

NucleoBond® Prep 100

26.04.2019

Guideline for the preparative scale plasmid purification

Kit specifications

The NucleoBond® Prep 100 kit is designed for the purification of plasmid from *E. coli* cultures for research use only applications.

Kit specifications at a glance

Culture volume	5-20 L
Max. pellet wet weight	90 g
Binding capacity	100 mg
Endotoxin level	< 0.1 EU/µg
Applications	Research use only, Transfection studies, gene therapy
Time/prep	20 h

Cell lysis

Optimal cell lysis depends on the correct ratio of cells to lysis buffer. It is highly recommended to measure the optical density of the bacterial culture at 600 nm (OD₆₀₀) just before harvesting the cells. The OD₆₀₀, multiplied with the harvested culture volume in milliliters, returns the ODV number which is equivalent to the number of cells in a pellet. For example, 10 liters of a culture with an OD₆₀₀ of 4 will result in an ODV of 40,000:

$$ODV = \text{harvested Volume [mL]} * OD(600) = (e.g.): 10,000 * 4 = 40,000$$

Divide this number by 50 to calculate the volume in milliliters of buffers S1-EF, S2-EF and S3-EF each, which is needed for an optimal lysis. To follow the above example:

$$\text{Volume (each S1 - EF, S2 - EF, S3 - EF) [mL]} = ODV/50 = (e.g.): 40,000/50 = 800$$

Especially when using optimized fermentation conditions, it is possible to reach high optical densities. In combination with a large culture volume harvest it is possible to overload the lysis buffer. An OD₆₀₀ of for example 8 would result in an ODV of 80,000 when harvesting cells from 10 liters of culture medium. An ODV of 80,000 would require at least 1600 mL of each lysis buffer which is not supplied.

Contact MACHEREY-NAGEL (tech-bio@mn-net.com) or your local distributor if additional lysis buffer is required! Alternatively, reduce input amount of cells to 90 g wet weight or ODV 50,000. It is not recommended to overload the lysis buffer with excess cells as effects could be reduced yield or even a complete failure of the cell lysis.

Resuspend cells vigorously and completely in Lysis Buffer S1-EF. Check Lysis buffer S2-EF for precipitates like cloudy white flocculates or a firm crust at the bottom of the bottle before use and dissolve any precipitates by heating the buffer to 37°C for at least 10 min.



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

Germany and international:
 Tel.: +49 24 21 969-0
 Fax: +49 24 21 969-199
 E-mail: info@mn-net.com

Switzerland:
MACHEREY-NAGEL AG
 Tel.: +41 62 388 55 00
 Fax: +41 62 388 55 05
 E-mail: sales-ch@mn-net.com

France:
MACHEREY-NAGEL EURL
 Tel.: +33 388 68 22 68
 Fax: +33 388 51 76 88
 E-mail: sales-fr@mn-net.com

USA:
MACHEREY-NAGEL Inc.
 Tel.: +1 484 821 0984
 Fax: +1 484 821 1272
 E-mail: sales-us@mn-net.com

Lysate clarification

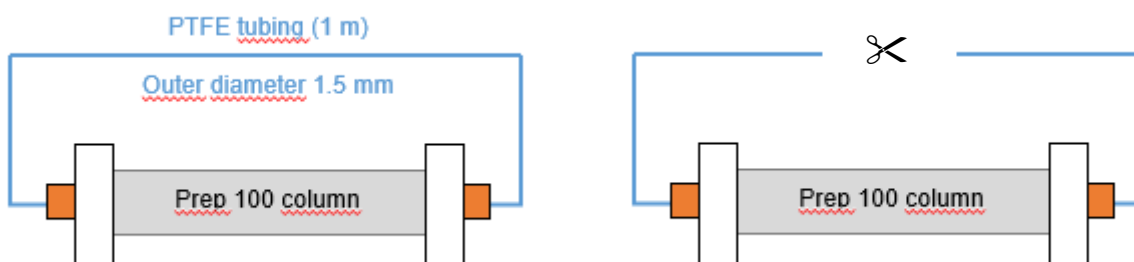
The lysis yields large amounts of precipitate. Use the supplied sieving fabric for a quick first separation and the supplied folded filters for a removal of fine precipitates according to step 4 in the NucleoBond® Prep 100 handbook. Place the filters and sieving fabric into funnels with an upper diameter of about 150 mm and a height of approximately 200 mm. The table below gives a few examples of suitable funnels and is no complete summary.

