



Plasmid DNA Purification

User manual

NucleoSpin® Multi-96 Plus Plasmid

August 2002/Rev. 01

MACHEREY-NAGEL



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1 Kit contents

Cat. No.	NucleoSpin® Multi-96 Plus Plasmid		
	1 x 96 preps 740 625.1	4 x 96 preps 740 625.4	24 x 96 preps ¹ 740 625.24
Buffer A1 ²	35 ml	150 ml	6 x 150 ml
Buffer A2	35 ml	150 ml	6 x 150 ml
Buffer A3	2 x 35 ml	200 ml	6 x 200 ml
Buffer AW	75 ml	240 ml	6 x 240 ml
Buffer A4 (concentrate) ²	3 x 20 ml	200 ml	6 x 200 ml
Buffer AE	25 ml	75 ml	6 x 75 ml
RNase A (lyophilized) ²	15 mg	60 mg	6 x 60 mg
NucleoSpin® Plasmid Binding Plate (transparent)	1	4	24
NucleoSpin® Plasmid Filter Plate (purple)	1	4	24
Square-well block ³	1	4	24
Gas-permeable foil	1	4	24
Self-adhering PE foil	5	20	120
MN Wash plate (including six paper sheets)	1	4	24
Elution plate U-bottom (including one self- adhering PE foil)	1	4	24
Protocol	1	1	6 x 1

¹ The kit for 24 x 96 preparations (Cat.No. 740 625.24) consists of 6 x Cat. No. 740 625.4

² For preparation of working solutions and storage conditions see section 3.

³ Every square-well block (culture plate) includes a gas permeable foil.

2 Product description

2.1 The basic principle

The **NucleoSpin® Multi-96 Plus 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 used directly for further applications.

2.2 Kit specifications

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

If using less than 96 samples the rubber pad or self-adhering PE foil (see ordering information) should be used in order to cover up non used wells to guarantee a proper vacuum.

- The kit is for use with the NucleoVac 96 vacuum manifold (see ordering information) or similar suitable vacuum manifolds (see section 2.3).
- This kit provides reagents and consumables for purification of up to 15-20 µg of highly pure plasmid DNA per well suitable for direct use in standard molecular biology applications like automated fluorescent sequencing, PCR, or restriction analysis.
- Using **NucleoSpin® Multi-96 Plus Plasmid** allows simultaneous processing of up to 96 samples typically within less than 45 minutes.
- 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 kb), 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.

Table 1: Kit specifications at a glance	
NucleoSpin® Multi-96 Plus Plasmid	
Culture volume	1.5 – 5 ml
Average yield	5-15 µg
Elution volume	75-150 µl
Binding capacity	20 µg
Vectors	< 15 kb
Time/prep	45 min/1 x 96

2.3 Suitability for other common vacuum manifolds

The **NucleoSpin® Multi-96 Plus Plasmid** kit can be used with other common vacuum manifolds. For further details see list below.

Vacuum manifold	Suitability	Additional equipment
Qiagen/QIAvac 96 ¹	yes	MN Frame (see ordering information)
BioRad/Aurum vacuum manifold	no	
Eppendorf/Perfect VAC Manifold	no	
Millipore/MultiScreen	no	

¹ In general the QIAvac 96 is suitable for the use with NucleoSpin® Plasmid Binding Plates. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN wash plate and Elution plate U-bottom in order to ensure best performance.

2.4 Elution procedure

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

Dispensed elution buffer	75 μ l	100 μ l	125 μ l	150 μ l	175 μ l
Recovered elution buffer containing plasmid DNA	30 \pm 5 μ l	55 \pm 5 μ l	80 \pm 5 μ l	105 \pm 5 μ l	130 \pm 5 μ l

