



PCR clean-up

User manual

NucleoSpin® 8 PCR Clean-up

NucleoSpin® 8 PCR Clean-up Core Kit

September 2011 / Rev. 02

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1 Components

1.1 Kit contents

NucleoSpin® 8 PCR Clean-up		
REF	12 x 8 preps 740668	60 x 8 preps 740668.5
Binding Buffer NT	30 mL	2 x 75 mL
Wash Buffer NT3 (Concentrate) ¹	100 mL	200 mL
Elution Buffer NE ²	25 mL	125 mL
NucleoSpin® PCR Clean-up Binding Strips (yellow rings)	12	60
MN Wash Plates ³	1	5
Rack of Tube Strips ⁴	1	5
Elution Plate U-bottom ⁵	1	5
User manual	1	1

¹ For preparation of working solutions and storage conditions see section 3.

² Composition of Elution Buffer NE: 5 mM Tris/HCl, pH 8.5

³ Including six paper sheets

⁴ Set of 1 rack, 12 strips with 8 tubes each, and 12 cap strips

⁵ Including one Self-adhering PE foil

NucleoSpin® 8 PCR Clean-up Core Kit	
REF	48 x 8 preps 740463.4
Binding Buffer NT	2 x 75 mL
Wash Buffer NT3 (Concentrate) ¹	200 mL
Elution Buffer NE ²	125 mL
NucleoSpin® PCR Clean-up Binding Strips (yellow rings)	48
User manual	1

1.2 Reagents to be supplied by user

- 96–100 % ethanol

¹ For preparation of working solutions and storage conditions see section 3.

² Composition of Elution Buffer NE: 5 mM Tris/HCl, pH 8.5

2 Product description

2.1 The basic principle

The **NucleoSpin® 8 PCR Clean-up** kit is designed for vacuum based clean-up of DNA after amplification in PCR reactions or after enzymatic treatment. As described in the **NucleoSpin® 8 PCR Clean-up** procedure, the addition of chaotropic salt (Buffer NT) leads to a reversible adsorption of the DNA to the silica membrane in the **NucleoSpin® 8 PCR Clean-up Binding Strips**. Primers, salts, nucleotides, and proteins (polymerases, BSA) are removed in subsequent washing steps using Buffer NT3. Finally, highly pure DNA is eluted in Elution Buffer NE (5 mM Tris / HCl, pH 8.5) or water (pH 8.5), and can be used directly for further applications.

2.2 Kit specifications

- **NucleoSpin® 8 PCR Clean-up** is designed for DNA clean-up, such as purification of PCR products, in the convenient 8-well strip format. The 8-well strip format allows the highest flexibility in sample throughput.
- The kit is designed for vacuum use with the NucleoVac 96 Vacuum Manifold and Starter Set A (see ordering information, section 6.2).
- This kit provides reagents and consumables for purification of up to 15 µg highly pure PCR products.
- DNA recovery of 75–90 % is obtained for DNA fragments of 65–10,000 bp. Primers, primer-dimers, nucleotides, salts, and polymerase are removed effectively.
- The final concentration of the eluted DNA is 50–150 ng/µL, depending on elution buffer volume used.
- Typically, the A_{260}/A_{260} ratio is > 1.8.
- Eluted PCR products are ready-to-use for several applications including automated fluorescent sequencing, cloning, or microarray technology.
- Using the **NucleoSpin® 8 PCR Clean-up** kit allows simultaneous processing of up to 48 samples typically within 30 minutes.

Kit specifications at a glance

Parameter	NucleoSpin® 8 PCR Clean-up
Format	8-well strips
Processing	Manual and automated, vacuum
Sample material	< 100 µL PCR reaction mixture
Fragment size	65 bp–10 kbp
Typical recovery	75–95 %
A_{260}/A_{280}	1.70–1.80
Elution volume	75–150 µL
Preparation time	30 min/6 strips
Binding capacity	15 µg

2.3 Required hardware

This kit is intended for use under vacuum. A support protocol for elution under centrifugation is included (see section 5.2).

