lysate was incubated according to the protocol.</li> </ul>

### 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
NucleoSnap® Finisher Midi (for use with NucleoBond® Xtra Midi, NucleoBond® PC 100)	740434.10	10 preps
	740434.50	50 preps
NucleoSnap® Finisher Maxi (for use with NucleoBond® Xtra Maxi, NucleoBond® PC 500)	740435.10	10 preps
	740435.50	50 preps
NucleoSpin® Finisher Midi (for use with NucleoBond® Xtra Midi, NucleoBond® PC 100)	740439.10	10 preps
	740439.50	50 preps
NucleoBond® Finalizer (for use with NucleoBond® PC 100, PC 500, PC 500 EF, NucleoBond® Xtra Midi, Midi EF)	740519.20	20 filters
		2 syringe sets
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

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

Visit [www.mn-net.com](http://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!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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 remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or

components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07 / 2010, Rev. 03

Please contact:

MACHEREY-NAGEL GmbH & Co. KG

Tel.: +49 24 21 969-270

e-mail: tech-bio@mn-net.com

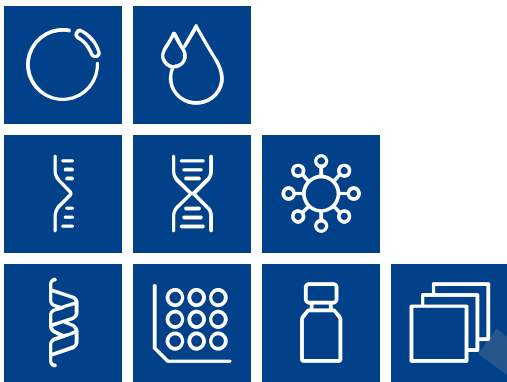
---

Trademarks:

DH5 $\alpha$ <sup>®</sup> is a trademark of Life Technologies, Inc.

NucleoBond<sup>®</sup> trademarks of MACHEREY-NAGEL GmbH & Co. KG

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.



[www.mn-net.com](http://www.mn-net.com)

**MACHEREY-NAGEL**



MACHEREY-NAGEL GmbH & Co. KG  
Neumann-Neander-Str. 6-8  
52355 Düren · Germany

DE	Tel.: +49 24 21 969-0	<a href="mailto:info@mn-net.com">info@mn-net.com</a>
CH	Tel.: +41 62 388 55 00	<a href="mailto:sales-ch@mn-net.com">sales-ch@mn-net.com</a>
FR	Tel.: +33 388 68 22 68	<a href="mailto:sales-fr@mn-net.com">sales-fr@mn-net.com</a>
US	Tel.: +1 484 821 0984	<a href="mailto:sales-us@mn-net.com">sales-us@mn-net.com</a>

A025744/0114