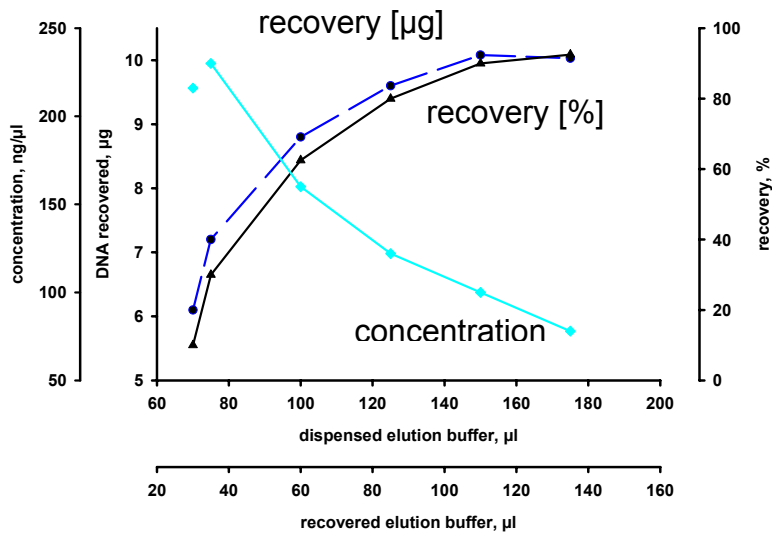


Fig. 1: Recovery rate and concentration depend on elution volume. 10 μ g of pBluescript were purified with **NucleoSpin[®] Robot-96 Plasmid*** and eluted with the indicated elution buffer volumes. High recovery is achieved with 120 μ l elution buffer (dispensed), as concentration drops.

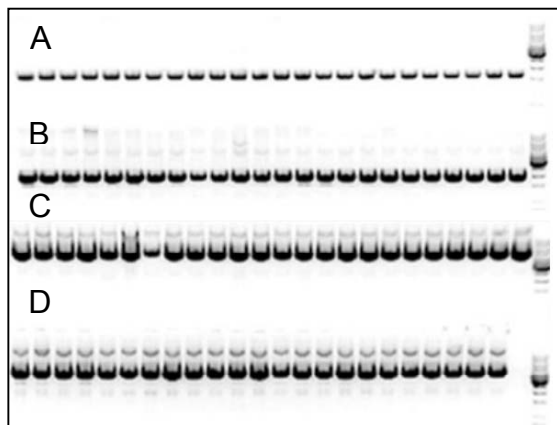


Fig. 2: Purity and yields of pBluescript KS+ (2.96 kbp, **A**), pUC 18 derivate (3.65 kbp, **B**), pcDNA3.1 (8.6 kbp, **C**), and pCMV β (7.2 kbp, **D**) using **NucleoSpin[®] Robot-96 Plasmid***. 15 μ l out of 125 μ l eluate were analyzed on a 1% agarose gel.

* NucleoSpin[®] Robot-96 Plasmid and NucleoSpin Multi-96 Plus Plasmid are absolutely identical in performance.

3 Storage conditions, preparation of working solutions, and setup of vacuum source

Attention:

Buffers A3 and AW contain guanidinium hydrochloride! Wear gloves and goggles!

- All kit components of the **NucleoSpin® Multi-96 Plus Plasmid** kit can be stored at room temperature (20-25°C) and are stable up to one year.
- Always keep buffer bottles tightly closed, especially if buffers are prewarmed during the preparation
- Sodium dodecyl sulfate (SDS) in buffer A2 may precipitate if stored at temperatures below 20°C. If a precipitate is observed in buffer A2, incubate the bottle at 30-40°C for some minutes and mix well.

Before starting any **NucleoSpin® Multi-96 Plus Plasmid** protocol prepare the following:

- Before first use of the kit, add RNase A to buffer A1: Add 1 ml of buffer A1 to the RNase A vial, vortex, transfer redissolved RNase A to the A1 bottle and shake well. After addition of RNase A to buffer A1 store this buffer at 4°C.
- Add indicated volume of 96-100% ethanol to buffer A4 concentrate.

