Bioanalysis

MACHEREY-NAGEL

User manual

PCR clean-up

- NucleoSpin® 96 PCR Clean-up
- NucleoSpin® 96 PCR Clean-up Core Kit

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1 Components

1.1 Kit contents

	NucleoSpin® 96 PCR Clean-up			
	1 x 96 preps	**2 x 96 preps**	**4 x 96 preps**	**24 x 96 preps[1]**
REF	**740658.1**	**740658.2**	**740658.4**	**740658.24**
Binding Buffer NT	25 mL	75 mL	2 x 75 mL	12 x 75 mL
Wash Buffer NT3 (Concentrate)[2]	100 mL	2 x 100 mL	2 x 100 mL	12 x 100 mL
Elution Buffer NE[3]	30 mL	60 mL	125 mL	6 x 125 mL
NucleoSpin® PCR Clean-up Binding Plate (yellow rings)	1	2	4	24
MN Wash Plate[4]	1	2	4	24
Elution Plate U-bottom[5]	1	2	4	24
User manual	1	1	1	6

[1] The kit for 24 x 96 preparations (REF 740658.24) consists of 6 x REF 740658.4.

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

[3] Composition of Elution Buffer NE: 5 mM Tris/HCl, pH 8.5

[4] Including six paper sheets

[5] Including one Self adhering PE foil

	NucleoSpin® 96 PCR Clean-up Core Kit
	4 x 96 preps
REF	**740464.4**
Binding Buffer NT	2 x 75 mL
Wash Buffer NT3 (Concentrate)[1]	2 x 100 mL
Elution Buffer NE[2]	125 mL
NucleoSpin® PCR Clean-up Binding Plate (yellow rings)	4
User manual	1

1.2 Reagents to be supplied by user

- 96–100 % ethanol

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

[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® 96 PCR Clean-up kit allows direct clean up of PCR reaction mixtures. Within the procedure the addition of chaotropic salt (Buffer NT) allows a reversible adsorption of the PCR products to the silica membrane of the NucleoSpin® 96 PCR Clean-up Binding Plates. High purity of the PCR-products is achieved by complete removal of primers, primer-dimers, salts, nucleotides, and proteins (e.g., polymerases, BSA) in subsequent washing steps using Buffer NT3. Highly pure PCR products are finally eluted with 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® 96 PCR Clean-up** is designed for the fast 96-well purification of PCR products in the microtiter plate format (e.g., desalination, removal of enzymes, nucleotides and / or labeling reagents like biotin or radioactive ATP).
- The kit is for use with the NucleoVac 96 vacuum manifold (see ordering information) or similar suitable vacuum manifolds (see section 2.4).
- If using less than 96 samples the rubber pad or Self adhering PE Foil (see ordering information) must be used in order to cover non used wells to maintain sufficient vacuum.
- The kit provides reagents and consumables for purification of up to 15 µg highly pure PCR products.
- Eluted PCR products are ready to use for several applications including automated fluorescent sequencing, cloning, or microarray technology.
- The **NucleoSpin® 96 PCR Clean-up** kit allows for the simultaneous processing of up to 96 samples typically within 45 minutes.

Kit specifications at a glance	
Parameter	NucleoSpin® 96 PCR Clean-up
Format	96-well plates
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	45 min/plate
Binding capacity	15 µg

2.3 Required hardware

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

This kit is intended for use under vacuum. A support protocol for centrifuge processing or elution under centrifugation is included (see section 5.2 and 5.3)

Centrifugation

For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accomodate the NucleoSpin® PCR Clean-up Binding Plate stacked on a Round- or MN Square-well Block and reach accelerations of 5,600–6,000 x *g* is required (bucket height: 85 mm). Regarding waste collection, suitable consumables (e.g., MN Square-well Blocks) are necessary and they are not included in the kit. For the most convenient handling, without the need of emptying and reusing the MN Square-well Blocks, we recommend using two MN Square-well Blocks if two 96-well plates are processed at once (see ordering information). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the amount of MN Square-well Blocks needed.

