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Endotoxin-free Plasmid DNA Purification

User Manual

NucleoBond® Xtra Midi EF

NucleoBond® Xtra Maxi EF

NucleoBond® Xtra Midi Plus EF

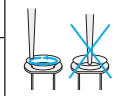
NucleoBond® Xtra Maxi Plus EF

November 2010 / Rev. 04

MACHEREY-NAGEL

Endotoxin-free Plasmid DNA Purification (NucleoBond® Xtra Midi EF / Maxi EF)

Protocol-at-a-glance (Rev. 04)

		Midi		Maxi	
1 – 3	**Cultivate and harvest bacterial cells**	4,500 – 6,000 x *g* 15 min at 4°C			
4 – 5	**Cell lysis** (Important: Check Buffer LYS-EF for precipitated SDS)	High-copy / low-copy 8 mL / 16 mL Buffer RES-EF 8 mL / 16 mL Buffer LYS-EF 5 min, RT		High-copy / low-copy 12 mL / 24 mL Buffer RES-EF 12 mL / 24 mL Buffer LYS-EF 5 min, RT	
6	**Equilibration of the column and filter**	15 mL Buffer EQU-EF		35 mL Buffer EQU-EF	
7	**Neutralization**	8 mL / 16 mL Buffer NEU-EF Incubate 5 min on ice		12 mL / 24 mL Buffer NEU-EF Incubate 5 min on ice	
8	**Clarification and loading of the lysate**	Invert the tube 3 times Load lysate on NucleoBond® Xtra Column Filter			
9	**1st Washing**	5 mL Buffer FIL-EF		10 mL Buffer FIL-EF	
10	**Discard NucleoBond® Xtra Column Filter**	Discard NucleoBond® Xtra Column Filter		Discard NucleoBond® Xtra Column Filter	
11	**2nd Washing**	35 mL Buffer ENDO-EF		90 mL Buffer ENDO-EF	
12	**3rd Washing**	15 mL Buffer WASH-EF		45 mL Buffer WASH-EF	
13	**Elution**	5 mL Buffer ELU-EF		15 mL Buffer ELU-EF	
14	**Precipitation**	**NucleoBond® Xtra Midi EF**	**NucleoBond® Xtra Midi Plus EF**	**NucleoBond® Xtra Maxi EF**	**NucleoBond® Xtra Maxi Plus EF**
		3.5 mL Isopropanol 5 – 15,000 x *g* 30 min at 4°C	3.5 mL Isopropanol Load NucleoBond® Finalizer	10.5 mL Isopropanol 5 – 15,000 x *g* 30 min at 4°C	10.5 mL Isopropanol Load NucleoBond® Finalizer Large
15	**Wash and dry DNA pellet**	2 mL 70% ethanol 5 – 15,000 x *g* 5 min at RT 5 – 10 min	2 mL 70% ethanol / ≥3 x air until dry	5 mL 70% ethanol 5 – 15,000 x *g* 5 min at RT 5 – 10 min	5 mL 70% ethanol / ≥6 x air until dry
16	**Reconstitute DNA**	Appropriate volume of TE-EF or H_2O-EF	200 – 800 µL TE-EF or H_2O-EF	Appropriate volume of TE-EF or H_2O-EF	400 – 1000 µL TE-EF or H_2O-EF

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1 Components

1.1 Kit contents

	NucleoBond® Xtra Midi EF		NucleoBond® Xtra Midi Plus EF	
	10 preps	50 preps	10 preps	50 preps
REF	740420.10	740420.50	740422.10	740422.50
Buffer RES-EF	100 mL	500 mL	100 mL	500 mL
Buffer LYS-EF	4 x 25 mL	500 mL	4 x 25 mL	500 mL
Buffer NEU-EF	100 mL	500 mL	100 mL	500 mL
Buffer EQU-EF	200 mL	1000 mL	200 mL	1000 mL
Buffer FIL-EF	2 x 30 mL	2 x 150 ml	2 x 30 mL	2 x 150 mL
Buffer ENDO-EF	2 x 200 mL	2 x 1000 mL	400 mL	2 x 1000 mL
Buffer WASH-EF	200 mL	1000 mL	200 mL	1000 mL
Buffer ELU-EF	2 x 32 mL	300 mL	2 x 32 mL	300 mL
Buffer TE-EF	30 mL	2 x 30 mL	30 mL	2 x 30 mL
H_2O-EF (for redissolving)	30 mL	2 x 30 mL	30 mL	2 x 30 mL
H_2O-EF (for 70% ethanol)	9 mL	2 x 30 mL	9 mL	2 x 30 mL
RNase A* (lyophilized)	6 mg	30 mg	6 mg	30 mg
NucleoBond® Xtra Midi Columns incl. NucleoBond® Xtra Midi Column Filters	10	50	10	50
NucleoBond® Finalizers	–	–	10	50
30 mL Syringes	–	–	10	50
1 mL Syringes	–	–	10	50
Plastic Washers	5	10	5	10
User Manual	1	1	1	1

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

1.1 Kit contents *continued*

	NuceoBond® Xtra Maxi EF		NucleoBond® Xtra Maxi Plus EF	
	10 preps	**50 preps**	**10 preps**	**50 preps**
REF	**740424.10**	**740424.50**	**740426.10**	**740426.50**
Buffer RES-EF	150 mL	750 mL	150 mL	750 mL
Buffer LYS-EF	150 mL	750 mL	150 mL	750 mL
Buffer NEU-EF	150 mL	750 mL	150 mL	750 mL
Buffer EQU-EF	400 mL	2 x 1000 mL	400 mL	2 x 1000 mL
Buffer FIL-EF	150 mL	600 mL	150 mL	600 mL
Buffer ENDO-EF	1000 mL	5 x 1000 mL	1000 mL	5 x 1000 mL
Buffer WASH-EF	500 mL	500 mL 2 x 1000 mL	500 mL	500 mL 2 x 1000 mL
Buffer ELU-EF	180 mL	900 mL	180 mL	900 mL
Buffer TE-EF	30 mL	2 x 30 mL	30 mL	2 x 30 mL
H_2O-EF (for redissolving)	30 mL	2 x 30 mL	30 mL	2 x 30 mL
H_2O-EF (for 70% ethanol)	30 mL	3 x 30 mL	30 mL	3 x 30 mL
RNase A* (lyophilized)	10 mg	50 mg	10 mg	50 mg
NucleoBond® Xtra Midi Columns incl. NucleoBond® Xtra Midi Column Filters	10	50	10	50
NucleoBond® Finalizers Large	–	–	10	50
30 mL Syringes	–	–	10	50
1 mL Syringes	–	–	10	50
Plastic Washers	5	10	5	10
User Manual	1	1	1	1

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

1.2 Reagents and equipment to be supplied by user

Reagents:

- Isopropanol (room-temperatured)
- 70% ethanol (room-temperatured)

Equipment:

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- Refrigerated centrifuge capable of reaching ≥5,000 x *g* with rotor for the appropriate centrifuge tubes or bottles (not necessary for NucleoBond® Xtra Midi/Maxi Plus EF kits)
- New pyrogen- or endotoxin-free plastic ware and pipette tips. If glass ware is to be used, heat over night at 180°C to destroy endotoxins. Autoclaving does not inactivate endotoxins and is not recommended if the autoclave is also used for inactivation of bacterial cultures.
- NucleoBond® Xtra Combi Rack (see ordering information) or equivalent holder

2 Kit specifications

- **NucleoBond® Xtra EF** kits are suitable for ultra fast purification of plasmids, cosmids, and very large constructs (P1 constructs, BACs, PACs) ranging from 3 kbp up to 300 kbp.
- The purified DNA is virtually free of endotoxins (<0.05 EU/µg plasmid DNA).
- **NucleoBond® Xtra Columns** are polypropylene columns containing **NucleoBond® Xtra Silica Resin** packed between two inert filter elements. The columns are available in Midi and Maxi sizes with typical DNA yields of 250 µg and 1000 µg, respectively.
- All **NucleoBond® Xtra Columns** are resistant to organic solvents such as alcohol, chloroform, and phenol and are suitable for buffers containing denaturing agents like formamide, urea, or common detergents like Triton X-100 or NP-40.
- **NucleoBond® Xtra Silica Resin** can be used over a wide pH range (pH 2.5 – 9.0), and can remain in contact with buffers for several hours without any change in its chromatographic properties.
- The **NucleoBond® Xtra Column Filters** are specially designed depth filters that fit into the **NucleoBond® Xtra Columns**. The filters are inserted ready-to-use in the **NucleoBond® Xtra Columns** and allow a time-saving simultaneous clearing of bacterial lysate and loading of cleared lysate onto the **NucleoBond® Xtra Column**. Furthermore, the use of the column filters avoids the time-consuming centrifugation step for lysate clearing.
- The **NucleoBond® Xtra Column Filters** allow complete removal of precipitate even with large lysate volumes without clogging and avoid shearing of large DNA constructs, such as PACs or BACs by the gentle depth filter effect.
- The **NucleoBond® Xtra Midi Plus EF** and **NucleoBond® Xtra Maxi Plus EF** kits additionally contain the **NucleoBond® Finalizer** and **NucleoBond® Finalizer Large**, respectively. These tools for a fast concentration and desalination of eluates are suitable for most plasmids and cosmids ranging from 2 – 50 kbp with recovery efficiencies from 40 – 90% (depending on elution volume).
- **NucleoBond® Finalizer** is a polypropylene syringe filter containing a special silica membrane. The **NucleoBond® Finalizer** provides a binding capacity of 500 µg, whereas the **NucleoBond® Finalizer Large** can hold up to 2000 µg plasmid DNA.
- Due to the small dead volumes of the **NucleoBond® Finalizers** the plasmid DNA can be eluted with a concentration up to 3 µg/µL (see section 5.12, Figure 4 and 5 for dependence of concentration on elution volume).
- All **NucleoBond® Finalizers** are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of endotoxins.