	NucleoSpin® Multi-96 Plus Plasmid		
Cat. No.	1 x 96 preps 740 625.1	4 x 96 preps 740 625.4	24 x 96 preps 740 625.24
Buffer A4 (concentrate)	3 x 20 ml add 80 ml ethanol to each bottle	200 ml add 800 ml ethanol	6 x 200 ml add 800 ml ethanol to each bottle

Establish a reliable vacuum source for the NucleoVac 96 vacuum manifold. The manifold may be used with vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of 200-400 mbar (pressure difference). The use of the NucleoVac Vacuum Regulator (Cat. No. 740 641) is recommended. Alternatively, adjust vacuum that during the purification the sample flows through the column with a rate of 1-2 drops per second.

4 Growing of bacteria cultures

4.1 General considerations

Yield and quality of plasmid DNA depends on e.g. the type of growing media and antibiotics, the bacterial host, plasmid type, size, or copy number. Therefore, these factors should be taken into consideration. As growing medium we recommend LB medium. Overnight-cultures in flasks reach under vigorous shaking usually an OD₆₀₀ of 3-6. If too much bacterial material is used, lysis and precipitation steps are inefficient and finally **NucleoSpin® Multi-96 Plus Plasmid columns** are overloaded causing decreased yield and plasmid quality.

4.2 Selection of culture media

The cultivation of cells is recommended at 37°C in LB (Luria-Bertani) medium at constant shaking (200-250 rpm). Alternatively, rich media like 2xYT 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 (≤ 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.

4.3 Cultivation of bacteria in a square-well block

Autoclave the square-well block supplied with the kit or use a sterile plate. 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 the gas-permeable foil supplied with the culture plate. 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 DH5 α or XL1 Blue is recommended. Growth times of 16-24 h are usually sufficient. However, for poorly growing bacteria, prolonged incubation times of up to 30 h may be required.

4.4 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:

Transfer bacterial cultures from the tubes to the square-well block supplied with the kit. 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.

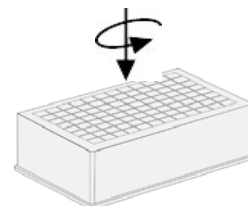
Do not use more than 5 ml LB culture or 3 ml rapid growing bacterial strain (using 2 x YT or TB medium) because lysis efficiency might be lower when using cell pellets which are too large.

5 General Procedure

1 Cultivate and harvest
bacterial cells

LB
2xYT
TB

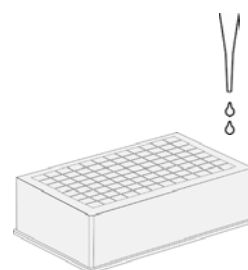
10 min
1,000 x g



2 Resuspend bacterial cells

250 µl A1

mix or shake

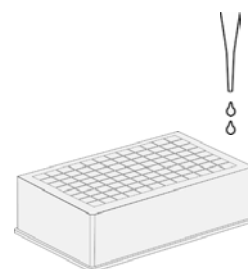


3 Lyse bacterial cells

250 µl A2

RT
2-5 min

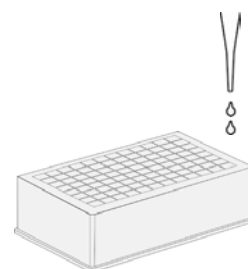
(optional: shake)



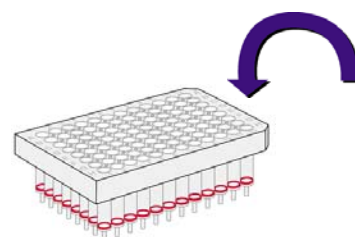
4 Neutralize

350 µl A3

(optional: mix or
shake)



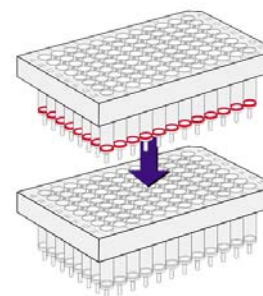
5 Transfer crude lysates onto
NucleoSpin® Plasmid Filter
Plate (purple)