Vacuum processing

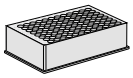
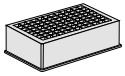
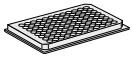
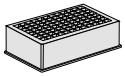
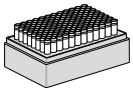
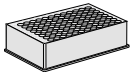
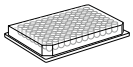
The NucleoSpin® 96 PCR Clean-up kit can be used with the NucleoVac 96 Vacuum Manifold. When using NucleoSpin® 96 PCR Clean-up with less than 96 samples, Self-adhering PE Foil should be used in order to close and protect non-used wells of the NucleoSpin® 96 PCR Clean-up Binding Plate and thus guarantee proper vacuum (see ordering information, section 6.2). The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of -0.2 to -0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information, section 6.2) is recommended. Alternatively, adjust the vacuum in a way that the sample flows through the column with a rate of 1–2 drops per second. Depending on the amount of sample being used, the vacuum times may need to be increased for complete filtration.

2.4 Recommended accessories for use of the NucleoSpin® 96 PCR Clean-up Core Kit

The **NucleoSpin® 96 PCR Clean-up Core Kit** provides buffers and NucleoSpin® Binding Plates only. Accessory plates (e.g., elution plates) are not provided with the core kit. The user can individually select additional consumables from a variety of suitable accessory plates according to his requirements for highest flexibility.

For use of **NucleoSpin® 96 PCR Clean-up Core Kit** follow the protocols (see section 5.1 or 5.2, respectively).

Recommended accessories for use of the **NucleoSpin® 96 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	4 x Round-well Block per 4 x 96 preps		Optional: If a premix of sample and Binding Buffer NT is favored.
	4 x Square-well Block		
Binding of DNA to the membrane and wash steps	4 x MN Wash Plate per 4 x 96 preps		MN Wash Plate minimizes the risk of cross contamination (vacuum processing only).
	MN Square-well Block		for centrifugation
Elution	4 x Rack of Tubes Strips with Cap Strips per 4 x 96 preps or	 	For elution under vacuum and centrifugation
	4 x Round-well Block with Cap Strips per 4 x 96 preps or	 	
	4 x Elution Plate U-bottom		For vacuum processing only

2.5 Automated processing on robotic platforms

NucleoSpin® 96 PCR Clean-up can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 96 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 Plate.

Drying of the NucleoSpin® PCR Clean-up Binding Plate under vacuum is sufficient because the bottom of the plate is protected from residues of wash buffer 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® 96 PCR Clean-up** kit (formerly known as NucleoSpin® 96 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 prewarmed (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

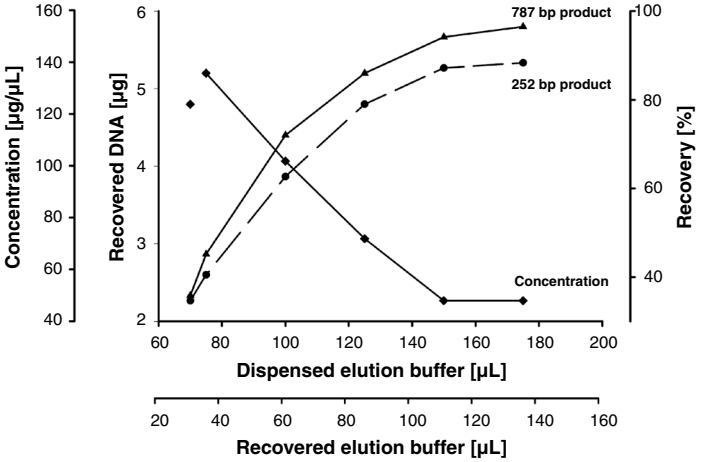


Figure 1 Recovery rate and concentration depend on elution volume. Two different PCR products (252 bp and 787 bp) have been purified with the NucleoSpin® 96 PCR Clean-up kit.

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® 96 PCR Clean-up / 96 PCR Clean-up Core Kits** should be stored at 15–25 °C and are stable until: see package label.

