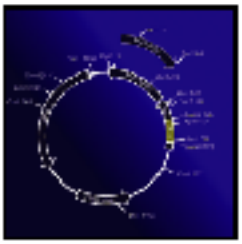


Plasmid DNA Purification

User manual

NucleoSpin® Plasmid

NucleoSpin® Plasmid QuickPure

July 2003/Rev. 01

MACHEREY-NAGEL

Protocol at a glance (Rev. 01)

Plasmid DNA Purification

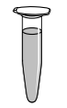
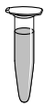
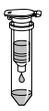
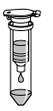
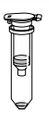
		Mini NucleoSpin® Plasmid	Mini NucleoSpin® Plasmid QuickPure
1	Cultivate and harvest bacteria cells	  30 s 11,000 x g	  30 s 11,000 x g
2	Cell Lysis	 Buffer A1 250 µl Buffer A2 250 µl Buffer A3 300 µl	 Buffer A1 250 µl Buffer A2 250 µl Buffer A3 300 µl
3	Clarification of lysate	  5 - 10 min 11,000 x g	  5 min 11,000 x g
4	Bind DNA	 load supernatant  1 min 11,000 x g	 load supernatant  1 min 11,000 x g
5	Wash silica membrane	 Buffer A4 600 µl  1 min 11,000 x g	 Buffer AQ 450 µl  4 min 11,000 x g
6	Dry silica membrane	  2 min 11,000 x g	Drying is performed during centrifugation of the single washing step
9	Elute highly pure RNA	 50 µl buffer AE RT 1 min  1 min 11,000 x g	 50 µl buffer AE RT 1 min  1 min 11,000 x g

Table of contents

1 Kit contents

	NucleoSpin® Plasmid		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740588.10**	**740588.50**	**740588.250**
Buffer A1	5 ml	15 ml	75 ml
Buffer A2	5 ml	15 ml	3 x 25 ml
Buffer A3	5 ml	20 ml	100 ml
Buffer AW	6 ml	30 ml	2 x 75 ml
Buffer A4* (concentrate)	2 ml	2 x 7 ml	2 x 20 ml
Buffer AE	5 ml	15 ml	75 ml
RNase A* (lyophilized)	2 mg	6 mg	30 mg
NucleoSpin® Plasmid columns	10	50	250
NucleoSpin® collection tubes (2 ml)	10	50	250
Protocol	1	1	1

* For preparation of solutions and storage conditions see section 3.

1 Kit contents *continued*

	NucleoSpin® Plasmid QuickPure		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740615.10**	**740615.50**	**740615.250**
Buffer A1	5 ml	15 ml	75 ml
Buffer A2	5 ml	15 ml	3 x 25 ml
Buffer A3	5 ml	20 ml	100 ml
Buffer AQ* (concentrate)	2 ml	7 ml	2 x 20 ml
Buffer AE	5 ml	15 ml	75 ml
RNase A* (lyophilized)	2 mg	6 mg	30 mg
NucleoSpin® Plasmid QuickPure columns	10	50	250
NucleoSpin® collection tubes (2 ml)	10	50	250
Protocol	1	1	1

* For preparation of solutions and storage conditions see section 3.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Plasmid** method, the pelleted bacteria are resuspended (buffer A1) and plasmid DNA is liberated from the *E. coli* host cells by SDS/alkaline lysis (buffer A2). Buffer A3 neutralizes the resulting lysate and creates appropriate conditions for binding of plasmid DNA to the silica membrane of the **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure column**. SDS precipitate and cell debris are then pelleted by a centrifugation step, the supernatant is loaded onto a **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure column**.

With the **NucleoSpin® Plasmid kit** contaminations like salts, metabolites, and soluble macromolecular cellular components are removed by simple washing with ethanolic buffer A4. Pure plasmid DNA is finally eluted under low ionic strength conditions with slightly alkaline buffer AE (5 mM Tris-Cl, pH 8.5). If host strains with high levels of nucleases are used, an additional washing step with prewarmed buffer AW is recommended. Additional washing with AW will also increase the reading length of automated fluorescent DNA sequencing reactions.