6 Clear crude lysates by vacuum filtration directly into the NucleoSpin® Plasmid Binding Plate (transparent)

**ca. – 0.2 to – 0.4 bar*
(1 min to 5 min)**

optional: incubate 1 to 3 min before applying vacuum

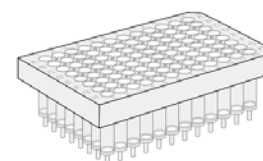


7 Reassemble vacuum manifold

(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)**

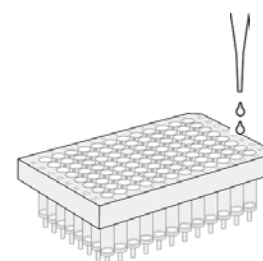


9 Wash silica membrane

600 µl AW

2 x 900 µl A4

**ca. – 0.4 bar*
(1 min)**



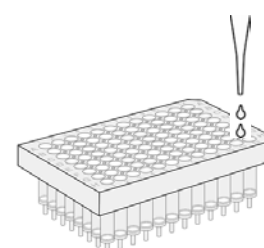
* reduction of atmospheric pressure

10 Remove MN wash plate

11 Dry NucleoSpin® Plasmid Binding Plate applying vacuum

**maximum vacuum
10 min – 15 min**

optional: dry the outlets of the NucleoSpin® Plasmid Binding Plate by placing it on a sheet of filter paper.

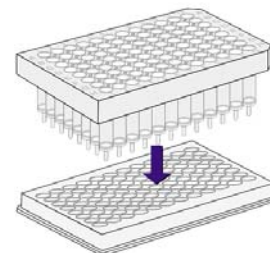


12 Insert collection plate
(microtiter plate, u-bottom)

13 Elute highly pure plasmid
DNA

75-150 µl AE

ca. – 0.4 bar*
(1 min)



5.1 Standard protocol for purification of high-copy plasmid DNA

Visit MN on the internet at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support. All MN protocols can be downloaded from our website.

1 Cultivate and harvest bacterial cells

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

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

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clean paper sheet (supplied) or soft tissue.

Optional:

Transfer bacteria cultures grown in tubes to square-well block as described in section 4. Alternatively perform the next three steps in the tubes.

2 Resuspend bacterial cells

Resuspend the bacterial pellet in 250 µl of buffer A1 by vortexing the sealed plate.

Alternatively resuspend bacterial pellet by pipetting up and down. Resuspend bacterial cells completely. No clumps should be visible.

3 Lyse bacterial cells

Add 250 µl of buffer A2 to the suspension.

Mix the suspension gently and immediately by inverting the sealed plate 6-8 times. Incubate at room temperature for a maximum of 5 min.

Note:

Do not vortex; doing so will release contaminating chromosomal DNA from the cellular debris into the suspension. Do not allow the lysis reaction to proceed for more than 5 minutes.

4 Neutralize

Add 350 µl of buffer A3 to the suspension.

Mix the suspension gently and immediately by inverting the sealed plate 6-8 times.

Optional:

Incubate on ice for 5 min for optimal forming of precipitate.

Prepare the NucleoVac 96 vacuum manifold:

Prepare manifold for filtration of crude lysates:

Insert spacers *MTP/Multi-96 Plate*, notched side up, into the grooves located on the short sides of the manifold. Insert waste container into manifold base. Place the NucleoSpin® Plasmid Binding Plate (transparent) into the manifold base. Insert the NucleoSpin® Plasmid Filter Plate (purple) into the lid and place lid onto the manifold.

5 Transfer crude lysates onto the NucleoSpin® Plasmid Filter Plate (purple).

Apply vacuum by opening the valve and press down NucleoSpin® Plasmid Filter Plate slightly until flow through starts. Adjust vacuum as necessary to establish a flow rate of 1-2 drops per second.

6 Clear crude lysate by vacuum filtration

When the crude lysate has passed the NucleoSpin® Plasmid Filter Plate, close the valve and ventilate the manifold. Remove and discard NucleoSpin® Plasmid Filter Plate. Open the manifold lid. Remove the NucleoSpin® Plasmid Binding Plate (white) with cleared lysates and insert it into the manifold lid.

7 Reassemble vacuum manifold:

Rest the MN Wash plate on the spacers (with the sealed foil on its top side) inside the manifold base. Take the manifold lid with inserted NucleoSpin® Plasmid Binding Plate and close the manifold base.