Examples of suitable funnels	
Supplier	REF
Carl Roth (www.carlroth.com)	YA51.1
VWR (www.vwr.com)	BRND147035
Merck (sigmaaldrich.com)	CLS6120P150-12EA

Instead of gravity flow filtration, centrifugation or vacuum filtration are further options for lysate clearing. When using centrifugation, be careful not to transfer debris into the cleared lysate pool. Vacuum filtration can be performed with the **NucleoBond® Bottle Top Filters Type 2** (REF 740553.5, Pack of 5). See section 5.3 of the user manual for details. One Bottle Top Filter Type 2 can clear about ODV 15,000 (e. g.: 5 L culture with an OD₆₀₀ = 3) before clogging.

Column setup

The supplied NucleoBond® AX prep 100 column contains the established NucleoBond® anion exchange resin. In contrast to the gravity flow kits the buffer and lysate supply for this column is driven by a pump against gravity. The column is supplied already connected to a PTFE tubing. The outer diameter of this tubing is 1,5 +/- 0,15 mm which corresponds to 1/16", the inner diameter is 1.0 mm (about 1/32"). The tubing is circular closed and needs to be cut in order to connect the column.

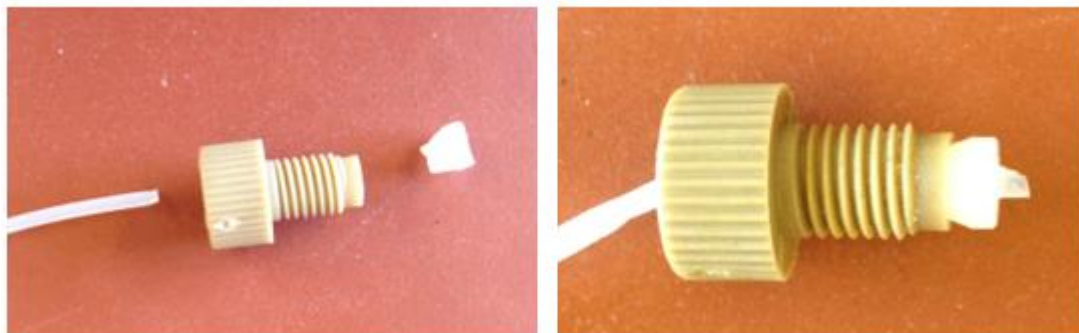


The resulting open end which will be connected with an Äkta or peristaltic pump (and via the pump with the lysate/ buffer reservoir) needs to be connected with nuts and ferrules and additional tubing which is not supplied and depending on the type of pump. **FPLC** and **HPLC pumps** can be connected directly via suitable connectors for 1/16":

Examples of suitable connectors for Äkta/ HPLC	
Supplier	REF
Vici AG (www.vici-jour.com)	JR-20124-10 (nut) JR-041-10 (ferrule)
SCP (www.scp GmbH.de)	58.1.2.062.40 (nut) 59.2.1.062.00 (ferrule)
Biotech (www.biotechfluidics.com)	XP-141 (nut and ferrule)



Combine nut, ferrule and tubing according to the following figure.



