Automated Plasmid DNA Purification

User manual

NucleoSpin® Robot-96 Plasmid Core kit

August 2002/Rev. 01

MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoSpin® Robot-96 Plasmid Core Kit
	24 x 96 preps[1]
Cat.No.	**740 616.24**
Buffer A1	6 x 200 ml
Buffer A2	6 x 200 ml
Buffer A3	6 x 200 ml
Buffer A4 (concentrate)[2]	6 x (4 x 100 ml)
Buffer AE	6 x (2 x 50 ml)
RNase A[2]	6 x 80 mg
NucleoSpin® Plasmid Binding Plate (transparent)	24
NucleoSpin® Plasmid Filter Plate (purple)	24
MN Wash plate (including six paper sheets)	24
Protocol	6 x 1

[1] The **NucleoSpin® Robot-96 Plasmid** Core Kit consists of 6 boxes with 4 x 96 preps each.
[2] For preparation of working solutions and storage conditions see section 3.

2 Product description

2.1 The basic principle

The **NucleoSpin® Robot-96 Plasmid** procedure is a modified version of the Birnboim and Doly alkaline lysis plasmid miniprep protocol. Bacterial cultures are harvested by an initial centrifugation step. After resuspension of the pelleted bacteria (buffer A1) and alkaline cell lysis (buffer A2), a neutralization and binding buffer (buffer A3) containing large amounts of chaotropic ions is added. Resulting bacterial crude lysates are cleared by vacuum filtration with the **NucleoSpin® Plasmid Filter Plate.** The cleared lysates containing the plasmid DNA are collected into the **NucleoSpin® Plasmid Binding Plate.** The chaotropic salt leads to a reversible adsorption of the plasmid DNA to the **NucleoSpin® silica membrane** during the second vacuum-filtration step. High purity of the final plasmid DNA preparation is achieved by complete removal of cellular contaminants, salts, detergents, and other compounds in subsequent washing steps. Highly pure plasmid DNA is finally eluted with elution buffer AE (5 mM Tris/HCl, pH 8.5) or water (pH 8.0-8.5), and can be directly used for further applications.

2.2 Kit specifications

- **NucleoSpin® Robot-96 Plasmid** is designed for the automated 96-well small-scale purification of high-copy plasmid DNA from *E. coli* in the microtiter plate format.

- The kit is for use with *common laboratory automation workstations* (see section 2.3).

- This kit provides reagents and basic consumables (NucleoSpin® Filter Plate, NucleoSpin® Plasmid Binding Plate, MN Wash Plate) for purification of up to 15-20 µg of highly pure plasmid DNA suitable for direct use in standard molecular biology applications like automated fluorescent sequencing, PCR, or restriction analysis.

- Using the **NucleoSpin® Robot-96 Plasmid** kit allows simultaneous processing of up to 96 samples typically within less than 90 minutes. Actual processing time depends on configuration of workstation used.

- Typically yields of 5-15 µg plasmid DNA can be purified from 1.5 ml overnight cultures.

- Yield depends on copy number and size of plasmid (<15 kbp), selected culture medium, and bacterial host strain.

- Membrane capacity is about 20 µg. The final concentration of eluted DNA is 50-200 ng/µl (depending on elution buffer volume and bacterial culture).

- Typically, the $A_{260/280}$ ratio is > 1.8. Eluted DNA is ready-to-use for e.g. automated fluorescent sequencing (e.g. ABI 3700, 3100, 377, 373, LICOR, MegaBace, ALF), restriction analysis, and PCR.

Kit specifications at a glance	
	NucleoSpin® Robot-96 Plasmid
Culture volume	1.5 ml
Average yield	5-15 µg
Elution volume	75-150 µl
Binding capacity	20 µg
Vectors	< 15 kb
Time/prep	90 min/96 preps

2.3 Elution procedure

See table for correlation between dispensed elution buffer volume and typical recoveries following the standard protocol.

The default volume of dispensed elution buffer in the available programs is 125 µl.