8 Bind DNA to silica membrane

Apply vacuum by opening the valve and press down the NucleoSpin® Plasmid Binding Plate slightly until flow through starts. Adjust vacuum as necessary to establish a flow rate of 1-2 drops per second.

When the cleared lysate has drained off, ventilate the vacuum manifold by closing the valve.

9 Wash silica membrane

Optional wash step:

Add 600 µl of buffer AW to each column of the NucleoSpin® Plasmid Binding Plate. Apply vacuum by opening the valve. Press down the NucleoSpin® Plasmid Binding Plate slightly. Allow the buffer to pass the columns.

Note:

Perform this additional wash step if your bacterial host strain is known to have high endogenous nuclease activity (e.g. E. coli HB 101, BMH 71-18 mutS, JM, or any wild-type strains) or if you intend to improve the sequencing results.

Ventilate the vacuum manifold by closing the valve. Add 900 µl of buffer A4 (with ethanol) to each column of the NucleoSpin® Plasmid Binding Plate. Apply vacuum and allow the buffer to pass the columns. Ventilate the manifold.

Repeat this washing-step once.

10 Remove MN Wash plate

After the final washing step close the valve, ventilate the vacuum manifold, and remove the NucleoSpin® Plasmid Binding Plate.

11 Dry NucleoSpin® Plasmid Binding Plate

Remove any residual washing buffer from the NucleoSpin® Plasmid Binding Plate. If necessary, tap the outlets of the plate onto a clean blotting paper (supplied with the kit) or soft tissue until no drops come out. Remove manifold lid, MN wash plate, and waste container from the vacuum manifold. Insert the NucleoSpin® Plasmid Binding Plate into the lid and close the manifold. Apply vacuum of 300-400 mbar for at least 10–15 min to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note:

The ethanol in buffer A4 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, close the valve and ventilate the vacuum manifold.

12 Insert Elution plate U-bottom

Rest the Elution plate U-bottom on the spacers inside the manifold base. For elution into microtiter plates spacers *MTP/Multi-96 Plate* are required which are already inserted into the manifold base from the previous steps. Reassemble the vacuum manifold as described before.

or

Elution into MN tube strips (not provided with the kit, see ordering information):

Insert spacers *Microtube rack*, notched side up, into the grooves located at the short sides of the vacuum manifold. Rest the microtube rack with the MN tube strips on the spacers inside the manifold base and reinstall the vacuum manifold as described before.

13 Elute highly pure plasmid DNA

Elute the DNA by adding 75 - 150 µl of Buffer AE (5 mM Tris-HCl, pH 8.5) or sterile dist. water (pH 7.5 - 8.5) to each column of the NucleoSpin® Plasmid Binding Plate.

The elution buffer should be dispensed carefully onto the center of the silica membrane. Incubate the buffer on the membrane for 1-3 minutes at room temperature. Apply vacuum by opening the valve. Press down the NucleoSpin® Plasmid Binding Plate slightly and collect the eluted DNA. After the elution buffer has passed the columns, close the valve and ventilate the vacuum manifold.

Remove the Elution plate U-bottom or MN tube strips containing eluted DNA and seal them with the adhesive cover foil (supplied with the Elution plate U-bottom) for further storage.

5.2 Support protocol - Elution of DNA using a centrifuge

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 giving a concentration of about 70 to 250 ng/µl (depending on elution buffer volume and the copy number of the plasmid).

- 1 Stop procedure after the final washing step with buffer A4: Take NucleoSpin® Plasmid Binding Plate from the manifold's top and tap it on a sheet of filter paper to remove residual wash buffer from the outlets.
-

- 2 Cover the 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)** to dry the membrane and the outlets of the binding plate.

Note:

We recommend to use a centrifuge (e.g. Hermle/MACHEREY-NAGEL: NucleoSwing Z513, Qiagen/Sigma 4-15c, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate™ rotor, Beckman Coulter, Allegra 25R) with a swing-out rotor which is capable of accommodating the NucleoSpin® Plasmid Plate/square-well block sandwich (bucket height: 85 mm). Do not use a microtiter plate as a support for the NucleoSpin® Plasmid Plate. Microtiter plates may crack when centrifuging at > 2,500 x g.