With the **NucleoSpin® Plasmid QuickPure kit**, the supernatant is loaded onto a **NucleoSpin® Plasmid QuickPure column**, contaminations like salts, metabolites, nucleases and soluble macromolecular cellular components are removed by only a single washing step with buffer AQ. Pure plasmid DNA is finally eluted under low ionic strength conditions with slightly alkaline buffer AE (5 mM Tris-Cl, pH 8.5).

2.2 About this User Manual

Experienced users who are performing the isolation of plasmid DNA using a **NucleoSpin® Plasmid** isolation kit may refer to the Protocol-at-a-glance instead of this User Manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this User Manual.

2.3 Kit specifications

- The **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure** kits are designed for the rapid, small-scale preparation of highly pure plasmid DNA (mini preps). Although the kits can be used for isolation of any plasmid, the size range for most effective purification is < 15 kb with the standard protocols.

- The prepared plasmid DNA is suitable for applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

- The **NucleoSpin® Plasmid** kit includes an additional washing buffer AW which is strongly recommended for the complete removal of high levels of endonucleases (also see section 5.1, step 5)

  For purification of low-copy plasmids like P1 constructs or cosmids with **NucleoSpin® Plasmid**, special instructions are given in section 2.4.

  Due to higher binding capacity of the **NucleoSpin® Plasmid** columns bacterial cultures grown in rich media (e.g. TB) as well as larger culture volumes (up to 10 ml) can be processed conveniently. Depending on culture volume and medium, centrifugation time for clarification of bacterial lysate can vary from 5 to 10 min.

- The **NucleoSpin® Plasmid QuickPure** column features a new specially treated silica membrane which allows to speed up the procedure by a combined washing/drying step. No additional steps are necessary if nuclease rich strains like HB101, ABLE and JM110 are used. The number of washing and drying steps is reduced from 3 to only 1! Therefore, the hands-on-time is less than 11 min.

Table 1: Kit specifications at a glance

parameters	**NucleoSpin® Plasmid (Mini)**	**NucleoSpin® Plasmid QuickPure (Mini)**
Culture volume	1-5 ml high copy 5-10 ml low copy	1-3 ml high copy
Elution volume	50 µl	50 µl
Binding capacity	60 µg	15 µg
Vectors	< 15 kb	< 15 kb
Time/prep*	25 min/18 preps	11 min/18 preps
Column type	mini spin column	mini spin column

* hands-on-time

2.4 Isolation of low copy-number plasmids, e.g. P1 or cosmids (refers only to NucleoSpin® Plasmid)

- Low-copy plasmids like P1 constructs, BACs (bacterial artificial chromosomes), or cosmids can be prepared with **NucleoSpin® Plasmid**. Due to the spinning forces the vector size is limited to < 15 kb.

- The use of at least 5 ml is recommended, although 10 ml of *E. coli* culture is optimal. Doubled volumes of buffers A1, A2, and A3 have to be used, and as a consequence, the cleared lysate must be loaded in successive steps onto one **NucleoSpin® Plasmid column**.

- The **NucleoSpin® Plasmid** kit does not include enough lysis buffer (A1, A2, A3 and RNase A) for the isolation of low copy plasmids. Extra buffer has to be ordered: **NucleoSpin® buffer set**, Cat. No.: 740953 (also see ordering information, section 6.2).

- Washing with buffer AW is recommended.

- To guarantee efficient elution, prewarm elution buffer AE to 70°C before adding it directly to the center of the silica membrane. Incubate the **NucleoSpin® Plasmid column** with buffer AE at room temperature (20-25°C) for 2 min before centrifugation.

2.5 Elution procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 70-90%) there are several modifications possible. Use elution buffer preheated to 70°C for the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90-100% of bound nucleic acids can be eluted.

- **High concentration:** Perform one elution step with 60% of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution (approx. 130%). Maximal yield of bound nucleic acids is about 80%.

- **High yield and high concentration**: Apply half of the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85-100% of bound nucleic acids are eluted with the standard elution volume at a high concentration.