Dispensed elution buffer	75 µl	100 µl	125 µl	150 µl	175 µl
Recovered elution buffer containing plasmid DNA	30±5µl	55±5 µl	80±5 µl	105±5 µl	130±5 µl

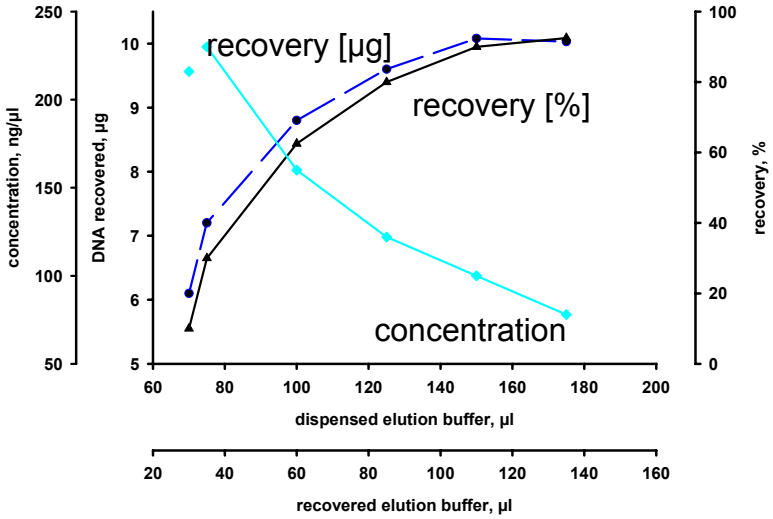


Fig. 1: Recovery rate and concentration depend on elution volume. 10 µg of pBluescript were purified with **NucleoSpin® Robot-96 Plasmid** and eluted with the indicated elution buffer volumes. High recovery is achieved with 120 µl elution buffer (dispensed), as concentration drops.

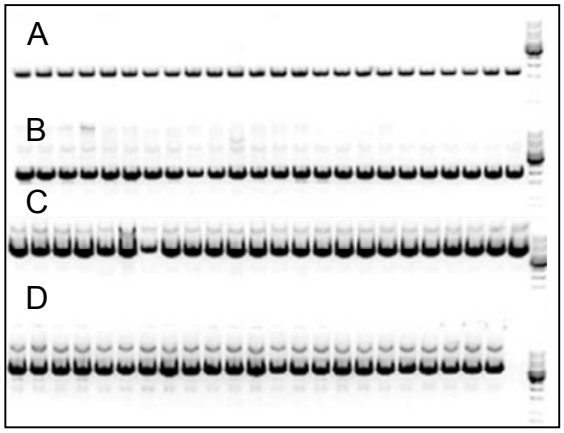


Fig. 2: Purity and yields of pBluescript KS+ (2.96 kbp, **A**), pUC 18 derivate (3.65 kbp, **B**), pcDNA3.1 (8.6 kbp, **C**), and pCMVβ (7.2 kbp, **D**) using **NucleoSpin® Robot-96 Plasmid**. 15 µl out of 125 µl eluate were analyzed on a 1% agarose gel.

2.4 Automation

NucleoSpin® Robot-96 Plasmid is designed for use on common laboratory automation workstations, such as:

Robot Supplier	**Robot**
Beckman-Coulter	Biomek 2000/FX
Cavro	MiniPrep series
Hamilton	Microlab STAR
MWG	RoboSmart/RoboPrep
Perkin Elmer	MultiPROBE II/II HT
Qiagen	BioRobot 9600/3000/8000
Tecan	Genesis RSP/RWS Separation System series
Zymark	SciClone ALH

Note: As other laboratory automation workstations are currently under evaluation please contact MN directly if your workstation is not on this list.

Visit MN on the internet at *www.mn-net.com* or contact your local MACHEREY-NAGEL distributor for availability of ready-to-run scripts and for technical support regarding hardware, software, setup instructions, and selection of the protocol. All MN protocols can be downloaded from our website.

3 Storage conditions and preparation of working solutions

Attention:

Buffer A3 contains guanidinium hydrochloride which is an irritant! Buffer A2 contains SDS and sodium hydroxide which are irritant and hazardous. Wear gloves and goggles when handling them!