A support protocol for complete processing under centrifugation is available from our technical service (tech-bio@mn-net.com).

Vacuum processing

For processing the 8-well strips under vacuum, the Starter Set A (see ordering information), containing Column Holders A and NucleoSpin® Dummy Strips is required.

For automation on laboratory platforms with standard 96-well plate manifolds, Starter Set A is also required.

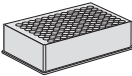
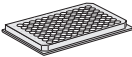
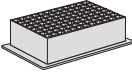
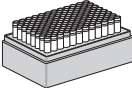

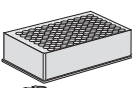

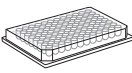
The **NucleoSpin® 8 PCR Clean-up** kits can be used **manually** with the NucleoVac 96 Vacuum Manifold (see ordering information).

2.4 Recommended accessories for use of the NucleoSpin® 8 PCR Clean-up Core Kit

The **NucleoSpin® 8 PCR Clean-up Core Kit** provides all necessary buffers and NucleoSpin® Binding Strips. Accessory plates (e.g., mixing plates, waste collection plates, elution plates or tubes) are not provided with the Core Kit. This condensed kit composition, along with a large variety of separately available accessories, allows for an optimal adjustment of the kit to individual user needs. The user can select additional consumables according to his/her requirements for highest flexibility.

For use of **NucleoSpin® 8 PCR Clean-up Core Kit** follow the protocols (see section 5.1 or 5.2, respectively).

Recommended accessories for use of the **NucleoSpin® 8 PCR Clean-up Core Kit** are available from MACHEREY-NAGEL. For ordering information please refer to section 6.2.

Protocol step	Suitable consumables, not supplied with the Core Kits	Remarks
Adjustment of binding conditions	8x Round-well Block per 48 x 8 preps 	Optional: If a premix of sample and Binding Buffer NT is favored.
Binding of DNA to the membrane and wash steps	8x MN Wash Plate per 48 x 8 preps 	MN Wash Plate minimizes the risk of cross contamination (vacuum processing only).
	2x MN Square-well Block 	For waste collection during centrifugation (reusable)
Elution	8x Rack of Tubes Strips with Cap Strips per 48 x 8 preps  or 	
	8x Round-well Block with Cap Strips per 48 x 8 preps  or 	
	8x Elution Plate U-bottom 	

2.5 Automated processing on robotic platforms

NucleoSpin® 8 PCR Clean-up can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8 PCR Clean-up** on a certain workstation, please contact MN. Full processing under vacuum enables complete automation without the need for centrifugation steps, regarding drying of the binding membrane and elution step.

The risk of cross-contamination is reduced by optimized vacuum settings during the elution step and by the improved shape of the outlets of the NucleoSpin® PCR Clean-up Binding Strips.

Drying the NucleoSpin® PCR Clean-up Binding Strips under vacuum is sufficient because the bottom of the strips is protected from buffer residues during the washing steps by the MN Wash Plate. As a result, we recommend trying to integrate the MN Wash Plate into the automated procedure. The MN Frame (see ordering information) can be used to position the disposable MN Wash Plate inside the vacuum chamber. This also reduces the risk of cross-contamination, as common metal adaptors tend to get contaminated by DNA. Thorough cleaning of the vacuum chamber is recommended after each run to prevent DNA-containing aerosols from forming.

Visit MN online at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol. Several application notes of the **NucleoSpin® 8 PCR Clean-up** kit (formerly known as NucleoSpin® 8 Extract II) on various liquid handling instruments can also be found at www.mn-net.com at Bioanalysis / Literature.