3 About this User Manual

The following section 4 provides you with a detailed description of the **NucleoBond® Xtra EF** purification system and important information about cell growth, cell lysis, and the subsequent purification steps. Sections 6 and 7 inform you about storage, buffer preparation, and safety instructions.

First-time users are strongly advised to read these chapters thoroughly before using this kit. Experienced users can directly proceed with the purification protocols (section 8) or just use the Protocol-at-a-glance for a quick reference.

Each procedural step in the purification protocol is arranged like the following example taken from section 8.1:

	Midi	Maxi
5	**Cell lysis (Buffer LYS-EF)**	
!	**Check Lysis Buffer LYS-EF for precipitated SDS prior to use**. If a white precipitate is visible, warm the buffer for several minutes at 30 – 40°C until precipitate is dissolved completely. Cool buffer down to room temperature (18 – 25°C).	
	Add **Lysis Buffer LYS-EF** to the suspension.	
	Mix gently by **inverting** the tube **5 times**. **Do not vortex** as this will shear and release contaminating chromosomal DNA from cellular debris into the suspension.	
	Incubate the mixture at room temperature (18 – 25°C) for **5 min**.	
	Note: Increase LYS-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis).	
	8 mL	**12 mL**

If you are performing a Midi prep to purify plasmid DNA you will find volumes or incubation times in the white boxes. For Maxi preps please refer to the black 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. The exclamation point marks information and hints that are essential for a successful preparation.

In the example shown above you are asked to check the Lysis Buffer LYS-EF prior to use and then to lyse the resuspended cell pellet in **8 mL** of **Buffer LYS-EF** when performing a Midi prep and in **12 mL** for a Maxi prep. Follow the handling instructions exactly and note the given hints for protocol alterations.

4 Endotoxins

4.1 Localization, molecular structure, and function of endotoxins

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

The structure of endotoxins can be divided into three domains:

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

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

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

4.2 Quantification of endotoxins

Endotoxins can be measured in highly sensitive photometric tests (e.g., “Limulus Amebocyte Lysate (LAL) Pyrochrome”, Lonza Cambrex BioWhittaker) and are expressed in endotoxin units (EU). For plasmid preparations the endotoxin level is given in EU per µg plasmid. A concentration of 0.1 EU/µg is usually considered endotoxin-free.

4.3 Removal of endotoxins

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

Due to their amphiphilic nature and their negative charge endotoxins behave like DNA and are co-purified with most common plasmid purification systems. Regular silica-membrane kits with a purification procedure based on chaotropic salt lead to plasmid DNA with an endotoxin level of >1000 EU/µg. Anion exchange kits like **NucleoBond® Xtra** reduce endotoxins to a level of <1 EU/µg. However, since this may be still too high for successful transfection of very sensitive cells like primary or neuronal cells, **NucleoBond® Xtra EF** was developed to reduce the endotoxin level to <0.05 EU/µg plasmid DNA using a patented procedure.

5 NucleoBond® Xtra EF purification system

5.1 Basic principle

The bacterial cells are lysed by an optimized set of newly formulated buffers based on the NaOH/SDS lysis method of Birnboim and Doly*.

After equilibration of the **NucleoBond® Xtra Column** together with the corresponding **NucleoBond® Xtra Column Filter**, the entire lysate is loaded by gravity flow and simultaneously cleared by the specially designed column filter.

Plasmid DNA is bound to the **NucleoBond® Xtra Silica Resin**.

After two efficient washing steps to remove endotoxins completely the plasmid DNA is eluted, precipitated, and easily dissolved in TE-EF, H_2O-EF, or any suitable endotoxin-free buffer for further use.

5.2 NucleoBond® Xtra anion-exchange columns

NucleoBond® Xtra is a patented silica-based anion-exchange resin, developed by MACHEREY-NAGEL. It is developed for routine separation of different classes of nucleic acids like oligonucleotides, RNA, and plasmids.

NucleoBond® Xtra Silica Resin consists of hydrophilic, macroporous silica beads functionalized with MAE (methyl-amino-ethanol). The dense coating of this functional group provides a high overall positive charge density under acidic pH conditions that permits the negatively charged phosphate backbone of plasmid DNA to bind with high specificity (Figure 1).

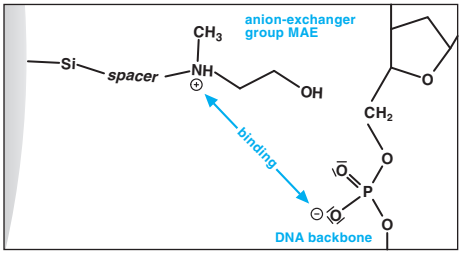


Figure 1: Ionic interaction of the positively charged methyl-hydroxyethyl-amino group with the negative phosphate oxygen of the DNA backbone.
In contrast to the widely used DEAE (diethylaminoethanol) group, the hydroxy group of methyl-hydroxyethyl-amin can be involved in additional hydrogen bonding interactions with the DNA.

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

Due to a specialized manufacturing process that is strictly controlled and monitored, the **NucleoBond® Xtra** silica beads are uniform in diameter and contain particularly large pores. These special properties allow optimized flow rates and sharp, well-defined elution profiles. **NucleoBond® Xtra** can separate distinct nucleic acid species from each other and from proteins, carbohydrates, and other unwanted cellular components over an exceptionally broad range of salt concentrations (Figure 2).

All contaminants from proteins to RNA and especially endotoxins are washed from the column, the positive charge of the resin is neutralized by a pH shift to slightly alkaline conditions, and pure plasmid DNA is eluted in a high-salt elution buffer.

The purified nucleic acid products are suitable for use in the most demanding molecular biology applications, including extremely sensitive transfections, *in vitro* transcription, automated or manual sequencing, cloning, hybridization, and PCR.

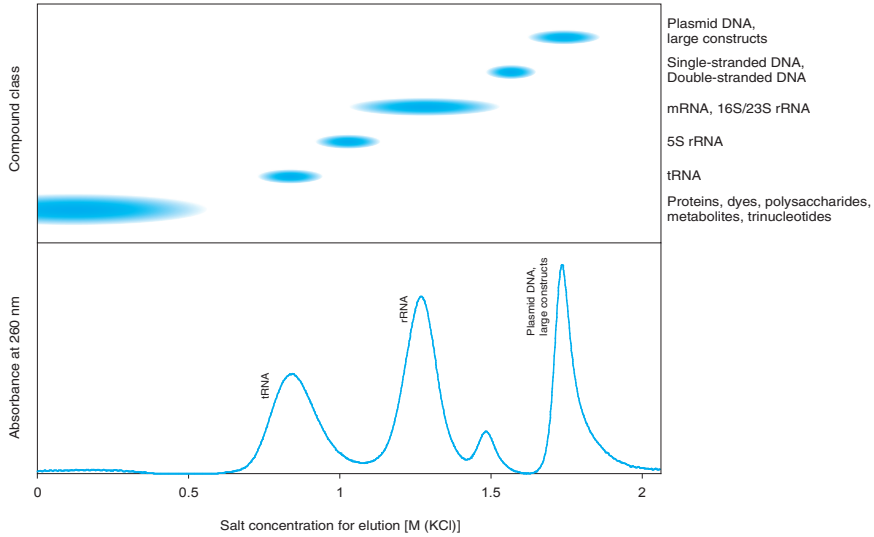


Figure 2: Elution profile of NucleoBond® Xtra Silica Resin at pH 7.0
The more interactions a nucleic acid can form between the phosphate backbone and the positively charged resin the later it is eluted with increasing salt concentration. Large nucleic acids carry more charges than short ones, double stranded DNA more than single stranded RNA.

5.3 Growth of bacterial cultures

Yield and quality of plasmid DNA highly depend on the **type of culture media** and antibiotics, the bacterial host strain, the plasmid type, size, and copy number, but also on the **growth conditions**.

For standard high-copy plasmids LB (Luria-Bertani) medium is recommended. The cell culture should be incubated at 37°C with constant shaking (200 – 250 rpm) preferably 12 – 16 h over night. Use flasks of at least three or four times the volume of the culture volume to provide a growth medium saturated with oxygen. Alternatively, rich media like 2xYT (Yeast/Tryptone), TB (Terrific Broth) or CircleGrow can be used. In this case bacteria grow faster, reach the stationary phase much earlier than in LB medium (≤12 h), and higher cell masses can be reached. However, this does not necessarily yield more plasmid DNA. Overgrowing a culture might lead to a higher percentage of dead or starving cells and the resulting plasmid DNA might be partially degraded or contaminated with chromosomal DNA. To find the optimal culture conditions, the culture medium and incubation times have to be optimized for each host strain/plasmid construct combination individually.

Cell cultures should be grown under **antibiotic selection** at all times to ensure plasmid propagation. Without this selective pressure, cells tend to lose a plasmid during cell division. Since bacteria grow much faster without the burden of a high-copy plasmid, they take over the culture rapidly and the plasmid yield goes down regardless of the cell mass. Table 1 gives information on concentrations of commonly used antibiotics.