- Before first use of the kit, add 1 ml of buffer A1 to the RNase A vial and vortex. Transfer the whole amount of redissolved RNase A to the buffer A1 bottle and mix thoroughly. Store buffer A1 containing RNase A at 4°C (buffer A1 including RNase A is stable for up to 6 months). **Equilibrate buffer A1 to room temperature before starting plasmid DNA preparation.**

- All other components of the **NucleoSpin® Robot-96 Plasmid** kit should be stored at room temperature (for a maximum of one year). Storage of buffer A2 at temperatures below 20°C may cause precipitation of SDS. If a salt precipitate is observed, incubate the bottle at 30-40°C for some minutes, and mix well until all of the precipitation is redissolved.

- Add indicated volume of 96% ethanol to buffer A4 concentrate before use.

	NucleoSpin® Robot-96 Plasmid Core kit
	24 x 96 preps
Cat. No.	**740616.24**
Buffer A4 (concentrate)	24 x 100 ml add 400 ml ethanol to each bottle

4 Growing of bacterial cultures

4.1 Selection of culture medium

The cultivation of cells is recommended at 37°C in LB (Luria-Bertani) medium at constant shaking (200-250 rpm). Alternatively, rich media like 2 x YT or TB (Terrific Broth) can be used. By using 2 x YT or TB, bacteria grow faster and reach the stationary phase much earlier than in LB medium ($\leq$ 12 h). This may lead to a higher percentage of dead or starving cells when starting the preparation. The resulting plasmid DNA from overgrown cultures may be partially degraded or contaminated with chromosomal DNA.

4.2 Cultivation of bacteria in a square-well block

Use a suitable 96-well square-well block for growing bacteria (available from MN, see ordering information). Add 1.2-1.5 ml of selected medium (with appropriate antibiotic, e.g. 100 µg/ml ampicillin) to each well of the square-well block. To avoid cross-contamination due to spillage during incubation, do not exceed a total culture volume of 1.5 ml. Inoculate each well with a single bacterial colony. Cover the square-well block with a suitable gas-permeable (see ordering information). Grow the culture in a suitable incubator at 37°C for 16-24 h with vigorous shaking (200-400 rpm). The square-well block may be fixed to the shaker with large-size flask clamps (for 2-l flasks) or tape.

Note:

The yield of plasmid DNA depends on growth conditions, bacterial strain, and cell density of the culture as well as on the size and copy number of the vector. Use of high-copy number plasmids such as pUC, pBluescript or pGEM, and *E. coli* strains like DH5α or XL1 Blue are recommended. Growth times of 16-24 h are usually sufficient. However, for poor growing bacteria, prolonged incubation times of up to 30 h may be required.

4.3 Cultivation of bacteria in tubes

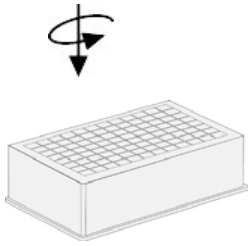
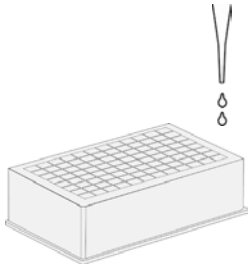
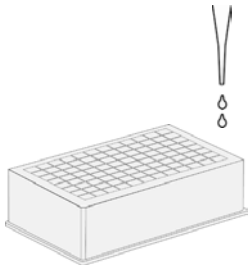
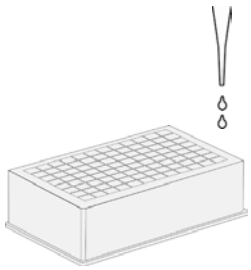
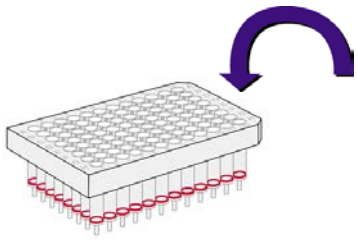
Use 1-5 ml of appropriate culture medium. Depending on the bacterial strain and copy number of the plasmid up to 5 ml LB medium or 3 ml 2 x YT or 3 ml TB medium can be used. Grow bacteria with vigorous shaking for 10-14 h.