3 Storage conditions, preparation of working solutions, and cultivation conditions

Attention:

Buffers A3 and AW contain guanidine hydrochloride! Wear gloves and goggles when using this kit!

- All kit components 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® Plasmid/NucleoSpin® Plasmid QuickPure** protocol prepare the following:

- Before first use of the kit, add 1 ml of buffer A1 to the RNase A vial, vortex, and pipette all of the resulting solution into the buffer A1 bottle. Store buffer A1 containing RNase at 4°C. The solution will be stable at this temperature up to six months.

- Add the indicated volume of 96-100% ethanol to buffer A4/buffer AQ.

	NucleoSpin® Plasmid		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740588.10**	**740588.50**	**740588.250**
Buffer A4 (concentrate)	2 ml add 8 ml ethanol	2 x 7 ml add 28 ml ethanol to each bottle	2 x 20 ml add 80 ml ethanol to each bottle
RNase A (lyophilized)	2 mg add 1 ml buffer A1	6 mg add 1ml buffer A1	30 mg add 1ml buffer A1

	NucleoSpin® Plasmid QuickPure		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740615.10**	**740615.50**	**740615.250**
Buffer AQ (concentrate)	2 ml add 8 ml ethanol	7 ml add 28 ml ethanol	2 x 20 ml add 80 ml ethanol to each bottle
RNase A (lyophilized)	2 mg add 1 ml buffer A1	6 mg add 1ml buffer A1	30 mg add 1ml buffer A1

4 Growing of bacterial cultures

4.1 General considerations

Yield and quality of plasmid DNA depend 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 OD_{600} of 3-6. If too much bacterial material is used, lysis and precipitation steps are inefficient and finally **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure 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 (Yeast/Tryptone) or TB (Terrific Broth) can be used. By using 2xYT 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.

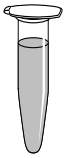
4.3 Difficult-to-lyse strains

Optimal results can be obtained with **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure** when isolating plasmid DNA from *E. coli* strains. However, for certain bacterial species it is possible to use the kit without modification (e.g. certain types of Enterobacteria). Most bacterial strains – especially Gram-positive species – require preincubation with additional lytic enzymes. Isolate plasmid DNA from **difficult-to-lyse strains** by first resuspending the pellet in buffer A1 containing an additional enzyme e.g. lysozyme (2 mg/ml final concentration). Incubate at 37°C for 30 minutes, then continue with the addition of buffer A2, and proceed with the **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure** protocol.

5 Protocols for plasmid DNA preparation

5.1 Standard protocol: Isolation of plasmid DNA from *E. coli* with NucleoSpin® Plasmid

1 Cultivate and harvest bacterial cells



Using **1–5 ml** of a saturated ***E. coli*** **LB culture,** pellet cells in a standard benchtop microcentrifuge for **30 s** at **11,000 x g.** Discard the supernatant.

Remove as much of the supernatant as possible. The binding capacity of the silica membrane for plasmid DNA (60 µg) allows processing of even larger culture volumes of up to 10 ml. However, depending on the cells used, the membrane may clog when using volumes > 5 ml.

30 s
11,000 x g

2 Cell lysis

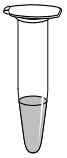
Add **250 µl buffer A1.** Resuspend the cell pellet by vigorous vortexing.

+ 250 µl A1

resuspend

No cell clumps should remain in the suspension before the addition of buffer A2.

Add **250 µl buffer A2.** Mix gently by inverting the tube **6-8 times.** Do not vortex. Incubate at **room temperature** for a maximum of **5 min.**



+ 250 µl A2

mix

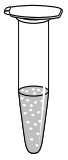
At least when using less than 3 ml of bacterial culture the cell suspension should become clear as cell lysis occurs. Do not vortex: chromosomal DNA might be released by mechanical shearing.

Add **300 µl buffer A3.** Mix gently by inverting the tube **6-8 times.** Do not vortex.

+ 300 µl A3

mix

3 Clarification of lysate

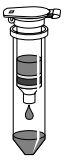


Centrifuge for **5 - 10 min** at **11,000 x g** at room temperature.

Depending on culture volume and medium, centrifugation time can vary from 5 – 10 min (see section 2.3).