2.6 Elution procedures

Elution of purified PCR products: The efficiency of the DNA elution depends on the pH of the elution buffer. Elution is most effective at pH 8.0–8.5. When using nuclease-free water for elution, the pH value should be checked and, if necessary, adjusted to 8.0–8.5. Lower pH of the selected elution buffer may lead to lower recoveries. Yield of larger DNA fragments (> 5–10 kbp) can be increased by using pre-warmed (70 °C) elution buffer (also see table below). An elution volume of 75–125 µL Buffer NE, as well as a 3–5 min incubation at room temperature (18–25 °C) of the elution buffer on the silica membrane are recommended.

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

The recommended dispense volume of elution buffer is 125 µL.

Correlation between dispensed elution buffer volume and typical recovery

Dispensed elution buffer	75 µL	100 µL	125 µL	150 µL	175 µL
Recovered elution buffer containing PCR-products	30 ± 5 µL	55 ± 5 µL	80 ± 5 µL	105 ± 5 µL	130 ± 5 µL

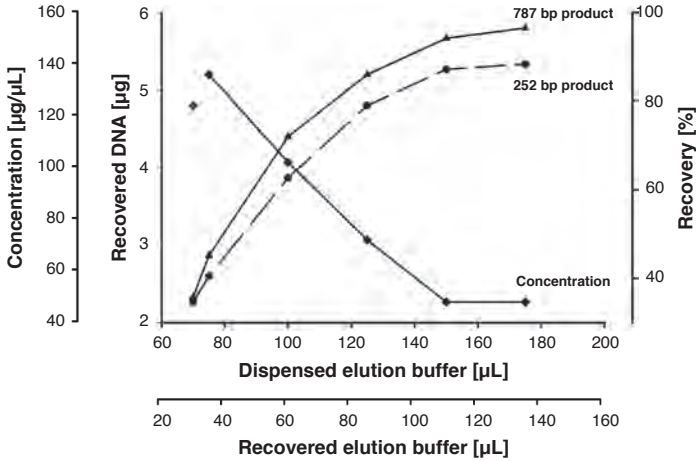


Figure 1: Recovery rate and concentration depend on elution volume.

Two different PCR products (252 bp and 787 bp) have been purified with the NucleoSpin® 8 PCR Clean-up kit.

Average DNA recovery rate depends on the size of PCR product	
Size of PCR product	Average DNA recovery rate
64 bp	60–80 %
164 bp	70–85 %
200 bp	70–85 %
490 bp	85–95 %
982 bp	85–95 %
1500 bp	80 %
2000 bp	75 %
4000 bp	50–60 %

3 Storage conditions and preparation of working solutions

Attention:

Storage conditions:

- **NucleoSpin® 8 PCR Clean-up/8 PCR Clean-up Core Kits** should be stored at room temperature (18–25 °C) and are stable for up to one year.

Before starting any **NucleoSpin® 8 PCR Clean-up/8 PCR Clean-up Core Kit** purification prepare the following:

- **Wash Buffer NT3:** Add the indicated volume of ethanol (96–100%) to **Buffer NT3 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer NT3 is stable at room temperature (18–25 °C) for up to one year.

NucleoSpin® 8 PCR Clean-up		
REF	12 x 8 preps 740668	60 x 8 preps 740668.5
Wash Buffer NT3 (Concentrate)	100 mL	200 mL
	Add 400 mL ethanol	Add 800 mL ethanol

NucleoSpin® 8 PCR Clean-up Core Kit	
REF	48 x 8 preps 740463.4
Wash Buffer NT3 (Concentrate)	200 mL
	Add 800 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® 8 PCR Clean-up** and the **NucleoSpin® 8 PCR Clean-up Core Kits** contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section.*

4.1 Risk and safety phrases

Component	Hazard contents	Hazard symbol	Risk phrases	Safety phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>Gefahrstoff-symbol</i>	<i>R-Sätze</i>	<i>S-Sätze</i>
NT	Guanidinium thiocyanate <i>Guanidiniumthiocyanat</i>	✘ Xn*	R 20/21/22-32-52/53	S 13-61

Risk phrases

- R 20/21/22 Harmful by inhalation, in contact with skin, and if swallowed.
Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.
- R 32 Contact with acids liberates very toxic gas.
Entwickelt bei Berührung mit Säure sehr giftige Gase.
- R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.