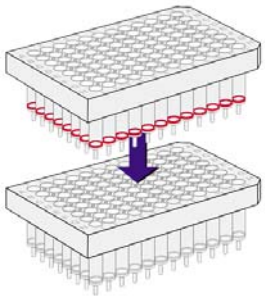
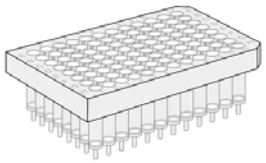
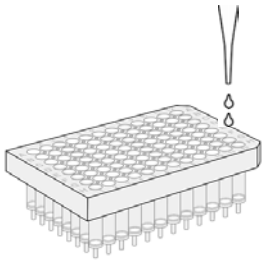
Optional:

If the robot workstation does not allow the use of selected culture tubes, transfer bacterial culture from the tubes into a suitable square-well block (available from MN, see ordering information). For this, transfer 1.5 ml of the culture to each well of the square-well block. Harvest the cultures by centrifugation. Discard supernatant. Usually 1.5 ml of culture are sufficient for DNA preparation. However, if necessary, add an additional 1.0–1.5 ml of the bacterial culture to each well of the square-well block, centrifuge again, and discard the supernatant.

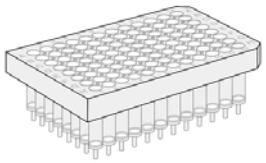
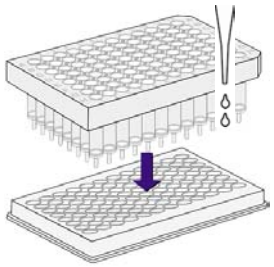
Do not use more than 5 ml LB culture or 3 ml rapid growing bacterial strain (using 2 x YT or TB medium) because lysis efficiency might be lower when using cell pellets which are too large.

5 General Procedure

1	**Cultivate** and **harvest** bacterial cells	**LB** **2 x YT** **TB** **10 min** **1,000 x g**	
2	**Resuspend** bacterial cells	**250 µl A1** **mix or shake**	
3	**Lyse** bacterial cells	**250 µl A2** **RT** **2-5 min** **(optional: shake)**	
4	**Neutralize**	**350 µl A3** **(optional: mix or shake)**	
5	**Transfer** of crude lysates to NucleoSpin® Plasmid Filter Plate (purple)		

6	**Clear crude lysates by vacuum filtration** directly into the NucleoSpin® Plasmid Binding Plate (transparent) *optional: incubate 1 to 3 min before applying vacuum*	**ca. - 0.2 – -0.4 bar * (1 min to 5 min)**	
7	**Reassemble** vacuum manifold Discard the NucleoSpin® Plasmid Filter Plate		
8	**Bind** DNA to silica membrane of the NucleoSpin® Plasmid Binding Plate by applying vacuum	**ca. – 0.4 bar*** **(1 min)**	
9	**Wash** silica membrane	**2 x 900 µl A4** **ca. – 0.4 bar * (1 min)**	
10	**Remove** MN Wash plate		

* reduction of atmospheric pressure

11	**Dry** NucleoSpin® Plasmid Binding Plate by applying vacuum *optional: dry the outlets of the NucleoSpin® Plasmid Binding Plate by placing it on a sheet of filter paper before applying vacuum*	**10 min – 15 min, maximum vacuum***	
12	**Insert** elution pale U-bottom (e.g. available from MN)		
13	**Elute** highly pure plasmid DNA *optional: incubate 1 - 3 min*	**75-150 µl AE** **ca. – 0.4 bar * (1 min)**	

* reduction of atmospheric pressure

5.1 Standard protocol for automated purification of high-copy plasmid DNA using common laboratory automation workstations

Note:

The list numbers in this protocol do not correspond with the list numbers in section 5 “General procedure”.

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1 Centrifuge square-well block containing the bacterial culture for 10 min at 1,000 x *g*.

It is strictly recommended to centrifuge the bacterial culture under these conditions. Centrifugation at higher *g*-forces may produce tight pellets which are more difficult to resuspend.

Optional:
If centrifugation at higher g-forces is used, a shaker integrated on the robot worktable will be necessary for complete resuspension of the bacterial pellet after addition of buffer A1.

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2 Discard supernatant. Remove residual medium by placing the square-well block upside down on clean paper sheet or soft tissue.

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3 Place square-well culture block on a suitable vortexer to facilitate the complete resuspension of bacterial pellets with buffer A1.

Place square-well block in the desired position of the robot worktable.

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4 Prepare buffer A1 by adding RNase A. Prepare buffer A4 by adding ethanol (see section 3 for details).