Table 1: 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.

The ***E. coli* host strain** mostly influences the quality of the plasmid DNA. Whereas strains like DH5α® or XL1-Blue usually produce high quality super-coiled plasmid DNA, other strains like for example HB101 with high levels of endonuclease activity might yield lower quality plasmid giving poor results in downstream applications like enzymatic restriction or sequencing.

The **type of plasmid**, especially the **size and the origin of replication (ori)** has a crucial influence on DNA yield. In general, the larger the plasmid or the cloned insert is, the lower is the expected DNA yield due to a lower copy number. Even a high-copy construct based on a ColE1 ori can behave like a low-copy vector in case of a large or unfavorable insert. In addition, the ori itself influences the yield by factor 10 – 100. Thus plasmids based on for example pBR322 or pACYC, cosmids or BACs are maintained at copy numbers <20 down to even only 1, whereas vectors based on for example pUC, pBluescript, or pGEM can be present in several hundred copies per cell.

Therefore, all the above mentioned factors should be taken into consideration if a particular DNA yield has to be achieved. Culture volume and lysis procedures have to be adjusted accordingly.

5.4 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.

* 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.

5.5 Culture volume for high-copy plasmids

Due to the influence of growth media (TB, CircleGrow, 2xYT), growth conditions (shaking, temperature), host strain or type of plasmid insert etc. the final amount of cells in a bacterial culture can vary over a wide range. By rule of thumb, 1 liter of *E. coli* culture with an OD_{600} of 1 consists of 1 x 10^{12} cells and yields about 1.5 – 1.8 g cell wet weight. Overnight cultures grown in LB medium usually reach an OD_{600} of 3 – 6 under vigorous shaking in flasks. Fermentation cultures even reach an OD_{600} of 10 and more. The expected DNA yield for a high-copy plasmid is approximately 1 mg per gram cell wet weight.

It is therefore important to **adjust the cell mass rather than the culture volume** for the best plasmid purification results. But since the cell mass or cell wet weight is tedious to determine it was replaced in this manual by the mathematical product of optical density at 600 nm (OD_{600}) and culture volume (Vol) – two variables that are much easier to measure.

$$\mathbf{ODV = OD_{600} \times Vol\ [\ mL\ ]}$$

Note that for a correct OD determination the culture samples have to be diluted if OD_{600} exceeds 0.5 in order to increase proportionally with cell mass. For a well grown *E. coli* culture a 1:10 dilution with fresh culture medium is recommended. The measured OD_{600} is then multiplied with the dilution factor 10 to result in a theoretical OD_{600} value. This OD_{600} is used in Table 2 to determine the appropriate culture volume. Table 2 shows recommended ODVs and the corresponding pairs of OD_{600} and culture volume that can be easily handled using the standard kit protocol lysis buffer volumes. For example, if the OD_{600} of your *E. coli* culture is 6, use 66 mL culture for a Midi prep or 200 mL for a Maxi prep.

Table 2: Recommended culture volumes for high-copy plasmids

NucleoBond® Xtra	Pellet wet weight	Rec. ODV	OD_{600} = 2	OD_{600} = 4	OD_{600} = 6	OD_{600} = 8	OD_{600} = 10
Midi	0.75 g	400	200 mL	100 mL	66 mL	50 mL	40 mL
Maxi	2.25 g	1200	600 mL	300 mL	200 mL	150 mL	120 mL

5.6 Culture volume for low-copy plasmids

NucleoBond® Xtra EF kits are designed for isolation of high-copy plasmids (up to several hundred copies/cell) as well as low-copy plasmids (<20 copies/cell). However, when purifying low-copy plasmids, the cell mass and the lysis buffer volumes should be increased at least by factor 2 to provide enough DNA to utilize the columns' binding capacity. Table 3 shows recommended ODVs and the corresponding pairs of OD_{600} and culture volume for low-copy plasmid cell cultures (for detailed information on calculating ODV = OD_{600} x Vol refer to section 5.5). For example, if the OD_{600} of your *E. coli* culture is 6, use 133 mL culture for a Midi prep or 400 mL for a Maxi prep.

Table 3: Recommended culture volumes for low-copy plasmids

NucleoBond® Xtra	Pellet wet weight	Rec. ODV	OD_{600}= 2	OD_{600}= 4	OD_{600} = 6	OD_{600} = 8	OD_{600} = 10
Midi	1.5 g	800	400 mL	200 mL	133 mL	100 mL	80 mL
Maxi	4.5 g	2400	1200 mL	600 mL	400 mL	300 mL	240 mL

For higher yields, it is advantageous to increase the cell culture and lysis buffer volumes even more, for example by factor 3 – 5. In this case additional lysis buffer can be ordered separately (for ordering information). Furthermore, a centrifuge should be used for lysate clarification instead of the provided **NucleoBond® Xtra Column Filters** since their capacity for precipitate is limited. Alternatively, chloramphenicol amplification can be considered to increase the plasmid copy number (see section 5.4).

5.7 Cell lysis

The bacterial cell pellet is resuspended in Buffer RES-EF and lysed by a sodium hydroxide/SDS treatment with Buffer LYS-EF. Proteins, as well as chromosomal and plasmid DNA are denatured under these conditions. RNA is degraded by DNase-free RNase A. Neutralization Buffer NEU-EF, containing potassium acetate, is then added to the lysate, causing SDS to precipitate as KDS (potassium dodecyl sulfate) and pulling down proteins, chromosomal DNA, and other cellular debris. The potassium acetate buffer also neutralizes the lysate. Plasmid DNA can revert to its native supercoiled structure and remains in solution.

The **NucleoBond® Xtra EF** buffer volumes (standard protocol) are adjusted to ensure optimal lysis for culture volumes, appropriate for high-copy plasmids according to section 5.5, Table 2. Using too much cell material leads to inefficient cell lysis and precipitation and might reduce plasmid yield and purity. Therefore, lysis buffer volumes should be increased when applying larger culture volumes in case of for example low-copy vector purification (section 5.6, Table 3).

By rule of thumb, calculate the necessary lysis buffer volumes for RES-EF, LYS-EF, and NEU-EF as follows:

$$\text{Vol [mL]} = \text{Culture Volume [mL]} \times OD_{600} / 50$$

For example, if 200 mL of a low-copy bacterial culture (OD_{600} = 4) is to be lysed, the appropriate volumes of Lysis Buffers RES-EF, LYS-EF, and NEU-EF are 16 mL each. If more lysis buffer is needed than is provided with the kit, an additional buffer set including buffers RES-EF, LYS-EF, NEU-EF, and RNase A can be ordered separately (see ordering information).

By using sufficient amounts of lysis buffer, lysis time can be limited to 3 – 4 minutes and should not exceed 5 minutes. Prolonged exposure to alkaline conditions can irreversibly denature and degrade plasmid DNA and liberate contaminating chromosomal DNA into the lysate.

5.8 Difficult-to-lyse strains

For plasmid purification of for example 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 RES-EF containing **2 mg / mL lysozyme** and incubate at **37 °C** for **30 minutes**. Proceed then with the lysis procedure according to the **NucleoBond® Xtra EF** standard protocol.

5.9 Setup of NucleoBond® Xtra Columns

Ideally the **NucleoBond® Xtra Midi** or **Maxi Columns** are placed into a **NucleoBond® Xtra Combi Rack** (see ordering information). They are held either by the collar ring of the cartridges or by the Plastic Washers included in the kit to individually adjust the height of each column (see Figure 3). The Plastic Washers can also be used to hold the columns on top of suitable collection tubes or flasks. The **NucleoBond® Xtra Combi Rack** can be used in combination with **NucleoBond® PC 100, 500**, and **2000** as well. Note that the **NucleoBond® Xtra Midi Columns** can also be placed in the **NucleoBond® Rack Large** (REF 740563).

A **B**

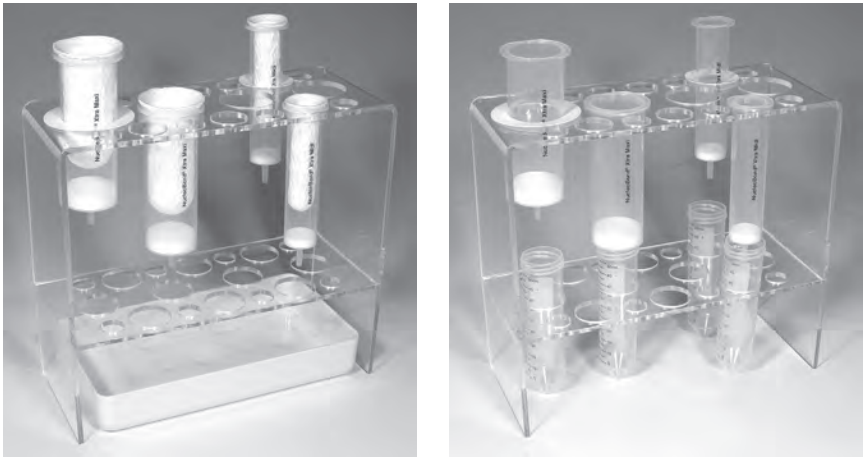
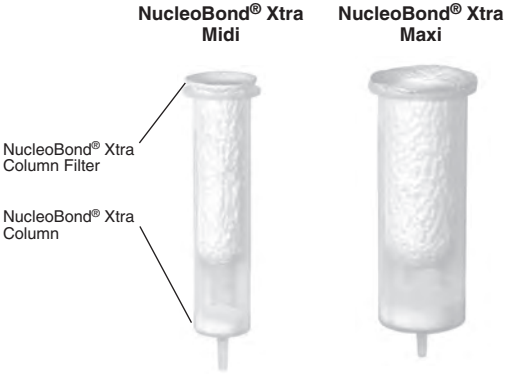


Figure 3: Setup of NucleoBond® Xtra Midi / Maxi Columns with the NucleoBond® Xtra Combi Rack
A: Setup for clarification, loading, and washing; B: Setup for elution.