- 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 AE (50-75 µl)** directly onto the silica membrane. Incubate for **1-3 min**.

Note:

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

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

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
	<p><i>Cell pellet not properly resuspended</i></p> <ul style="list-style-type: none"> 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.
Incomplete lysis of bacterial cells	<p><i>SDS in buffer A2 precipitated</i></p> <ul style="list-style-type: none"> 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. <p><i>Too many bacterial cells used</i></p> <ul style="list-style-type: none"> 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.
	<p><i>No or not enough antibiotic used during cultivation</i></p> <ul style="list-style-type: none"> Cells harbouring the plasmid of interest may become overgrown by non-transformed cells. Add appropriate amounts of freshly prepared stock solutions to all media, solid and liquid. <p><i>Bacterial cultures are too old</i></p> <ul style="list-style-type: none"> See suggestions in section 4 'Growing of bacterial cultures'.
Poor plasmid yield	<p><i>High-copy number plasmid was not used</i></p> <ul style="list-style-type: none"> Use high-copy number plasmid. <p><i>Incomplete lysis of bacterial cells</i></p> <ul style="list-style-type: none"> See 'Possible cause and suggestions' above. <p><i>No ethanol added to buffer A4 concentrate, ethanol evaporated</i></p> <ul style="list-style-type: none"> Add indicated volume of ethanol to buffer A4 concentrate and mix. Keep bottle tightly closed to prevent evaporation of ethanol. Replace buffer A4 in open trough reservoirs.

Problem	Possible cause and suggestions
Poor plasmid yield (continued)	<p><i>Elution conditions are not optimal</i></p> <ul style="list-style-type: none">• 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	<p><i>Excessive mixing steps after addition of lysis buffers A2 and A3, or before transfer of crude lysate to the NucleoSpin® Plasmid Binding Plate. Excessive mixing will cause shearing of the chromosomal DNA, leading to co-purification during the preparation of plasmid DNA.</i></p> <ul style="list-style-type: none">• Mix by gentle inversion of the sealed culture block. <p><i>Culture volume was too high</i></p> <ul style="list-style-type: none">• Reduce culture volume if lysate is too viscous for gentle and complete mixing. <p><i>Bacterial culture overgrown</i></p> <ul style="list-style-type: none">• Overgrown bacterial cultures contain lysed cells and degraded DNA. See suggestions in section 4 'Growing of bacterial cultures'. <p><i>Lysis was too long</i></p> <ul style="list-style-type: none">• Lysis step must not exceed 5 min.
RNA in the eluate	<p><i>RNA was not degraded completely</i></p> <ul style="list-style-type: none">• 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 the ethanolic buffer A4 after the final washing step. Dry the NucleoSpin® Plasmid 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 buffer AE 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 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 gels.
-

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Multi-96 Plus Plasmid	740 625.1	For 1 x 96 preps
NucleoSpin® Multi-96 Plus Plasmid	740 625.4	For 4 x 96 preps
NucleoSpin® Multi-96 Plus Plasmid	740 625.24	For 24 x 96 preps
Resuspension buffer A1 (without RNase A)	740 911.1	1 l
Lysis buffer A2	740 912.1	1 l
Neutralisation buffer A3	740 913.1	1 l
Wash buffer A4 (concentrate for 1 l buffer)	740 914.1	200 ml
Wash buffer AW	740 916.1	1 l
Elution buffer AE	740 917.1	1 l
RNase A (lyophilized)	740 505	100 mg
RNase A (lyophilized)	740 505.50	50 mg
Square-well block	740 670	20
MN tube strips	740 637	5 sets
Gas-permeable foil	740 675	50
Self-adhering PE foil	740 676	50
NucleoVac 96 vacuum manifold	740 681	1
NucleoVac Vacuum Regulator	740 641	1
MN Frame	740680	1
NucleoSwing Z 513	740 610	1
NucleoSwing Z 513 K (refrigerated)	740 610 K	1

6.3 References

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

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6.4 Product Use Restriction / Warranty

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