Safety phrases


- S 13 Keep away from food, drink, and animal feedstuffs.
Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.
- S 61 Avoid release to the environment. Refer to special instructions / safety data sheet.
Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen / Sicherheitsdatenblatt zu Rate ziehen.

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
NT	Guanidinium thiocyanate 30–60 % <i>Guanidiniumthiocyanat 30–60 %</i>	 Warning <i>Achtung</i>	302, 412, 031	260, 273, 301+312, 330

Hazard phrases

- H 031 Contact with acids liberates toxic gas.
Entwickelt bei Berührung mit Säure giftige Gase.
- H 302 Harmful if swallowed.
Giftig bei Verschlucken.
- H 412 Harmful to aquatic life with long lasting effects.
Schädlich für Wasserorganismen, mit langfristiger Wirkung.

Precaution phrases

- P 260 Do not breathe dust / fume / gas / mist / vapours / spray.
Staub / Rauch / Gas / Nebel / Dampf / Aerosol nicht einatmen.
- P 273 Avoid release to the environment.
Freisetzung in die Umwelt vermeiden. Mund ausspülen.
- P 301+312 IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell.
Bei Verschlucken: Bei Unwohlsein Giftinformationszentrum oder Arzt anrufen.
- P 330 Rinse mouth.
Mund ausspülen.

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (www.mn-net.com).

5 Protocols

5.1 NucleoSpin® 8 PCR Clean-up – manual vacuum processing

- For hardware requirements refer to section 2.3.
- For detailed information regarding the vacuum manifold set-up see page 17.
- For detailed information on each step see page 18.
- For use of the NucleoSpin® 8 PCR Clean-up Core Kit (REF 740463.4), refer to section 2.4 regarding recommended accessories.

Before starting the preparation:

- Check if Buffer NT3 was prepared according to section 3.
- Set up the vacuum manifold according to the scheme.

Protocol-at-a-glance

1	Adjust the volume of the reaction mixture to 100 µL using Tris buffer (pH 7.0–7.5), nuclease-free water (pH 7.0–7.5), or use Buffer NE	For PCR samples < 100 µL
2	Dispense binding buffer to NucleoSpin® PCR Clean-up Binding Strips	200 µL NT
3	Transfer PCR samples to NucleoSpin® PCR Clean-up Binding Strips and mix	100 µL diluted PCR sample
4	Bind DNA to silica membrane of the NucleoSpin® PCR Clean-up Binding Strips by applying vacuum	-0.2 bar* (1 min)
5	Wash silica membrane	2 x 900 µL NT3 -0.2 bar* (1 min)
6	Remove MN Wash Plate	

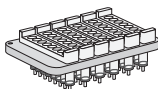
* Reduction of atmospheric pressure

7	Dry NucleoSpin® PCR Clean-up Binding Strips by applying vacuum <i>Optional: Dry the outlets of the NucleoSpin® PCR Clean-up Binding Strips by placing it on a sheet of filter paper before applying vacuum</i>	Full vacuum 10–15 min (run pump continuously)
8	Insert Rack of Tube Strips	
9	Elute DNA <i>Optional: Incubate 1–3 min</i>	75–150 µL NE -0.4 to -0.6 bar* (1 min)

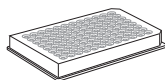
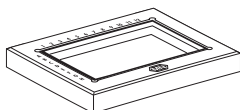
* Reduction of atmospheric pressure

Setup of vacuum manifold:

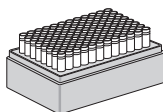
Binding / Washing / Elution steps



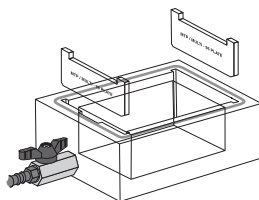
Column Holder A with NucleoSpin® Binding Strips inserted. Unused rows have to be filled with NucleoSpin® Dummy Strips



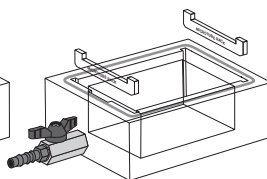
MN Wash Plate



Rack of Tube Strips



Manifold base with spacers 'MTP/Multi-96 Plate' inserted



Manifold base with spacers for 'Microtube Rack' inserted

Binding / Washing step

Elution step

* Reduction of atmospheric pressure

Detailed protocol

For processing of NucleoSpin® 8 PCR Clean-up under vacuum the NucleoVac 96 Vacuum Manifold is required.

Before starting the preparation:

- Check if Buffer NT3 was prepared according to section 3.
-

1 Adjust the volume of reaction mixture

For PCR reaction volumes below 100 µL: Before starting the purification procedure, add Tris buffer (10 mM, pH 7.0), **nuclease-free water** (pH 7.0–7.5), or **Elution Buffer NE** to adjust the reaction mixture to a final volume of 100 µL.

Note: If less than 100 µL of PCR reaction mixture is used the volume of Binding Buffer NT has to be adjusted. It is mandatory that the ratio of Buffer NT : PCR reaction mixture is 2 : 1.

Prepare the NucleoVac 96 Vacuum Manifold

Insert an appropriate number of NucleoSpin® PCR Clean-up Binding Strips (yellow rings) into a Column Holder A. Close any unused openings of the Column Holder A with NucleoSpin® Dummy Strips.

Note: Make sure that the NucleoSpin® PCR Clean-up Binding Strips are inserted tightly into the column holder. Uneven or not properly inserted strips may prevent sealing when vacuum is applied to the manifold.

Insert spacers 'MTP/Multi-96 Plate', notched side up, into the grooves located on the short sides of the manifold. Insert deep waste reservoir into the center of the manifold. Place the MN Wash Plate on top of the spacers. Insert Column Holder A with inserted NucleoSpin® PCR Clean-up Binding Strips into the manifold lid and place lid on the manifold base. Check and adjust the vacuum (pressure difference -0.2 bar). Release the vacuum.

2 Dispense binding buffer to the NucleoSpin® PCR Clean-up Binding Strips

Add **200 µL Buffer NT** to each well of the NucleoSpin® PCR Clean-up Binding Strips.

* Reduction of atmospheric pressure

3 Transfer PCR samples to the NucleoSpin® PCR Clean-up Binding Strips and mix (column wise processing is recommended)

Mix by pipetting up and down 5 times.

Optional: Pre-mix PCR reaction and Buffer NT in a Square-well Block etc. (not supplied).

4 Bind DNA to silica membrane

Apply vacuum by opening the valve and press down the Column Holder A slightly until flow-through starts. Allow the samples to pass the columns and release vacuum by closing the valve (**-0.2 to -0.4 bar***, **1 min**).

5 Wash silica membrane

1st wash

Add **900 µL Buffer NT3** (with ethanol added) to each well of the NucleoSpin® PCR Clean-up Binding Strips.

Apply vacuum by opening the valve. Press down the Column Holder A slightly until flow-through starts. Allow the buffer to pass the columns. Release the vacuum (**-0.2 to -0.4 bar***, **1 min**).

2nd wash

Repeat this washing step once.