5 - 10 min
11,000 x g

4 Bind DNA

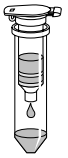


load supernatant

Place a NucleoSpin® Plasmid column in a 2 ml collecting tube and load the supernatant from step 3 onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through.

1 min 11,000 x g

5 Wash silica membrane



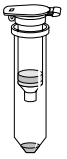
+ 600 µl A4

Place the NucleoSpin® Plasmid column back into the 2 ml collecting tube and add **600 µl buffer A4** (with ethanol, see section 3). Centrifuge for **1 min** at **11,000 x g.** Discard flow-through.

If plasmid DNA is prepared from host strains containing high levels of nucleases (e.g. HB101 or strains of the JM series), ***it is strongly recommended*** *to perform an additional washing step with* ***500 µl prewarmed buffer AW (50°C)*** *and a centrifugation step (****1 min; 11,000 x g****) before proceeding with washing buffer A4. Additional washing with buffer AW will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions.*

1 min 11,000 x g

6 Dry silica membrane

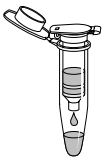


To dry the silica membrane completely, reinsert the NucleoSpin® Plasmid column into the 2 ml collecting tube. Centrifuge for **2 min** at **11,000 x g**.

2 min 11,000 x g

Residual ethanolic washing buffer, which might inhibit enzymatic reactions, is removed by this centrifugation step completely.

7 Elute highly pure DNA



+ 50 µl AE

Place the NucleoSpin® Plasmid column in a 1.5 ml microcentrifuge tube and add **50 µl buffer AE.** Incubate **1 min** at **room temperature.** Centrifuge for **1 min** at **11,000 x g.**

1 min 11,000 x g

By repeating this step, the overall yield will increase by 15-20 % (also see section 2.7). Elution can be done with TE buffer or water as well. However, we recommend the use of a weakly buffered, slightly alkaline buffer containing no EDTA, especially if the plasmid DNA is intended for sequencing reactions. If water is used, the pH should be checked and adjusted to pH 8-8.5; absorption of CO_2 leads to a decrease in pH of unbuffered solutions.

5.2 Support protocol: Isolation of M13 DNA with NucleoSpin® Plasmid

For this protocol, the additional phage lysis buffer M1 is required. Buffer M1 can be ordered separately: For ordering information please contact MACHEREY-NAGEL (see cover page).

1 Cultivate and harvest bacterial cells

Grow an M13-infected *E. coli* culture and centrifuge the bacterial cells at **4,000 × g** for **10 min** at **4°C**.

2 Cell lysis

Transfer the supernatant to a new microcentrifuge tube. For each **1 ml supernatant** (containing suspended phage particles), add **10 µl glacial acetic acid.** Mix by inverting the tube 6–8 times. Incubate at room temperature for 2 min.

Place a NucleoSpin® Plasmid column into a 2 ml collecting tube and load sample. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through.

Maximal loading volume of a NucleoSpin® Plasmid column is 700 µl. If larger volumes are to be processed, load samples in successive steps. Do not load the column more than 3 times.

Place the NucleoSpin® Plasmid column back into the collecting tube and add **700 µl** phage lysis **buffer M1**. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through.

Place the NucleoSpin® Plasmid column back into the 2 ml collecting tube, add **700 µl buffer M1** and incubate for **1 min** at **room temperature**. **Centrifuge** for **1 min** at **11,000 x g**. Discard flow-through.

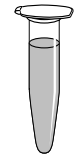
3 Clarification of the lysate

Not necessary.

Proceed with step 4 of the standard protocol (section 5.1).

5.3 Standard protocol: Isolation of plasmid DNA from *E. coli* with NucleoSpin® Plasmid QuickPure

1 Cultivate and harvest bacterial cells



Using **1–3 ml** of a saturated ***E. coli*** **LB culture,** pellet cells in a standard benchtop microcentrifuge for **30 s** at **11,000 x g.** Discard the supernatant.

30 s
11,000 x g

Remove as much of the supernatant as possible.

2 Cell lysis

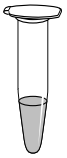
Add **250 µl buffer A1.** Resuspend the cell pellet by vigorous vortexing.