5.10 Filtration and loading of the lysate

After the alkaline lysis, the sample has to be cleared from cell debris and precipitate to ensure high plasmid purity and a fast column flow rate. This is achieved by passing the solution through a **NucleoBond® Xtra Column Filter** which is provided already inserted into the **NucleoBond® Xtra Column**.



The **NucleoBond® Xtra Column Filters** are designed to eliminate the centrifugation step after alkaline lysis. They are pre-wet during column equilibration and allow a time-saving simultaneous clearing of bacterial lysate and loading of the **NucleoBond® Xtra Column**.

Compared to lysate clearing by centrifugation or syringe filters the **NucleoBond® Xtra Column Filter** furthermore avoids shearing of large DNA constructs such as PACs or BACs by the gentle depth filter effect (filtration occurs on the surface of the filter as well as inside the filter matrix). Its special material and design lead to very rapid passage of the lysate through the filter and even very large lysate volumes can be applied without the risk of clogging. This is especially important for for example low-copy plasmid purification. However, if more than the recommended cell mass (see section 5.5, Table 2, section 5.6, Table 3) was lysed, it might be advantageous to use a centrifuge for lysate clarification rather than the provided column filters due to their limited precipitate capacity.

5.11 Washing of the column

The high salt concentration of the lysate prevents proteins and RNA from binding to the **NucleoBond® Xtra Column** (see section 5.2, Figure 2). However, to remove all traces of contaminants and to purge the dead volume of the **NucleoBond® Xtra Column Filters** it is important to wash the column and the filter in three subsequent washing steps.

First apply **Filter Wash Buffer FIL-EF** to the funnel rim of the filter to wash all residual lysate out of the filter onto the column. Do not just pour the buffer inside the filter. Then pull out and discard the column filter or remove the filter by turning the column upside down.

It is essential to wash the **NucleoBond® Xtra Column without filter two more times** with **Wash Buffer ENDO-EF and WASH-EF** to remove all traces of endotoxins. This ensures highest yields with best achievable purity.

5.12 Elution and concentration of plasmid DNA

Elution is carried out under high-salt conditions and by a shift of pH from 7.0 to 9.0. Under these alkaline conditions the positive charge of the anion-exchange resin is neutralized and plasmid DNA is released. For any downstream application it is necessary to precipitate the DNA and to remove salt and all traces of alcohol since they disturb or inhibit enzymatic activity needed for restriction or sequencing reactions.

All **NucleoBond® Xtra EF** eluates already contain enough salt for an isopropanol pre-cipitation of DNA. Therefore the precipitation is started by directly adding 0.7 volumes of isopropanol. To prevent co-precipitation of salt, use **room-temperature (18 – 25°C) isopropanol** only and do not let the plasmid DNA solution drop into a vial with isopropanol but **add isopropanol to the final eluate and mix immediately**.

Afterwards either follow the centrifugation protocol given after the **NucleoBond® Xtra EF** purification protocol or follow the support protocol for the **NucleoBond® Finalizers** in section 8.3 to eliminate the time-consuming centrifugation steps for precipitation (use of **NucleoBond® Finalizers** is only recommended for vector sizes smaller than 50 kbp).

The **NucleoBond® Finalizers** are designed for quick concentration and desalination of plasmid and cosmid DNA eluates that are obtained by anion-exchange chromatography based DNA purification. The sample is precipitated with isopropanol as mentioned above and loaded onto a special silica membrane by means of a syringe. After an ethanolic washing step the membrane is dried by pressing air through the filter. Elution of pure DNA is carried out with H_2O-EF or slightly alkaline low salt buffer like TE-EF (10 mM Tris/HCl, pH 7.5, 1 mM EDTA). Do not use pure water unless pH is definitively higher than 7.0.

For maximum yield it is recommended to perform the elution step twice. The first elution step is done using fresh buffer whereas in the second elution step the eluate from the first elution is reapplied on the **NucleoBond® Finalizer** to allow complete solubilization of the plasmid.

DNA recovery highly depends on the used elution buffer volume. Large volumes result in a high recovery of up to 90% but in a lower DNA concentration. Small elution volumes on the other hand increase the concentration but at the cost of DNA yield.

If a small volume is chosen, make sure to recover as much eluate as possible from the syringe and **NucleoBond® Finalizer** by pressing air through the **NucleoBond® Finalizer** several times after elution and collecting every single droplet to minimize the dead volume.

Figure 4 and 5 illustrate exemplarily how DNA recovery and final DNA concentration depend on the buffer volume which is used for elution of DNA from the **NucleoBond® Finalizer** and **NucleoBond® Finalizer Large**, respectively.

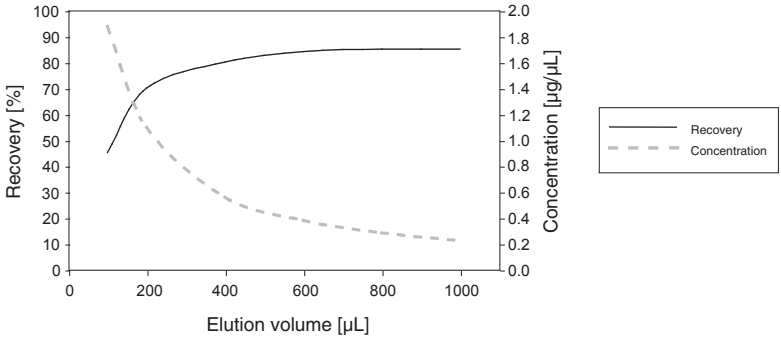


Figure 4: Final DNA recovery and concentration after NucleoBond® Finalizer application
A NucleoBond® Xtra Midi EF eluate containing 250 µg plasmid DNA (8 kbp) was loaded onto a NucleoBond® Finalizer and eluted two-fold with increasing volumes of TE-EF.

The **NucleoBond® Finalizer** is designed to hold a maximum of 500 µg DNA and is therefore ideally suited to be used in combination with **NucleoBond® Xtra Midi EF**. Maximum DNA recovery can be achieved by using >600 µL of elution buffer. For a higher concentration experienced users can lower the elution buffer volume to 400 – 200 µL.

Table 4 gives an overview about recovery and concentration of different amounts of plasmid DNA loaded onto a **NucleoBond® Finalizer**. DNA was eluted two-fold with increasing volumes of TE-EF. Please refer to this tables to select an elution buffer volume that meets your needs best.

Table 4: DNA recovery and concentration for the NucleoBond® Finalizer

Loaded DNA	Elution volume 100 µL	200 µL	400 µL	600 µL	800 µL	1000 µL
500 µg	**35 %**	**60 %**	**70 %**	**75 %**	**75 %**	**75 %**
	2.5 µg/µL	2.3 µg/µL	1.2 µg/µL	0.8 µg/µL	0.6 µg/µL	0.5 µg/µL
250 µg	**40 %**	**65 %**	**75 %**	**80 %**	**80%**	**80 %**
	1.9 µg/µL	1.1 µg/µL	0.6 µg/µL	0.4 µg/µL	0.3 µg/µL	0.2 µg/µL
100 µg	**45 %**	**70 %**	**80 %**	**85 %**	**85 %**	**85 %**
	0.7 µg/µL	0.4 µg/µL	0.2 µg/µL	0.1 µg/µL	0.1 µg/µL	0.1 µg/µL
50 µg	**30 %**	**75 %**	**85 %**	**90 %**	**90 %**	**90 %**
	0.3 µg/µL	0.2 µg/µL	0.1 µg/µL	0.1 µg/µL	0.1 µg/µL	<0.1 µg/µL

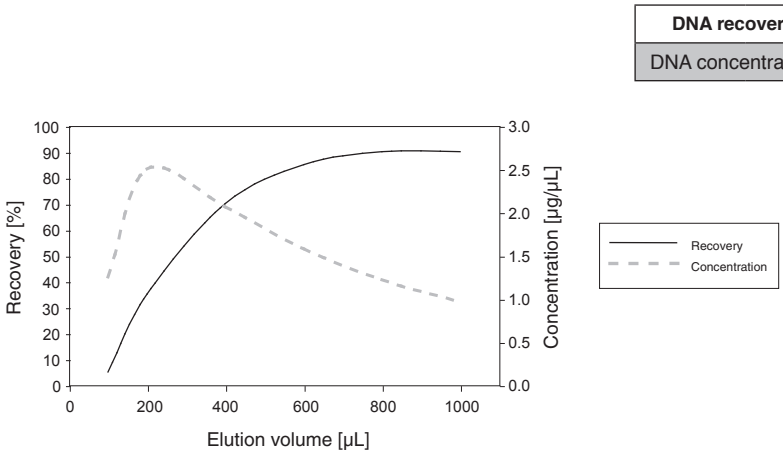


Figure 5: Final DNA recovery and concentration after NucleoBond® Finalizer Large application

A NucleoBond® Xtra Maxi EF eluate containing 1000 µg plasmid DNA (8 kbp) was loaded onto a NucleoBond® Finalizer Large and eluted two-fold with increasing volumes of TE-EF.