Before starting any **NucleoSpin® 96 PCR Clean-up / 96 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 15–25 °C for up to one year.

	NucleoSpin® 96 PCR Clean-up			
	1 x 96 preps	**4 x 96 preps**	**4 x 96 preps**	**24 x 96 preps**
REF	**740658.1**	**740658.2**	**740658.4**	**740658.24**
Wash Buffer NT3 (Concentrate)	100 mL Add 400 mL ethanol	2 x 100 mL Add 400 mL ethanol to each bottle	2 x 100 mL Add 400 mL ethanol to each bottle	12 x 100 mL Add 400 mL ethanol to each bottle

	NucleoSpin® 96 PCR Clean-up Core Kit
	4 x 96 preps
REF	**740464.4**
Wash Buffer NT3 (Concentrate)	2 x 100 mL Add 400 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® 96 PCR Clean-up kit** wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

Caution: Guanidinium thiocyanate in buffer NT can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 NucleoSpin® 96 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 14.
- For detailed information on each step see page 15.
- For use of the NucleoSpin® 96 PCR Clean-up Core Kit (REF 740464.4), 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 sheme

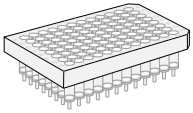
Protocol at a glance

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 Plate	**200 µL NT**
3	**Transfer** PCR samples to NucleoSpin® PCR Clean-up Binding Plate and mix	**100 µL diluted PCR sample**
4	**Bind** DNA to silica membrane of the NucleoSpin® PCR Clean-up Binding Plate by applying vacuum	**-0.2 bar to -0.4 bar* (1 min)**
5	**Wash** silica membrane	**2 x 900 µL NT3–0.2 bar to -0.4 bar* (1 min)**
6	**Remove** MN Wash Plate	
7	**Dry** NucleoSpin® PCR Clean-up Binding Plate by applying vacuum Optional: Dry the outlets of the NucleoSpin® PCR Clean-up Binding Plate by placing it on a sheet of filter paper before applying vacuum	**-0.3 to -0.4 bar* 10–15 min (run pump continuously)***
8	Insert Elution Plate U-bottom	
9	**Elute** DNA Optional: Incubate 1–3 min	**75–150 µL NE** **- 0.4 to - 0.6 bar* (1 min)**

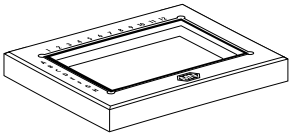
* Reduction of atmospheric pressure

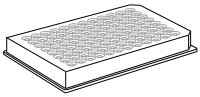
Setup of vacuum manifold:

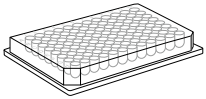
Binding / Washing / Elution steps



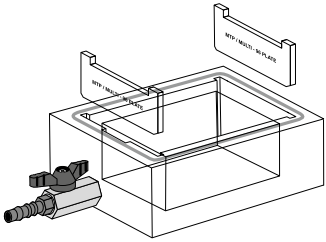
NucleoSpin® Binding Plate







MN Wash Plate Elution Plate



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

Binding / Washing step **Elution step**

Detailed protocol

For processing of NucleoSpin® 96 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 spacers labeled '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 MN Wash Plate on top of the spacers. Insert NucleoSpin® PCR Clean-up Binding Plate into the manifold lid and place lid on the manifold base. Close manifold base with the manifold lid. Close the vacuum manifold's valve, check and adjust the vacuum (-0.2 bar*).

2 Dispense binding buffer to the NucleoSpin® PCR Clean-up Binding Plate (column wise processing is recommended)

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

3 Transfer PCR samples to the NucleoSpin® PCR Clean-up Binding Plate and mix

Mix by pipetting up and down 5 times.

Optional: Premix 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 plate slightly until flowthrough starts. Allow the samples to pass the columns and release vacuum by closing the valve (**-0.2 to -0.4 bar*, 1 min**).