+ 250 µl A1

resuspend

No cell clumps should remain in the suspension before the addition of buffer A2.

Add **250 µl buffer A2.** Mix gently by inverting the tube **6-8 times.** Do not vortex. Incubate at **room temperature** for a maximum of **5 min.**



+ 250 µl A2

mix

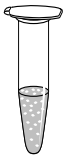
At least when using less than 3 ml of bacterial culture the cell suspension should become clear as cell lysis occurs. Do not vortex: chromosomal DNA might be released by mechanical shearing.

Add **300 µl buffer A3.** Mix gently by inverting the tube **6-8 times.** Do not vortex.

+ 300 µl A3

mix

3 Clarification of lysate

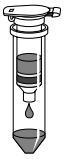


Centrifuge for **5 min** at **11,000 x g** at room temperature.

5 min
11,000 x g

4 Bind DNA

Place a NucleoSpin® Plasmid QuickPure column in a 2 ml collecting tube and load the supernatant from step 3 onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through.

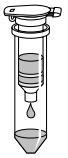


load supernatant

1 min 11,000 x g

5 Wash silica membrane

Place the NucleoSpin® Plasmid QuickPure column back into the 2 ml collecting tube and add **450 µl buffer AQ** (with ethanol, see section 3). Centrifuge for **4 min** at **11,000 x g.** Discard flow-through.



+ 450 µl AQ

4 min 11,000 x g

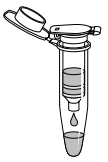
6 Dry silica membrane

The drying of the NucleoSpin® Plasmid QuickPure column is performed by the 4 min centrifugation in step 5.

7 Elute highly pure DNA

Place the NucleoSpin® Plasmid QuickPure column in a 1.5 ml microcentrifuge tube and add **50 µl buffer AE.** Incubate **1 min** at **room temperature.** Centrifuge for **1 min** at **11,000 x g.**

By repeating this step, the overall yield will increase by 15-20 % (also see section 2.3). Elution can be done with TE buffer or water as well. However, we recommend the use of a weakly buffered, slightly alkaline buffer containing no EDTA, especially if the plasmid DNA is intended for sequencing reactions. If water is used, the pH should be checked and adjusted to pH 8-8.5; absorption of CO_2 leads to a decrease in pH of unbuffered solutions.



+ 50 µl AE

RT 1 min

1 min 11,000 x g

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 buffer A2. *SDS in buffer A2 precipitated* • SDS in buffer A2 may precipitate upon storage. If a precipitate is formed, incubate buffer A2 at 30–40°C for 5 min and mix well. *Too many bacterial cells used* • We recommend LB as optimal growth medium. When using very rich media like TB (terrific broth), the cell density of the cultures may become too high.
Poor plasmid yield	*Incomplete lysis of bacterial cells* • See "Possible cause and suggestions" above. *Suboptimal precipitation of SDS and cell debris* • Precipitation of SDS and cell debris will be slightly more effective when centrifuging at 4°C instead of room temperature. *No or insufficient amounts of antibiotic used during cultivation* • Cells carrying the plasmid of interest may become overgrown by nontransformed cells, when inadequate levels of the appropriate antibiotic are used. Add appropriate amounts of freshly prepared stock solutions to all media; both solid and liquid. *Bacterial culture too old* • Do not incubate cultures for more than 16 h at 37°C under shaking. We recommend LB as the optimal growth medium; however, when using very rich media like TB (terrific broth), cultivation time should be reduced to < 12 h.