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5 Add buffers to the reservoirs or place the buffer bottles in the corresponding positions of the robot worktable. Place the plastic equipment like plates and the assembled vacuum manifold in the locations as specified in the individual robotic programs.

Optional:
The elution buffer AE (5 mM Tris/HCl, pH 8.5) may be substituted by nuclease-free water (check pH is 8.0–8.5 before use). This is recommended if the eluted DNA has to be concentrated for downstream applications or Tris salts interfere with downstream applications. A concentration of Tris higher than 10 mM can interfere with common sequencing chemistries.

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6 Select method or program for plasmid DNA purification.

Optional:
After transfer to the NucleoSpin® Plasmid Filter Plate, incubate crude lysates for 1-3 min. This incubation allows the formation of a compact white precipitate. This step is usually not required for culture volumes up to 1.5 ml.

Optional:
A washing step with buffer AW is recommended when using end(-) host strains. Buffer AW is not included in the NucleoSpin® Robot-96 Plasmid Core kit. See ordering information.

7 Completely dry the NucleoSpin® Plasmid plate by applying maximum vacuum.

IMPORTANT:
This step removes residual washing buffer A4 from the NucleoSpin® Plasmid Binding Plate. The removal is only effective when maximum vacuum is used, allowing maximum airflow to go through the wells. Residual ethanol from buffer A4 may inhibit subsequent enzymatic reactions.

8 Elution of purified plasmid DNA.
Dispense between a minimum of 75 µl and a maximum of 150 µl (see section 2.4) of elution buffer AE. Lower volumes of elution buffer will cause inhomogeneous results. For increased DNA concentration, a minimum elution volume of 75 µl can be used. By applying higher volumes of dispensed elution buffer, the concentration of resulting eluted DNA will decrease, but the efficiency of elution will increase (cp. Fig 1).

5.2 Support protocol - Elution of DNA using a centrifuge

Optional step:

Elution of purified DNA in a centrifuge may be necessary when higher concentrations of the final DNA are required for downstream applications. Using a centrifuge allows reduction of the dispensed volume to 50-75 µl.

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1 Stop the method after the final washing step with buffer A4. Remove NucleoSpin® Plasmid Binding Plate from the manifold´s top and tap on a sheet of filter paper to remove residual wash buffer from the outlets.

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2 Cover NucleoSpin® Plasmid Binding Plate with self-adhering PE foil. Place the plate on top of a square-well block or round-well block (see ordering information) and **centrifuge for 10 min at maximum speed (> 4,000 x *g*, optimal 5,800 x *g*).**

Note:

We recommend to use a centrifuge (e.g. Hermle/MACHEREY-NAGEL: NucleoSwing Z513, Qiagen/Sigma 4-15, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™ rotor) with a swing-out rotor which is capable of accommodating the NucleoSpin® Plasmid Binding Plate/square-well block sandwich (bucket hight: 85 mm). Do not use a microtiter plate as a support for the NucleoSpin® Plasmid Binding Plate. Microtiter plates may crack when centrifuging at > 2,500 x g.

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3 Insert the NucleoSpin® Plasmid Binding Plate to a new square-well or round-well block. Remove the self-adhering PE foil and dispense **elution buffer (50-75 µl)** directly onto the silica membrane. Incubate for **1-3 min at room temperature.**

Note:

Alternatively, a 96-well thermocycler plate can be inserted into the square-well block.

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4 Centrifuge for **2 min at maximum speed (> 4,000 x *g*, optimal 5,800 x *g*)** to collect the DNA.

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6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	*Cell pellet not properly resuspended* • It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of lysis buffer A2. If necessary, increase number of mixing cycles or duration of shaking. *SDS in buffer A2 precipitated* • SDS in buffer A2 may precipitate upon storage. If this happens, incubate A2 at 30–40°C for 5 min and mix well before use *Too many bacterial cells used* • Usage of LB as the growth medium is recommended. When using rich media like TB, cultures reach very high cell densities. Reduce culture volume to 1.0-1.5 ml.
Poor plasmid yield	*No or not enough antibiotic used during cultivation* • Cells harbouring the plasmid of interest may become overgrown by nontransformed cells. Add appropriate amounts of freshly prepared stock solutions to all media, solid and liquid. *Bacterial cultures are too old* • See suggestions in section 4 'Growing of bacterial cultures'. *High-copy number plasmid was not used* • Use high-copy number plasmid. *Incomplete lysis of bacterial cells* • See 'Possible cause and suggestions' above. *No ethanol added to buffer A4 concentrate, ethanol evaporated* • Add indicated volume of ethanol to buffer A4 concentrate and mix. Keep bottle tighty closed to prevent evaporation of ethanol. Replace buffer A4 in open trough reservoirs.