If the column is to be connected with a **peristaltic pump**, the diameter and type of the tubing must be increased to at least 1/8" and silicone or viton. This can be done via "unions" or adapter sets which connect a 1/16" PTFE tubing to a 1/8" peristaltic pump tubing. There are dedicated adapter sets available which connect a noncompressible PTFE tubing to a compressible silicone tubing for exactly this purpose.

Examples of suitable unions to increase tubing diameter for peristaltic pumps

Supplier	REF
Vici AG (www.vici-jour.com)	JR-PTAS (Peristaltic to PTFE tubing adapter set)
Darwin Microfluidics (darwin-microfluidics.com)	Peristaltic Tubing Adapter for 1/16" OD PTFE Tubing
Kinesis (kinesis.co.uk)	Connectors for peristaltic pump tubing

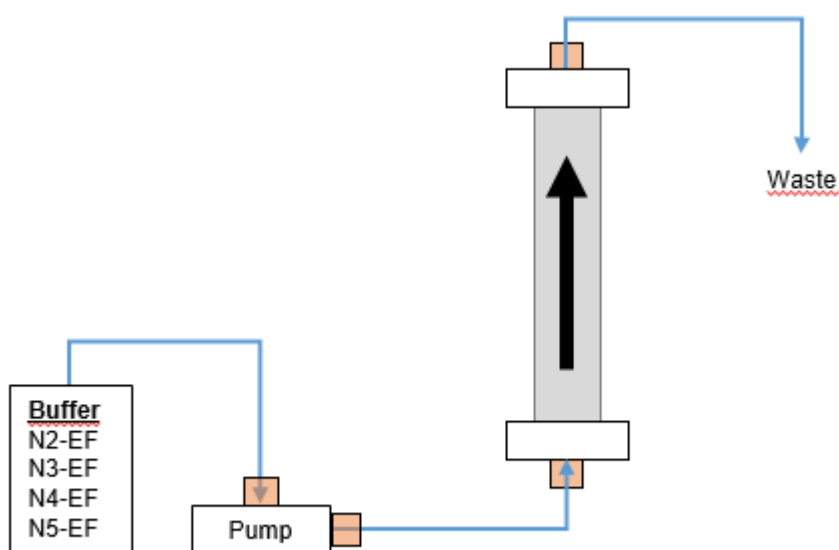
The exact outer diameter of the tubing depends on the pump head and is variable. Use at least a 1/8" silicone tubing for the peristaltic pump. Larger diameters are possible, but always combine pump head, silicone tubing and tubing adapter accordingly.

Examples of suitable 1/8" silicone tubing

Supplier	REF
Merck (www.sigmaaldrich.com)	T1664-25FT
LabSmith (www.products.labsmith.com)	1/8" OD Silicone Tubing



Schematic overview



Pump

Besides FPLC and HPLC pumps, which are very expensive, there is a vast variety of suitable peristaltic pumps. The pump should be able to support a flow rate of at least 4 mL/min. Equilibration and washing steps of the NucleoBond® Prep 100 kit recommend flow rates of up to 20 mL/min, but a lower flow rate at these steps will not have a negative impact on DNA quality, only on preparation time. Pressure within the column should not exceed 7 kg/cm² (100 PSI) during usage. Pumps and pump heads are usually designed to fit a specific need and are variable in terms of equipment. Contact a supplier directly for more information.

Examples of peristaltic pump suppliers

Supplier	Pump
Integra Biosciences (www. Integra-biosciences.com)	DOSE IT (portable peristaltic pump)
Heidolph (heidolph-instruments.com)	Hei-FLOW Series
CAPP (www.capp.dk)	Liquimaster
GE Lifescience	P-1

This list like all lists before is not complete and only gives a general overview.

Elution

The dead volume of the Prep 100 column will be about 200 mL. The first 200 mL of the eluate can be discarded as they only contain residual wash buffer. If possible, take fractions and check aliquots for DNA photometrically to limit the final elution volume as much as possible. It is also possible to use an in-line photometer to cut the DNA fraction from the entire lysate.