* Reduction of atmospheric pressure

5 Wash silica membrane

1st wash

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

Apply vacuum by opening the valve. Press down the NucleoSpin® PCR Clean-up Binding Plate slightly until flowthrough 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 NucleoSpin® PCR Clean-up Binding Plate. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

7 Dry NucleoSpin® PCR Clean-up Binding Plate

Remove any residual washing buffer from the NucleoSpin® PCR Clean-up Binding Plate. If necessary, tap the outlets of the NucleoSpin® PCR Clean-up Binding Plate onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the NucleoSpin® PCR Clean-up Binding Plate 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, close the release the vacuum.

8 Insert Elution Plate U-bottom

Insert 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 Rack of 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 Rack of Tube Strips on the spacers inside the manifold base and reassemble the vacuum manifold as described before.

* Reduction of atmospheric pressure

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 Plate.

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

* Reduction of atmospheric pressure

5.2 NucleoSpin® 96 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 Plate stacked on a rack of Tube Strips is required. It is also necessary that the centrifuge reached accelerations of 5,600–6,000 x *g* (bucket height: 85 mm).
- For centrifugation, a microtiterplate centrifuge is required which is able to accommodate the NucleoSpin® PCR Clean-up Binding Plate stacked on Rack of Tube Strips and reaches accelerations of 5,600–6,000 x *g* (bucket height: 85 mm).
- Suitable elution tubes: Rack of Tube Strips has to be ordered separately (see ordering information).

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

2 Place the NucleoSpin® PCR Clean-up Binding Plate 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 Plate. Microtiter plates may crack under centrifugation at > 1,500 x g.

3 Place the NucleoSpin® PCR Clean-up Binding Plate on top of a Rack of Tube Strips (not supplied with the kit, see ordering information). 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.

5.3 NucleoSpin® 96 PCR Clean-up – centrifuge processing

For use of the NucleoSpin® 96 PCR Clean-up kit in a centrifuge additional equipment is required:

- MN Square-well Block (REF 740476) or Square-well Block (REF 740481)
- Round-well Block Low (REF 740482)

Note: The Elution Plate, U-bottom supplied with the kit is for vacuum elution only.

Please note that there are only few centrifuges which can be used for handling of NucleoSpin® 96 PCR Clean-up kits. The centrifuge should be able to pick up a swing out rotor which is capable of accommodating the NucleoSpin® Plasmid Binding Plate / Square-well Block sandwich (bucket height: 85 mm) and reaches accelerations of 5,600 – 6,000 x *g*.

For transfer of the sample from the Round-well Block to the NucleoSpin® PCR Binding Plate we recommend usage of an electronic eight-channel pipetting device with extra long tips capable of holding more than 500 µl. A good choice is the Matrix Impact2 multichannel pipettor with 102-mm-long 1,250 µl tips (Matrix # 8251).

Procedure

1	**Perform PCR reaction according to the standard protocols.** **For PCR reaction volumes below 100 µL**: Before starting the purification procedure, add Tris buffer (10 mM, pH 7.0) to adjust the reaction mixture to a final volume of 100 µL. The amount of added mineral oil has not to be considered. *Note: Removal of mineral oil is not necessary.*
2	**Transfer 100 µL sample** to each well of the (MN) Square-well Block (not supplied with the kit).
3	Add **200 µL of Binding Buffer NT** into the wells of the (MN) Square-well Block and pipette up and down several times for mixing. (Alternatively, add to 1 vol of PCR reaction and 2 vol of Buffer NT into the PCR plate. Mix by pipetting up and down.)
4	Transfer all of the sample / Buffer NT mixture into the wells of the **NucleoSpin® PCR Clean-up Binding Plate**. Do not moisten the rims while dispensing samples. Moistened rims may cause cross contamination during centrifugation steps.
5	Place the (MN) Square-well Block (not provided with the kit) and **NucleoSpin® PCR Clean-up Binding Plate** onto the centrifuge carrier and place it into the rotor buckets. Centrifuge at **5,600 x *g*** for **2 min**. Typically, samples will pass through the columns within ≤ 1 min.
6	Add **900 µL of NT3** to each well of the **NucleoSpin® PCR Clean-up Binding Plate** and centrifuge again at **5,600 x *g*** for **1–2 min**. After centrifugation discard flowthrough collected in the (MN) Square-well Block. Repeat this washing step once.
7	Centrifuge for 5–10 min at **5,600 x *g*** in order to remove residual washing buffer from the silica membrane and for drying the membrane.