6 Remove MN Wash Plate

After the final washing step, close the valve, release the vacuum, and remove the Column Holder A with the inserted NucleoSpin® PCR Clean-up Binding Strips. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

* Reduction of atmospheric pressure

7 Dry NucleoSpin® PCR Clean-up Binding Strips

Remove any residual washing buffer from the NucleoSpin® PCR Clean-up Binding Strips. If necessary, tap the outlets of the NucleoSpin® PCR Clean-up Binding Strips onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until the droplets stop. Insert the Column Holder A, with the NucleoSpin® PCR Clean-up Binding Strips, into the lid and close the manifold. Apply vacuum of **-0.3 to -0.4 bar*** for **at least 10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer NT3 inhibits enzymatic reactions and has to be completely removed before eluting the DNA.

Finally, release the vacuum.

8 Insert Rack of Tube Strips

Insert spacers 'Microtube rack', notched side up, into the grooves located at the short sides of the vacuum manifold. Place the Rack of Tube Strips on the spacers inside the manifold base and insert the appropriate number of Tube Strips. Insert the column holder with the NucleoSpin® PCR Clean-up Binding Strips in the manifold lid and place it on the manifold base.

Alternatively, elution can be performed into a 96-well microtiter plate (supplied with the kit). Insert spacers 'MTP/Multi-96 Plate', notched side up, into the grooves located at the short sides of the vacuum manifold. Place the Elution Plate U-bottom on the spacers inside the manifold base. Insert the column holder with the NucleoSpin® PCR Clean-up Binding Strips in the manifold lid and place it on the manifold base.

9 Elute DNA

Add **75–150 µL Elution Buffer NE** (5 mM Tris-HCl, pH 8.5) **or water** (pH 8.5) to each well of the NucleoSpin® PCR Clean-up Binding Strips.

The buffer should be dispensed onto the center of the silica membrane. Incubate for **1–3 min at room temperature** (optionally), apply vacuum, and collect the eluted DNA. After the elution buffer has passed the columns, release the vacuum (**-0.4 to -0.6 bar***, **1–2 min**).

Remove the Rack of Tube Strips or Elution Plate U-bottom containing eluted DNA and seal them with the supplied Cap Strips (for the tube strips) or adhesive cover foil (for the elution plate) for further storage.

* Reduction of atmospheric pressure

5.2 NucleoSpin® 8 PCR Clean-up – elution of DNA using a centrifuge

Eluting the purified DNA in a centrifuge may be necessary when higher concentrations of the final DNA are required for downstream applications. Using a centrifuge allows the dispensed volume to be reduced down to 50–75 µL, giving a DNA concentration of about 70–200 ng/µL (depending on elution buffer volume and fragment length).

Required hardware:

- For centrifugation, a microtiterplate centrifuge that can accommodate the NucleoSpin® PCR Clean-up Binding Strips (inserted into a Column Holder C) stacked on a rack of Tube Strips is required. It is also necessary that the centrifuge reaches accelerations of 5,600–6,000 x *g* (bucket height: 85 mm).
- For processing the 8-well strips the Starter Set C (see ordering information), containing Column Holders C, MN Square-well Blocks, and Rack of Tube Strips is required.

1 Stop the method after the final washing step with Buffer NT3. Remove the NucleoSpin® PCR Clean-up Binding Strips from the manifold's top and tap on a sheet of filter paper to remove residual wash buffer from the outlets.

2 Place the Column Holder C, with the NucleoSpin® PCR Clean-up Binding Strips, on top of a MN Square-well Block (not supplied with the kit, see ordering information) and centrifuge for **10 min at maximum speed** (> 4,000 x *g*, optimal 5,800 x *g*).

*Note: Do not use a microtiter plate as a support for the NucleoSpin® PCR Clean-up Binding Strips. Microtiter plates may crack under centrifugation at > 1,500 x *g*.*

3 Place the Column Holder C, with the NucleoSpin® PCR Clean-up Binding Strips, on top of a Rack of Tube Strips. Dispense **Elution Buffer NE** (50–150 µL) directly onto the silica membrane and incubate for **1–3 min at room temperature**.