NucleoBond® Xtra Maxi EF eluates are easily concentrated with a **NucleoBond® Finalizer Large** which is able to bind up to 2000 µg plasmid DNA. Maximum DNA recovery can be achieved by using >800 µL of elution buffer. For a higher concentration experienced users can lower the elution buffer volume to 600 – 400 µL.

Table 5 gives an overview about recovery and concentration of different amounts of plasmid DNA loaded onto a **NucleoBond® Finalizer Large**. DNA was eluted two-fold with increasing volumes of TE-EF. Please refer to this tables to select an elution buffer volume that meets your needs best.

Table 5: DNA recovery and concentration for the NucleoBond® Finalizer Large

Loaded DNA	Elution volume 100 µL	200 µL	400 µL	600 µL	800 µL	1000 µL
1500 µg	**5 %**	**30 %**	**65 %**	**80 %**	**85 %**	**90 %**
	1.9 µg/µL	3.2 µg/µL	2.9 µg/µL	2.2 µg/µL	1.7 µg/µL	1.4 µg/µL
1000 µg	**5 %**	**35 %**	**70 %**	**85 %**	**90%**	**90 %**
	1.3 µg/µL	2.5 µg/µL	2.1 µg/µL	1.6 µg/µL	1.2 µg/µL	1.0 µg/µL
500 µg	**10 %**	**40 %**	**70 %**	**85 %**	**90 %**	**90 %**
	1.3 µg/µL	1.4 µg/µL	1.0 µg/µL	0.8 µg/µL	0.6 µg/µL	0.5 µg/µL
100 µg	**15 %**	**45 %**	**70 %**	**80 %**	**85 %**	**90 %**
	0.4 µg/µL	0.3 µg/µL	0.2 µg/µL	0.1 µg/µL	0.1 µg/µL	0.1 µg/µL

DNA recovery
DNA concentration

5.13 Determination of DNA yield and quality

The **yield** of a plasmid preparation should be estimated prior to and after the isopropanol precipitation in order to calculate the recovery after precipitation and to find the best volume to dissolve the pellet in. Simply use either **NucleoBond® Xtra EF** Elution Buffer ELU-EF or the respective low-salt buffer as a blank in your photometric measurement.

The nucleic acid **concentration** of the sample can be calculated from its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µg DNA/mL. Note that the absolute measured absorbance should lie between 0.1 and 0.7 in order to be in the linear part of Lambert-Beer's law. Dilute your sample in the respective buffer if necessary.

The plasmid **purity** can be checked by UV spectroscopy as well. A ratio of A_{260}/A_{280} between 1.80 – 1.90 and A_{260}/A_{230} around 2.0 indicates pure plasmid DNA. An A_{260}/A_{280} ratio above 2.0 is a sign for too much RNA in your preparation, an A_{260}/A_{280} ratio below 1.8 indicates protein contamination.

Endotoxin levels can be measured with commercially available kits, for example "Limulus Amebocyte Lysate (LAL) Pyrochrome", Lonza Cambrex BioWhittaker.

Plasmid **quality** can be checked by running the precipitated samples on a 1% agarose gel. This will give information on conformation and structural integrity of isolated plasmid DNA, i.e., it shows whether the sample is predominantly present in the favorable super-coiled form (ccc, usually the fastest band), as an open circle (oc) or even in a linear form (see section 9.1, Figure 6).

5.14 Convenient stopping points

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

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

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

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

6 Storage conditions and preparation of working solutions

Storage conditions and preparation of working solutions

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

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

- Dissolve the lyophilized RNase A by the addition of 1 mL of Buffer RES-EF. 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 RES-EF and shake well. Note the date of RNase A addition. The final concentration of RNase A is 60 µg/mL Buffer RES-EF. Store Buffer RES-EF with RNase A at 4 °C. The solution will be stable at this temperature for at least 6 months.
- Buffer LYS-EF should be stored at room temperature (18 – 25 °C) since the containing SDS might precipitate at temperatures below 20 °C. If precipitation is observed, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is completely redissolved.
- Add indicated volume of 96 – 100 % ethanol to the endotoxin-free water in the bottles labeled “70 % EtOH”.

	NucleoBond® Xtra Midi / Maxi EF			
REF	**740420.10** **740422.10**	**740424.10** **740426.10**	**740420.50** **740422.50**	**740424.50** **740426.50**
H_2O-EF (for 70 % ethanol)	9 mL Add 21 mL ethanol	30 mL Add 70 mL ethanol	2 x 30 mL Add 70 mL ethanol to each bottle	2 x 30 mL Add 70 mL ethanol to each bottle

7 Safety instructions – risk and safety phrases

The following components of the **NucleoBond® Xtra EF** 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	✖ Xi*	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24
LYS-EF	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 neccessary 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.

8 NucleoBond® Xtra EF plasmid purification

The following section includes the protocols for high-copy and low-copy plasmid purification as well as for concentration of NucleoBond® Xtra EF eluates with the NucleoBond® Finalizers.

8.1 High-copy plasmid purification (Midi, Maxi)

	Midi	Maxi

1 Prepare a starter culture

Inoculate a 3 – 5 mL starter culture of LB medium with a single colony picked from a freshly streaked agar plate. Make sure that plate and liquid culture contain the appropriate selective antibiotic to guarantee plasmid propagation (see section 5.3 for more information). Shake at 37°C and ~300 rpm for ~8 h.

2 Prepare a large overnight culture

! *Note: To utilize the entire large binding capacity of the NucleoBond® Xtra Columns it is important to provide enough plasmid DNA. If the culture is known to grow poorly or the plasmid does not quite behave like a high-copy plasmid, please consult section 5.6 for larger culture volumes. If you are not sure about the plasmid copy number and growth behavior of your host strain, increase the culture volume and decide later in step 3 how much cells to use for the preparation. The recommended culture volumes below are calculated for a final OD_{600} of around 4 (see section 5.5).*

Inoculate an overnight culture by diluting the starter culture 1/1000 into the given volumes of LB medium also containing the appropriate selective antibiotic. Grow the culture overnight at 37°C and ~300 rpm for 12 – 16 h.

100 mL	300 mL

3 Harvest bacterial cells

Measure the cell culture OD_{600} and determine the recommended culture volume.

$Vol\,[mL] = 400 / OD_{600}$	$Vol\,[ml] = 1200 / OD_{600}$

Pellet the cells by centrifugation at **4,500 – 6,000 x *g*** for **≥10 min** at **4 °C** and discard the supernatant completely.

Midi	Maxi

Note: It is of course possible to use larger culture volumes, for example if the plasmid does not behave like a typical high-copy vector (see section 5.6 for more information). In this case increase RES-EF, LYS-EF, and NEU-EF buffer volumes proportionally in steps 4, 5, and 7. Additional lysis buffer might have to be ordered separately (see ordering information for NucleoBond® Xtra EF Buffer Set I, section 9.2). If the culture volume is more than double the recommended culture volume, it is advantageous to use a centrifuge for the lysate clarification in step 8 rather than the NucleoBond® Xtra Column Filters.

4 Resuspension (Buffer RES-EF)

Resuspend the cell pellet completely in **Resuspension Buffer RES-EF + RNase A** by pipetting the cells up and down or vortexing the cells.

For an efficient cell lysis it is important that no clumps remain in the suspension.

Note: Increase RES-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis and section 5.8 regarding difficult-to-lyse strains).

8 mL	12 mL

5 Cell lysis (Buffer LYS-EF)

Check Lysis Buffer LYS-EF for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30 – 40 °C until precipitate is dissolved completely. Cool buffer down to room temperature (18 – 25 °C).

Add **Lysis Buffer LYS-EF** to the suspension.

Mix gently by **inverting** the tube **5 times**. **Do not vortex** as this will shear and release contaminating chromosomal DNA from cellular debris into the suspension.

Incubate the mixture at room temperature (18 – 25 °C) for **5 min**.

Note: Increase LYS-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis)

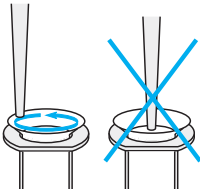
8 mL	12 mL

Midi	Maxi

6 Equilibration (Buffer EQU-EF)

Equilibrate a NucleoBond® Xtra Column together with the inserted column filter with **Equilibration Buffer EQU-EF**.

Apply the buffer onto the rim of the column filter as shown in the picture and make sure to wet the entire filter.



Allow the column to empty by gravity flow. The column does not run dry.

15 mL	35 mL

7 Neutralization (Buffer NEU-EF)

Add **Neutralization Buffer NEU-EF** to the suspension and immediately mix the lysate gently by **inverting** the tube **10 – 15 times. Do not vortex**.

! The flask or tube used for this step should not be filled more than two thirds to allow homogeneous mixing. Make sure to neutralize completely to precipitate all the protein and chromosomal DNA. The lysate should turn from a slimy, viscous consistency to a low viscosity, homogeneous suspension of an off-white flocculate.

Note: Increase NEU-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis).

8 mL	12 mL

! **Incubate crude lysate on ice.**

5 min	5 min

Midi	Maxi

8 Clarification and loading

Make sure to have a homogeneous suspension of the precipitate by **inverting the tube 3 times** directly before applying the lysate to the equilibrated NucleoBond® Xtra Column Filter to avoid clogging of the filter.