Problem	Possible cause and suggestions
Poor plasmid yield (continued)	*Suboptimal elution conditions* • If possible, use a slightly alkaline elution buffer like buffer AE (5 mM Tris-HCl, pH 8.5). If nuclease-free water is used, check the pH of the water. Elution efficiencies drop drastically with buffers < pH 7. *No high copy-number plasmid was used* • **For NucleoSpin® Plasmid:** If using low copy-number plasmids (e.g. plasmids bearing the P15A ori, cosmids, or P1 constructs), the culture volumes used should be increased at least up to 5 ml.
No plasmid yield	*Reagents not applied properly* • Add indicated volume of 96-100 % ethanol to buffer A4/buffer AQ concentrate and mix thoroughly (also see section 3). *Nuclease-rich host strains used* • Especially when working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation. • **For NucleoSpin® Plasmid:** If using nuclease-rich strains like *E. coli* HB101 or strains of the JM series, be sure to perform the optional AW washing step (step 5; section 5.1). Optimal endonuclease removal can be achieved by incubating the membrane with prewarmed buffer AW (50°C) for 2 min before centrifugation. *Inappropriate storage of plasmid DNA* • Quantitate DNA directly after preparation, e.g. by agarose gel electrophoresis. Store plasmid DNA dissolved in water at < -18°C or at < +5°C when dissolved in AE or TE buffer.
Poor plasmid quality	*Nicked plasmid DNA* • Cell suspension was incubated with alkaline lysis buffer A2 for more than 5 min. *Genomic DNA contamination* • Cell lysate was vortexed or mixed too vigorously after addition of buffer A2. Genomic DNA was sheared and thus liberated.

Problem	Possible cause and suggestions
Poor plasmid quality (continued)	*Smeared plasmid bands on agarose gel* • Especially when working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation. • **For NucleoSpin® Plasmid:** If using nuclease-rich strains like *E. coli* HB101 or strains of the JM series, be sure to perform the optional AW washing step (step 5; section 5.1). Optimal endonuclease removal can be achieved by incubating the membrane with prewarmed buffer AW (50°C) for 2 min before centrifugation.
Suboptimal performance of plasmid DNA in enzymatic reactions	*Carryover of ethanol* • **For NucleoSpin® Plasmid:** Be sure to centrifuge ≥1 min at 11,000 x g in step 6 in order to achieve complete removal of ethanolic buffer A4. • **For NucleoSpin® Plasmid QuickPure:** Be sure to centrifuge ≥4 min at 11,000 x g in step 5 in order to achieve complete removal of ethanolic buffer AQ *Elution of plasmid DNA with TE buffer* • EDTA may inhibit sequencing reactions. Repurify plasmid DNA and elute with buffer AE or water. Alternatively, the eluted plasmid DNA can be precipitated with ethanol and redissolved in buffer AE or water. *No additional washing with buffer AW performed* • **For NucleoSpin® Plasmid:** Additional washing with 500 µl buffer AW before washing with ethanolic buffer A4 will increase the reading length of sequencing reactions. *Not enough DNA used for sequencing reaction* • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions. *Plasmid DNA prepared from too much bacterial cell material* • Do not use more than 3 ml of a saturated *E. coli* culture if preparing plasmid DNA for automated fluorescent DNA sequencing.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Plasmid	740588.10	10
NucleoSpin® Plasmid	740588.50	50
NucleoSpin® Plasmid	740588.250	250
NucleoSpin® Plasmid QuickPure	740615.10	10
NucleoSpin® Plasmid QuickPure	740615.50	50
NucleoSpin® Plasmid QuickPure	740615.250	250
NucleoSpin® buffer set for the isolation of low-copy plasmids	740953	1 set
Resuspension buffer A1 (without RNase A)	740911.1	1 l
Lysis buffer A2	740912.1	1 l
Neutralization buffer A3	740913.1	1 l
Wash buffer A4 concentrate (for 100 ml buffer)	740914	20 ml
Wash buffer A4 concentrate (for 1 l buffer)	740914.1	200 ml
Wash buffer AW	740916	100 ml
Wash buffer AW	740916.1	1 l
Elution buffer AE	740917.1	1 l
RNase A	740505	100 mg
RNase A	740505.50	50 mg
NucleoSpin® collection tubes (2ml)	740600	1000

6.3 References

Birnboim, H.C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening of recombinant plasmid DNA. Nucleic Acids Res. **7:** 1513-1523.

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76:** 615-619.

6.4 Product Use Restriction / Warranty

NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure kits components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoSpin® Plasmid/NucleoSpin® Plasmid QuickPure** kits for a specific application range as the performance characteristic of this kits has not been verified to a specific organism.

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