

# Plasmid DNA Purification from small to large scale

## NucleoBond® Plasmid Purification Kits

- 1. NucleoBond® PC Kits** Five column sizes are available for purification of up to 10 mg of **ultra-pure high- and low copy plasmid DNA**
- 2. NucleoBond® PC EF Kits** Four column sizes are available for purification of up to 100 mg of **endotoxin-free plasmid DNA** for transfection of endotoxin-sensitive cells, gene therapy, and vaccination
- 3. NucleoBond® BAC 100 Kit** Increased volumes of lysis buffers and RNase A for purification of **large constructs, e.g. BACs, PACs, P1s, cosmids**

### 1. NucleoBond® PC Kits

NucleoBond® AX is a silica-based anion exchanger developed and manufactured by MACHEREY-NAGEL for routine separation of different classes of nucleic acids, covered by **European patent E.P. 0496822**. The extraordinary high charge density on its hydrophilic, macroporous surface results in a salt concentration range for binding and elution of nucleic acids which is much larger than on conventional anion exchangers.

All NucleoBond® AX columns are resistant to organic solvents like alcohol, chloroform and phenol. They are free of RNase and DNase.

NucleoBond® AX columns are easy to handle. Solutions are pipetted or just poured into the columns. Their flow through the exchanger bed is by gravity. The columns do not run dry.

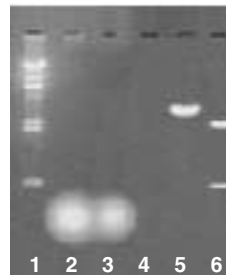


### Features

- ✓ anion-exchange chromatography
- ✓ fast and versatile separation system
- ✓ working range from nanograms to (milli) grams DNA
- ✓ recovery of plasmid DNA > 90%
- ✓ **NucleoBond® folded filters eliminate the centrifugation step – no shearing even for large constructs**
- ✓ approved and easy procedures
- ✓ structural integrity > 92% ccc monomer

#### Purification of plasmid DNA (pUC19) using NucleoBond® AX 100

As can be seen in the figure, this procedure yields high pure plasmid DNA that is free of contaminants like genomic DNA or RNA.

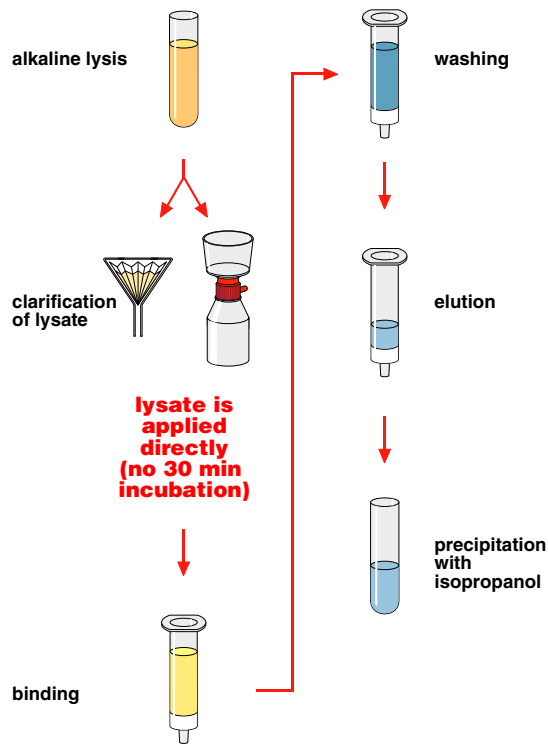


1 % agarose gel (TAE)

- 1: marker
- 2: flow-through
- 3: 1<sup>st</sup> washing step
- 4: 2<sup>nd</sup> washing step
- 5: elution
- 6: purified plasmid digested with *EcoRI* / *SspI*

## 2. NucleoBond® PC EF Kits

### Procedure



### Features

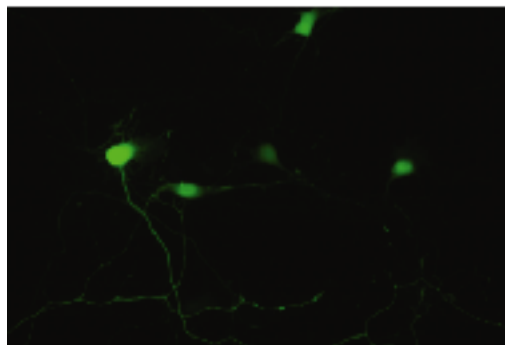
- ✓ covered by US patent # US 6, 428703 B1
- ✓ no additional steps required – minimal hands-on-time
- ✓ endotoxin-free plasmids for transfection, gene therapy, and vaccination
- ✓ DNA with less than 0,05 EU/µg DNA
- ✓ columns available for up to 500 µg, 2000 µg, 10 mg, and 100 mg
- ✓ folded filters/bottle top filters included for clarification of bacterial lysate

### Application Data

#### Endotoxin-free DNA for transfection of primary neurons

Expression of GFP (Green Fluorescent Protein) in mature hippocampal neurons (>7 days in culture) grown at low density on poly-L-lysine coated glass coverslips.

The neurons were transiently transfected with a cDNA (EF-free plasmid DNA was purified with NucleoBond® PC 10000 EF) encoding GFP using a modified calcium phosphate protocol (Koehrmann et al, 1999) and the expression pattern was viewed 16 hours post-transfection.



## 3. NucleoBond® BAC 100 Kit

### Customer Testimonial

*"Only one of the kits we tried worked; that was the NucleoBond® kit. The vector DNA purified with this kit gave us a transformation efficiency that was comparable to that reported in the literature using BAC vector DNA prepared by two or three rounds of CsCl gradient centrifugation."*

Teh-hui Kao, Ph.D. and Andrew G. McCubbin, Ph.D.,  
Department of Biochemistry and Molecular Biology, Eberly College of Science  
Penn State University (USA)

Trademarks: NucleoBond® is a registered trademark of MACHERY-NAGEL.

# MACHERY-NAGEL

MACHERY-NAGEL GmbH & Co. KG · P.O. Box 10 13 52 · D-52313 Düren · Germany · Tel. (0 24 21) 969-0 · Fax (0 24 21) 969 199  
Switzerland: MACHERY-NAGEL AG · P.O. Box · CH-4702 Oensingen · Tel. (0 62) 388 55 00 · Fax (0 62) 388 55 05  
France: MACHERY-NAGEL S.à.r.l. · 1, rue Gutenber · B.P. 135 · F-67722 Hoerd · Tel. 0388 682268 · Fax 0388 517688