The lysate is simultaneously cleared and loaded onto the column. Refill the filter if more lysate has to be loaded than the filter is able to hold. Allow the column to empty by gravity flow.

Note: The lysate appears turbid after passing the filter. The turbidity is not caused by precipitate passing the filter, it does not clog the column, it rather indicates correct working of the procedure.

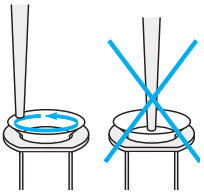
Alternative: The precipitate can be removed by centrifugation at ≥5,000 x *g* for at least 10 min, for example if more than double the recommended cell mass was used. If the supernatant still contains suspended matter transfer it to a new tube and repeat the centrifugation, preferably at higher speed, or apply the lysate to the equilibrated NucleoBond® Xtra Column Filter.

This clarification step is extremely important since residual precipitate may clog the NucleoBond® Xtra Column. To load the column you can either apply the cleared lysate to the equilibrated filter or remove the unused filter beforehand. Allow the column to empty by gravity flow.

Note: You may want to save all or part of the flow-through for analysis (see section 9.1).

9 Wash column filter and column (Buffer FIL-EF), 1st wash

Wash the NucleoBond® Xtra Column Filter and NucleoBond® Xtra Column with **Filter Wash Buffer FIL-EF**. Allow the column to empty by gravity flow.



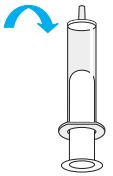
Apply the buffer to the funnel shaped rim of the filter and make sure it is washing out the lysate which is remaining in the filter. Omitting this step or just pouring the buffer directly inside the funnel may reduce plasmid yield.

5 mL	10 mL

Midi	Maxi

10 Discard column filter

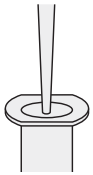
Either pull out the NucleoBond® Xtra Column Filter or discard it by turning the column upside down.



11 Wash column (Buffer ENDO-EF), 2nd wash

! Wash the NucleoBond® Xtra Column with **Wash Buffer ENDO-EF**. It is important to remove the column filter before applying the wash buffer to avoid low purity.

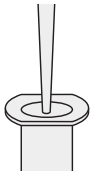
Allow the column to empty by gravity flow.



35 mL	90 mL

12 Wash column (Buffer WASH-EF), 3rd wash

Wash the NucleoBond® Xtra Column with **Wash Buffer WASH-EF**. Allow the column to empty by gravity flow.



15 mL	45 mL

13 Elution (Buffer ELU-EF)

Elute the plasmid DNA with **Elution Buffer ELU-EF**. Collect the eluate in a 15 mL or 50 mL centrifuge tube (not provided).

Note: Preheating Buffer ELU-EF to 50°C prior to elution may improve yields for large constructs such as BACs.

! Proceed with **step 14** for the centrifugation protocol after isopropanol precipitation or continue with **section 8.3** for plasmid concentration and desalination using the NucleoBond® Finalizer (NucleoBond® Xtra Midi Plus EF) or NucleoBond® Finalizer Large (NucleoBond® Xtra Maxi Plus EF).

Optional: Determine plasmid yield by UV spectrophotometry in order to adjust desired concentration of DNA in step 16 and calculate the recovery after precipitation.

5 mL	15 mL

Midi	Maxi

14 Precipitation

Note: It is highly recommended to determine plasmid yield by measuring A_{260} before precipitating the DNA (see section 5.13). This helps to choose the best buffer volume in step 16 and allows calculation of the recovery after precipitation.

Add **room-temperature isopropanol** to precipitate the eluted plasmid DNA. Vortex well and let the mixture sit for **2 minutes**.

Centrifuge at **≥5,000 x *g*** for **≥15 min** at **≤room temperature**, preferably at **15,000 x *g*** for **30 min** at **4 °C**. Carefully discard the supernatant.

3.5 mL	10.5 mL

15 Wash and dry DNA pellet (endotoxin-free 70 % EtOH)

Add endotoxin-free **room-temperature 70% ethanol** to the pellet and centrifuge at **≥5,000 x *g***, preferably **≥15,000 x *g*** for **5 min** at **room temperature** (18 – 25 °C).

2 mL	5 mL

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

Note: Plasmid DNA might be harder to dissolve when over-dried.

10 – 15 min	15 – 30 min

16 Reconstitute DNA (Buffer TE-EF or H_2O-EF)

Dissolve the DNA pellet in an appropriate volume of endotoxin-free **Buffer TE-EF** or **H_2O-EF**. Depending on the type of centrifugation tube, dissolve under gentle pipetting up and down or 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 (see section 5.13).

8.2 Low-copy plasmid purification (Midi, Maxi)

The lysis buffer volumes provided in the kit are adjusted for high-copy plasmid purification. Therefore, additional buffer has to be ordered separately for routine purification of low-copy plasmids (see ordering information).

Midi	Maxi

1 Prepare a starter culture

Inoculate a 3 – 5 mL starter culture of LB medium with a single colony picked from a freshly streaked agar plate. Make sure that plate and liquid culture contain the appropriate selective antibiotic to guarantee plasmid propagation (see section 5.3 for more information). Shake at 37°C and ~300 rpm for ~8 h.

2 Prepare a large overnight culture

! *Note: To utilize the entire large binding capacity of the NucleoBond® Xtra Columns it is important to provide enough plasmid DNA. For the standard low-copy procedure the culture volumes were doubled compared to the high-copy vector protocol. However, due to a plasmid content that is 10 – 100 times lower, this might be insufficient. If you need large amounts of low-copy plasmids, further increase the culture volume by factor 3 – 5 (see section 5.6 for more information) and decide in step 3 how much cells to use for the preparation.* The culture volumes recommended below are calculated for a final OD_{600} of around 4 (see section 5.6).

Inoculate an overnight culture by diluting the starter culture 1/1000 into the given volumes of LB medium also containing the appropriate selective antibiotic. Grow the culture overnight at 37°C and ~300 rpm for 12 – 16 h.

200 mL	600 mL

3 Harvest bacterial cells

Measure the cell culture OD_{600} and determine the recommended culture volume.

Vol [mL] = 800 / OD_{600}	Vol [mL] = 2400 / OD_{600}

Pellet the cells by centrifugation at **4,500 – 6,000 x *g*** for **≥10 min** at **4 °C** and discard the supernatant completely.

Midi	Maxi

Note: It is of course possible to use larger culture volumes, for example if a large amount of low-copy plasmid is needed (see section 5.6 for more information). In this case increase RES-EF, LYS-EF, and NEU-EF buffer volumes proportionally in steps 4, 5 and 7. Additional lysis buffer volumes might have to be ordered separately (see ordering information for NucleoBond® Xtra EF Buffer Set I, section 9.2). Use a centrifuge for the lysate clarification rather than the NucleoBond® Xtra Column Filters.

4 Resuspension (Buffer RES-EF)

Resuspend the cell pellet completely in **Resuspension Buffer RES-EF + RNase A** by pipetting up and down or vortexing the cells.

For an efficient cell lysis it is important that no clumps remain in the suspension.

Note: Increase RES-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis and section 5.8 regarding difficult-to-lyse strains).

16 mL	24 mL

5 Cell lysis (Buffer LYS-EF)

! **Check Lysis Buffer LYS-EF for precipitated SDS prior to use.** If a white precipitate is visible, warm the buffer for several minutes at 30 – 40 °C until precipitate is dissolved completely. Cool buffer down to room temperature (18 – 25 °C).

Add **Lysis Buffer LYS-EF** to the suspension.

Mix gently by **inverting** the tube **5 times**. **Do not vortex** as this will shear and release contaminating chromosomal DNA from cellular debris into the suspension.

Incubate the mixture at room temperature (18 – 25 °C) for **5 min**.

Note: Increase LYS-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis).

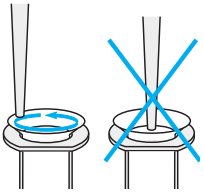
16 mL	24 mL

Midi	Maxi

6 Equilibration (Buffer EQU-EF)

Equilibrate a NucleoBond® Xtra Column together with the inserted column filter with **Equilibration Buffer EQU-EF**.

Apply the buffer onto the rim of the column filter as shown in the picture and make sure to wet the entire filter.



Allow the column to empty by gravity flow. The column does not run dry.

15 mL	35 mL

7 Neutralization (Buffer NEU-EF)

Add **Neutralization Buffer NEU-EF** to the suspension and immediately mix the lysate gently by **inverting** the tube **10 – 15 times. Do not vortex**.

! The flask or tube used for this step should not be filled more than two thirds to allow homogeneous mixing. Make sure to neutralize completely to precipitate all the protein and chromosomal DNA. The lysate should turn from a slimy, viscous consistency to a low viscosity, homogeneous suspension of an off-white flocculate.

Note: Increase NEU-EF buffer volume proportionally if more than the recommended cell mass is used (see section 5.7 for information on optimal cell lysis).

16 mL	24 mL

! **Incubate crude lysate on ice.**

5 min	5 min

Proceed with step 8 of the high-copy plasmid purification protocol (section 8.1) if not significantly more than the recommended lysis buffer volumes were used.

Otherwise it might be advantageous to centrifuge the precipitate first in order to avoid clogging of the NucleoBond® Xtra Column Filters.

8.3 Concentration of NucleoBond® Xtra EF eluates with the NucleoBond® Finalizers

Note: Use of the **NucleoBond® Finalizers** is only recommended for vector sizes smaller than 50 kbp.