8 Place the **NucleoSpin® PCR Clean-up Binding Plate** on a Round-well Block, low (not provided with the kit).

9 Dispense **75–150 µL Elution Buffer NE** or water to each well of the **NucleoSpin® PCR Clean-up Binding Plate**. Dispense buffer directly onto the membrane. Incubate at room temperature for **1 min**. Centrifuge at **5,600–6,000 x *g*** for **2–3 min**.

Optional: Prewarm Elution Buffer to 70°C before dispensing. This will increase recovery for PCR products > 1000 bp.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*No ethanol added to Buffer NT3 Concentrate, ethanol evaporated* • Add indicated volume of ethanol to Buffer NT3 Concentrate and mix. Keep bottle tightly closed to prevent evaporation of ethanol. *Elution conditions are not optimal* • 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. *Elution buffer volume is insufficient* • 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	*Carry-over of ethanol* • Be sure to remove all of ethanolic Buffer NT3 after the final washing step. Dry the NucleoSpin® PCR Clean-up Binding Plate for at least 10 min with maximum vacuum. *Elution of PCR products with TE buffer* • 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. *Not enough DNA used in sequencing reactions* • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.
Suboptimal performance of PCR product in sequencing reactions, problems with downstream applications (continued)	*Contamination of PCR product preparation with ethanol* • 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. *Eluted DNA contains residual primers/primer dimers* • 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® 96 PCR Clean-up	740658.1 740658.2 740658.4 740658.24	1 x 96 preps 2 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® 96 PCR Clean-up Core Kit	740464.4	4 x 96 preps
NucleoSpin® 8 PCR Clean-up	740668 740668.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 8 PCR Clean-up Core Kit	740463.4	48 x 8 preps
MN Wash Plate	740479 740479.24	4 24
Round-well Block with Cap Strips (set consists of 1 Round-well Block and 12 Cap Strips)	740475 740475.24	4 sets 24 sets
Rack of Tube Strips (1 set consists of 1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Cap Strips	740478 740478.24	48 288
MN Square-well Block	740476 740476.24	4 24
Round-well Block Low (set consists of Round-well Block Low and Self adhering Foil)	740487 740487.24	4 sets 24 sets
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

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

6.3 References

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

6.4 Product use restriction / warranty

NucleoSpin® 96 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.

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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.

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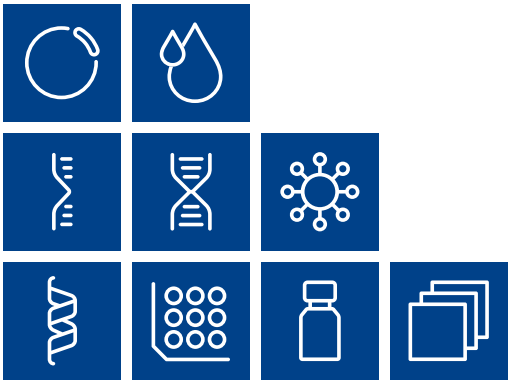

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RNA
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Protein
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Accessories
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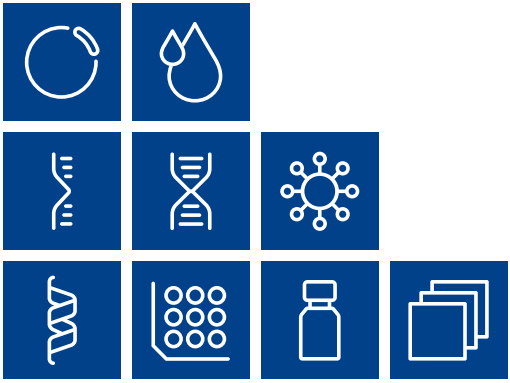

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