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

Remove the Rack of Tube Strips containing eluted DNA and close them with Cap Strips for further storage.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	<p><i>No ethanol added to Buffer NT3 Concentrate, ethanol evaporated</i></p> <ul style="list-style-type: none"> • Add indicated volume of ethanol to Buffer NT3 Concentrate and mix. Keep bottle tightly closed to prevent evaporation of ethanol.
	<p><i>Elution conditions are not optimal</i></p> <ul style="list-style-type: none"> • If possible, use a slightly alkaline elution buffer like Buffer NE (5 mM Tris-HCl, pH 8.5). When using nuclease-free water for elution, make sure the pH value is 8.5. Elution efficiencies drop dramatically at pH < 7.
	<p><i>Elution buffer volume is insufficient</i></p> <ul style="list-style-type: none"> • Optimal elution is achieved for an elution buffer volume of 100–150 μL. Do not use less than 75 μL elution buffer.
Suboptimal performance of PCR product in sequencing reactions, problems with downstream applications	<p><i>Carryover of ethanol</i></p> <ul style="list-style-type: none"> • Be sure to remove all of ethanolic Buffer NT3 after the final washing step. Dry the NucleoSpin® PCR Clean-up Binding Strips for at least 10 min with maximum vacuum.
	<p><i>Elution of PCR products with TE buffer</i></p> <ul style="list-style-type: none"> • EDTA may inhibit enzymatic reactions like DNA sequencing. Repurify the PCR products and elute with NE buffer or nuclease-free water. Alternatively, the DNA may be precipitated with ethanol and redissolved in buffer NE buffer or nuclease-free water.
	<p><i>Not enough DNA used in sequencing reactions</i></p> <ul style="list-style-type: none"> • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.

Problem	Possible cause and suggestions
Suboptimal performance of PCR product in sequencing reactions, problems with downstream applications (continued)	<i>Contamination of PCR product preparation with ethanol</i> <ul style="list-style-type: none"> Insufficient drying after final washing step with Buffer NT3. Remaining ethanol may cause problems with downstream applications like DNA sequencing or loading of samples onto agarose gel.
	<i>Eluted DNA contains residual primers/primer dimers</i> <ul style="list-style-type: none"> Minimize amount of primers in PCR reaction mixture. Make sure that the ratio of binding buffer NT:PCR reaction mixture is 2:1.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 8 PCR Clean-up	740668	12 x 8 preps
	740668.5	60 x 8 preps
NucleoSpin® 8 PCR Clean-up Core Kit	740463.4	48 x 8 preps
NucleoSpin® 96 PCR Clean-up	740658.1	1 x 96 preps
	740658.2	2 x 96 preps
	740658.4	4 x 96 preps
	740658.24	24 x 96preps
NucleoSpin® 96 PCR Clean-up Core Kit	740464.4	4 x 96 preps
MN Wash Plate	740479	4
	740479.24	24
Round-well Block with Cap Strips (set consists of 1 Round-well Block and 12 Cap Strips)	740475	4 sets
	740475.24	24 sets
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740477	4 sets
	740477.24	24 sets
Cap Strips	740478	48
	740478.24	288
MN Square-well Block	740476	4
	740476.24	24
Round-well Block Low (set consists of Round-well Block Low and Self-adhering Foil)	740487	4 sets
	740487.24	24 sets

Product	REF	Pack of
Elution Plate U-bottom (with Self-adhering Foil)	740486.24	24 sets
Cap Strips	740638	30
Self-adhering PE Foil	740676	50
MN Frame	740680	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Starter Set A (for processing NucleoSpin® 8-well strips on NucleoVac 96 Vacuum Manifold)	740682	1
Starter Set C (for processing NucleoSpin® 8-well strips under centrifugation)	740684	1

Visit www.mn-net.com for more detailed product information.

6.3 Reference

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

6.4 Product use restriction/warranty

NucleoSpin® 8 PCR Clean-up (Core Kit) components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

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