Problem	Possible cause and suggestions
Poor plasmid yield (continued)	*Elution conditions are not optimal* • If possible, use a slightly alkaline elution buffer like AE (5 mM Tris-HCl, pH 8.5). When using nuclease-free water for elution, make sure the pH value is within the range of pH 8.0–8.5. Elution efficiencies drop drastically with buffers < pH 7.
High level contamination with chromosomal DNA	*Excessive mixing steps after addition of lysis buffers A2 and A3, or before transfer of crude lysate to the NucleoSpin® Plasmid Filter Plate. Mixing will cause shearing of chromosomal DNA, leading to a co-purification during the preparation of plasmid DNA.* • Reduce number of mixing cycles, reduce shaker action. *Culture volume was too high* • Reduce culture volume if lysate is too viscous for gentle and complete mixing. *Bacterial culture overgrown* • Overgrown bacterial cultures contain lysed cells and degraded DNA. See suggestions in section 4 'Growing of bacterial cultures'. *Lysis was too long* • Lysis step must not exceed 5 min. *Tips* • Use widebore tips or disposable tips for transfer of crude lysate to the NucleoSpin® Plasmid Filter Plate to prevent shearing of the chromosomal DNA**.**
RNA in the eluate	*RNA was not degraded completely* • Ensure that RNase A is added to buffer A1 before use. • Reduce culture volume if necessary.

Problem	Possible cause and suggestions
Suboptimal performance of plasmid DNA in sequencing reactions, problems with downstream applications	*Carryover of ethanol* • Be sure to remove all of ethanolic buffer A4 after the final washing step. Dry the NucleoSpin® Plasmid Binding Plate for at least 10 min with maximum vacuum. *Elution of plasmid DNA with TE buffer* • EDTA may inhibit enzymatic reactions like DNA sequencing. Repurify the plasmid DNA and elute with AE buffer or nuclease-free water. Alternatively, the plasmid DNA may be precipitated with ethanol, and redissolved in AE buffer or nuclease-free water. E. coli *strains with high endogenous-nuclease levels are used as host* • Perform the washing step with buffer AW (not supplied) before washing with ethanolic buffer A4. *Not enough DNA used for sequencing reactions* • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions. *Contamination of final plasmid preparation with ethanol* • Insufficient drying after final washing step with buffer A4. Remaining ethanol may cause problems with downstream applications like DNA sequencing or loading of samples onto agarose gel.
Vacuum manifold	*NucleoSpin® Plasmid Filter Plate sticks to manifold* • Clean gasket. Remove any residual salt. Clean manifold top with water and ethanol. Do not use grease.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Robot-96 Plasmid Core Kit	740616.24	24 x 96 preps
NucleoSpin® Robot-96 Plasmid	740708.2	2 x 96 preps
NucleoSpin® Robot-96 Plasmid	740708.4	4 x 96 preps
NucleoSpin® Robot-96 Plasmid	740708.24	24 x 96 preps
Resuspension buffer A1 (without RNase A)	740911.1	1 l
Lysis buffer A2	740912.1	1 l
Neutralisation buffer A3	740913.1	1 l
Wash buffer A4 concentrate (for 1 l buffer)	740914.1	200 ml
Wash buffer AW	740916.1	1 l
Elution buffer AE	740917.1	1 l
RNase A (lyophilzed)	740 505	100 mg
RNase A (lyophilized)	740 505.50	50 mg
Square-well block	740670	20
Gas permeable foil	740674	50
Self adhering PE foil	740676	50
MN Frame	740680	1

6.3 References

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

Vogelstein, B. & Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA **76**, 615-619.

6.4 Product Use Restriction / Warranty

NucleoSpin® Robot-96 Plasmid Core kits components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user to verify the use of the **NucleoSpin® Robot-96 Plasmid Core** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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