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

1 Precipitate DNA

*Note: The **NucleoBond® Finalizer** only holds up to **500 µg** and the **NucleoBond® Finalizer Large** is limited to **2000 µg** of plasmid DNA. Loading more DNA might lead to clogging and complete loss of your sample. Thus, it is highly recommended to determine plasmid yield by measuring A_{260} before precipitating the DNA (see section 5.13). Furthermore, this helps to choose the best buffer volume in step 5 and allows calculation of the recovery after concentration.*

Add **0.7 volumes** of **room-temperature isopropanol** (not supplied with the kit). Vortex well and let the mixture sit for **2 minutes**.

(E.g., for 5 mL NucleoBond® Xtra Midi eluate add **3.5 mL** isopropanol, for 15 mL NucleoBond® Xtra Maxi eluate add **10.5 mL** isopropanol.)

3.5 mL for 5 mL eluate	10.5 mL for 15 mL eluate

2 Load precipitate

Remove the plunger from a **30 mL Syringe** and attach a NucleoBond® Finalizer to the outlet. Fill the precipitation mixture into the syringe, insert the plunger, hold the syringe in a vertical position, and press the mixture **slowly** through the NucleoBond® Finalizer using **constant force**. Discard the flow-through.

3 Wash precipitate (endotoxin-free 70 % EtOH)

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

Fill **endotoxin-free 70 % ethanol** into the syringe, insert the plunger, hold the syringe in a vertical position, and press the ethanol **slowly** through the NucleoBond® Finalizer. Discard the flow-through.

2 mL	5 mL

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

4 Dry filter membrane

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer. Press air through the NucleoBond® Finalizer as strongly as possible while **touching a tissue** with the tip of the NucleoBond® Finalizer to soak up ethanol.

Repeat this step at least as often as indicated below until **no more ethanol** leaks from the NucleoBond® Finalizer.

Note: A new dry syringe can be used to speed up the procedure (not provided).

≥3 times until dry	≥6 times until dry

Optional: You can incubate the NucleoBond® Finalizer for 10 minutes at 80°C to minimize ethanol carry-over. However, the final recovery may be reduced by over-drying the DNA.

5 Elute DNA (Buffer TE-EF or H_2O-EF)

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger of a **1 mL Syringe** and attach the NucleoBond® Finalizer to the syringe outlet.

Note: Refer to section 5.12, Table 4 (Midi) or 5 (Maxi) to choose the appropriate volume of elution buffer.

Pipette an appropriate volume of endotoxin-free **Redissolving Buffer TE-EF** or **H_2O-EF** into the syringe (see section 5.12). Do not use pure water unless pH is definitively >7. Place the NucleoBond® Finalizer outlet in a vertical position over a fresh collection tube (not provided) and **elute plasmid DNA** very slowly, drop by drop by inserting the plunger.

200 – 800 µL	400 – 1000 µL

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

Transfer the first eluate back into the syringe and elute into the same collection tube a second time.

Load first eluate completely	Load first eluate completely

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger to aspirate air, reattach the NucleoBond® Finalizer and **press the air out again to force out as much eluate as possible**.

Determine plasmid yield by UV spectroscopy and confirm plasmid integrity by agarose gel electrophoresis (see section 5.13).

9 Appendix

9.1 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 (Table 1, section 5.3). **Second**, aliquots of the cleared lysate, the flow-through, the combined washing steps (Buffer FIL-EF, ENDO-EF and WASH-EF), and the eluate should be kept for further analysis by agarose gel electrophoresis.

Refer to Table 6 to choose a fraction volume yielding approximately 5 µg of plasmid DNA assuming 250 µg and 1000 µg were loaded onto the **NucleoBond® Xtra Midi** and **Maxi Column**, respectively. Precipitate the nucleic acids by adding 0.7 volumes of isopropanol, centrifuge the sample, wash the pellet using 70% ethanol, centrifuge again, air dry for 10 min, dissolve the DNA in 100 µL TE buffer, pH 8.0, and run 20 µL on a 1% agarose gel.

Table 6: NucleoBond® Xtra EF eluate volumes required for an analytical check

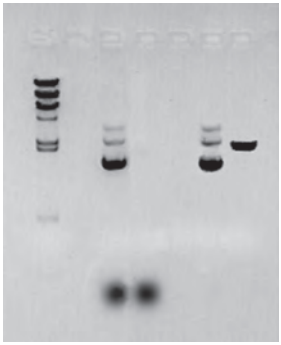
Sample	Purification step	Volume required [µL]	
		Midi	Maxi
I	Cleared lysate of protocol step 8	500	200
II	Column flow-through after protocol step 8	500	200
III	Wash flow-through after protocol step 9, 11, and 12	500	400
IV	Eluate after protocol step 13	100	100

The exemplary gel picture (Figure 6) 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 (lanes 1 and 2). It might also occur in the wash fraction but must be absent in the eluate.

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



M: Marker λ *Hin*dIII

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

2: II, lysate 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 6: Exemplary analytical check of NucleoBond® Xtra Midi purification samples
Plasmid: pUC18, bacterial strain: *E. coli* DH5α®. 20 µL of each precipitated sample has been analyzed on a 1% agarose gel. Equal amounts of plasmid DNA before (lane 1) and after (lane 4) purification using NucleoBond® Xtra Midi are shown with a recovery of >90%.

Problem	Possible cause and suggestions
No or low plasmid DNA yield	*Plasmid did not propagate* • Check plasmid content in the cleared lysate (see Figure 6). Use colonies from fresh plates for inoculation and add selective antibiotic to plates and media. • Estimate plasmid content prior to large purifications by a quick NucleoSpin® Plasmid or NucleoSpin® Plasmid QuickPure preparation. *Alkaline lysis was inefficient* • Too much cell mass was used. Refer to section 5.5 – 5.7 regarding recommended culture volumes and lysis buffer volumes. Check plasmid content in the cleared lysate (see Figure 6). • Check Buffer LYS-EF for SDS precipitation before use, especially after storage below 20°C. If necessary incubate the bottle for several minutes at 30 – 40°C and mix well until SDS is redissolved. *SDS- or other precipitates are present in the sample* • Load the crude lysate onto the NucleoBond® Xtra Column Filter inserted in the NucleoBond® Xtra Column. This ensures complete removal of SDS precipitates. Incubation of cleared lysates for longer periods of time might lead to formation of new precipitate. If precipitate is visible, it is recommended to filter or centrifuge the lysate again directly before loading it onto the NucleoBond® Xtra Column. *Sample/lysate is too viscous* • Too much cell mass was used. Refer to section 5.5 – 5.7 regarding recommended culture volumes and lysis buffer volumes. • Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA. Otherwise, filtration efficiency and flow rate go down and SDS prevents DNA from binding to the column. *pH or salt concentrations of buffers are too high* • Check plasmid content in the wash fractions (see Figure 6). Keep all buffers tightly closed. Check and adjust pH of Buffer FIL-EF (pH 6.5), ENDO-EF (pH 6.5), WASH-EF (pH 7.0), and ELU-EF (pH 9.0) with HCl or NaOH if necessary.

Problem	Possible cause and suggestions
NucleoBond® Xtra Column Filter clogs during filtration	*Culture volumes are too large* • Refer to section 5.5 – 5.7 regarding recommended culture volumes and larger lysis buffer volumes. *Precipitate was not resuspended before loading* • Invert crude lysate at least 3 times directly before loading. *Incomplete precipitation step* • Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA
NucleoBond® Xtra Column is blocked or very slow	*Sample is too viscous* • Do NOT attempt to purify lysate prepared from a culture volume larger than recommended for any given column size with standard lysis buffer volumes. Incomplete lysis not only blocks the column but can also significantly reduce yields. Refer to section 5.5 and 5.6 for recommended culture volumes and section 5.7 for larger culture volumes and adjusted lysis buffer volumes. • Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA. *Lysate was not cleared completely* • Use NucleoBond® Xtra Column Filter or centrifuge at higher speed or for a longer period of time. • Precipitates occur during storage. Clear lysate again before loading the column.
Genomic DNA contamination of plasmid DNA	*Lysis treatment was too harsh* • Make sure not to lyse in Buffer LYS-EF for more than 5 min. *Lysate was mixed too vigorously or vortexed after lysis* • Invert tube for only 5 times. Do not vortex after addition of Buffer LYS-EF. • Use larger tubes or reduce culture volumes for easier mixing.

Problem	Possible cause and suggestions
RNA contamination of plasmid DNA	*RNase digestion was inefficient* • RNase was not added to Buffer RES-EF or stored improperly. Add new RNase to Buffer RES-EF. See section 9.2 for ordering information. *pH or salt concentration of wash buffer is too low* • Check RNA content in the wash fractions (see Figure 6). Keep all buffers tightly closed. Check pH of Buffer FIL-EF, ENDO-EF, and WASH-EF and adjust pH to 7.0 with HCl or NaOH if necessary. *Wash step with Buffer WASH was not sufficient* • Double or triple washing step with Buffer WASH-EF. Additional Buffer WASH-EF can be ordered separately (see ordering information).
Low purity (A_{260}/A_{280} <1.8)	*NucleoBond® Xtra Column Filter was not removed before second washing step* • Protein content too high due to inefficient washing. Remove the NucleoBond® Xtra Column Filter **before** performing the second washing step with Buffer ENDO-EF. *Buffer ENDO-EF or WASH-EF was used instead of Buffer FIL-EF for the first wash* • Buffer FIL-EF has to be used to wash out the NucleoBond® Xtra Column Filter to avoid SDS and protein carry-over. *Only minimal amounts of DNA were loaded onto the column* • Too much free binding capacity requires more extensive washing – double washing step with Buffer ENDO-EF. • Reduce lysis time <5 min.
No nucleic acid pellet formed after precipitation	*Pellet was lost* • Handle the precipitate with care. Decant solutions carefully. Determine DNA yield in Buffer ELU-EF in order to calculate the amount of plasmid DNA that should be recovered after precipitation. *Plasmid DNA might be smeared over the wall of the tube* • Dissolve DNA with an appropriate volume of endotoxin-free reconstitution buffer by rolling the tube for at least 30 min.

Problem	Possible cause and suggestions
No nucleic acid pellet formed after precipitation *(continued)*	*Nucleic acid did not precipitate* • Check type and volumes of precipitating solvent. Make sure to use at least 0.7 volumes of isopropanol and mix thoroughly. • Centrifuge for longer periods of time at higher speed.
Nucleic acid pellet is opaque or white instead of clear and glassy	*Co-precipitation of salt* • Check isopropanol purity, and perform precipitation at room temperature (18 – 25°C) but centrifuge at 4°C. Do not let the eluate drip from the column into isopropanol but add isopropanol to the final eluate and mix immediately. • Try resuspending the pellet in Buffer ENDO-EF, and reload onto the same NucleoBond® Xtra Column. Wash the column several times with Buffer WASH-EF before loading.
Nucleic acid pellet does not resuspend in buffer	*Pellet was over-dried* • Try to dissolve at higher temperatures for a longer period of time (e.g., 2 h at 37°C or overnight at RT), preferably under constant spinning (3D-shaker). *Co-precipitation of salt or residual alcohol* • Wash the pellet again with 70% ethanol, or increase the reconstitution buffer volume. *Insoluble particles in redissolved DNA* • Centrifuge the redissolved DNA to pellet the insoluble particles and transfer supernatant to a new tube. Insoluble particles do not affect DNA quality. As an alternative, insoluble particles can easily be removed by using the NucleoBond® Finalizer (NucleoBond® Xtra Midi EF) or NucleoBond® Finalizer Large (NucleoBond® Xtra Maxi EF) which filter the particles out of the solution.
No or low plasmid DNA yield after NucleoBond® Finalizer precipitation	*Already no or low plasmid DNA after elution from the NucleoBond® Xtra Column* • Refer to detailed troubleshooting “No or low plasmid DNA yield”.

Problem	Possible cause and suggestions
No or low plasmid DNA yield after NucleoBond® Finalizer precipitation *(continued)*	*Dead volume too high* • If high concentration of plasmid DNA is the main aim, elution should be performed in small volumes. Naturally a portion of the eluate will be lost in the syringe and on the NucleoBond® Finalizer. To minimize these losses in the second elution step, try to transfer even the last droplet from the syringe to the NucleoBond® Finalizer, for example by tapping the NucleoBond® Finalizer and syringe onto the bench top. Then fill the syringe with air and press forcefully the last droplets out of the NucleoBond® Finalizer. Repeat this step several times. You might have to practice this procedure several times to achieve optimal results. An acceptable dead volume is smaller than 30 µL with NucleoBond® Finalizer and 60 µL with NucleoBond® Finalizer Large. *Elution volume too small* • Since there are dead volumes of about 30 µL (NucleoBond® Finalizer) and 60 µL (NucleoBond® Finalizer Large), reasonable elution volumes start with 200 µL (NucleoBond® Finalizer) and 400 µL (NucleoBond® Finalizer Large) respectively. Furthermore, smaller volumes are insufficient to wet the entire membrane and will drastically decrease your yield. Refer to section 5.12, Table 4 (NucleoBond® Finalizer) and 5 (NucleoBond® Finalizer Large) to estimate the recovery that can be expected depending on elution buffer volume. *Elution too fast* • Plasmid DNA needs time to dissolve. Eluate really very slowly, drop by drop. Repeat the elution procedure using the first eluate. *Forgot to elute a second time* • Repeating the elution procedure with the first eluate is crucial for optimal yields. However, eluting a third time shows no further improvement. *Plasmid size* • Precipitation efficiency is almost independent of plasmid size, but elution from the NucleoBond® Finalizers becomes more and more difficult with increasing size of the construct. If you face low yields with large cosmids you may try heating the NucleoBond® Finalizer, the syringes, and elution buffer to 70 °C.

Problem	Possible cause and suggestions
Low DNA concentration after NucleoBond® Finalizer precipitation	*Low overall yield* • Refer to detailed troubleshooting “No or low plasmid DNA yield” and lower your elution buffer volume. Refer to section 5.12, Table 4 and 5 to estimate the DNA concentrations that can be expected. *Fresh elution buffer was used for second elution step* • The second elution step is crucial for optimal yield but to achieve a high DNA concentration the eluate of the first elution step has to be used for the second elution. *Not enough DNA loaded* • Since there is a technical limitation to at least 200 µL (NucleoBond® Finalizer) and 400 µL (NucleoBond® Finalizer Large) of elution buffer due to membrane wetting and dead volume, a minimal amount of DNA has to be loaded to achieve a desired concentration. If possible try to pool several DNA precipitation batches since percentage of recovery and concentration significantly increase with higher amounts of loaded DNA.
Purified plasmid does not perform well in subsequent reactions	*Plasmid DNA is contaminated with chromosomal DNA or RNA* • Refer to the detailed troubleshooting above. *Plasmid DNA is contaminated with residual alcohol* • Plasmid DNA was not dried completely before redissolving. Precipitate DNA again by adding 1/10 volume of 3 M NaAc pH 5.0 and 0.7 volumes of isopropanol. Proceed with the precipitation protocol in this manual und dry DNA pellet completely. *DNA is degraded* • Make sure that your entire equipment (pipettes, centrifuge tubes, etc.) is clean and nuclease-free. • Do not lyse the sample with Buffer LYS-EF for more than 5 min.

Problem	Possible cause and suggestions
Purified plasmid does not perform well in subsequent reactions *(continued)*	*DNA is irreversibly denatured* • A denatured plasmid band runs faster on the gel than the supercoiled conformation. Do not lyse the sample after addition of Buffer LYS-EF for more than 5 min. *Endotoxin level is too high* • Refer to the detailed troubleshooting below.
Endotoxin level is too high	*Too much cell mass was used* • Decrease cell mass or better increase lysis buffer volumes. *Inefficient endotoxin removal* • Apply all washing buffers in the correct order: FIL-EF, ENDO-EF, WASH-EF. *Contamination of DNA product after purification* • Use only new pyrogen- or endotoxin-free plastic ware and pipette tips. Endotoxins tend to stick to glass ware and are hard to remove. If glass ware is to be used, heat over night at 180°C to destroy endotoxins. Autoclaving does not inactivate endotoxins and is not recommended if the autoclave is also used for inactivation of bacterial cultures. • Use only the tested endotoxin-free buffers provided with the kit especially for preparation of 70% ethanol and for DNA reconstitution.

9.2 Ordering information

Product	REF	Pack of
NucleoBond® Xtra Midi EF	740420.10/.50	10/50
NucleoBond® Xtra Midi Plus EF (incl. NucleoBond® Finalizer)	740412.10/.50	10/50
NucleoBond® Xtra Maxi EF	740424.10/.50	10/50
NucleoBond® Xtra Maxi Plus EF (incl. NucleoBond® Finalizer)	740426.10/.50	10/50
NucleoBond® Xtra Combi Rack	740415	1
NucleoBond® Xtra EF Buffer Set I (Buffer RES-EF, LYS-EF, NEU-EF, RNase A)	740427	1
Buffer WASH-EF	740392.1000	1000 mL
NucleoBond® Finalizer (for use with NucleoBond® Xtra Midi, Midi EF, NucleoBond® PC 100, PC 500, PC 500 EF)	740519.20	20 filters 2 syringe sets
NucleoBond® Finalizer Plus (for use with NucleoBond® Xtra Midi, Midi EF, NucleoBond® PC 100, PC 500, PC 500 EF)	740520.20	20 filters 20 syringe sets
NucleoBond® Finalizer Large (for use with NucleoBond® Xtra Maxi, Maxi EF, NucleoBond® PC 2000, PC 2000 EF)	740418.20	20 large filters 2 syringe sets
NucleoBond® Finalizer Large Plus (for use with NucleoBond® Xtra Maxi, Maxi EF, NucleoBond® PC 2000, PC 2000 EF)	740419.20	20 large filters 20 syringe sets
RNase A	740505	100 mg
RNase A	740505.50	50 mg

9.3 Product use restriction/warranty

NucleoBond® Xtra Midi/Maxi EF 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.

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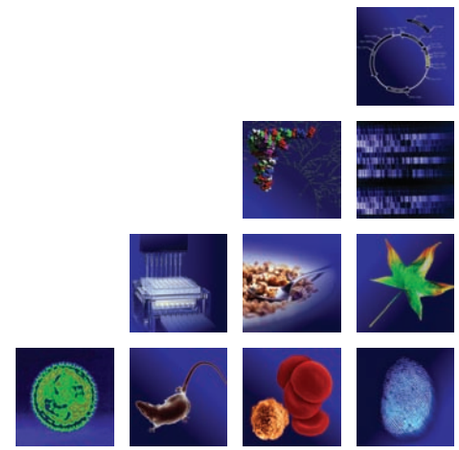

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