



PCR clean-up

User manual

NucleoSpin[®] 96 Extract II

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MACHERY-NAGEL



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1 Kit contents

Cat.No.	NucleoSpin® 96 Extract II			
	1 x 96 preps 740 658.1	2 x 96 preps 740 658.2	4 x 96 preps 740 658.4	24 x 96 preps* 740 658.24
Buffer NT	30 ml	75 ml	2 x 75 ml	12 x 75 ml
Buffer NT3 (concentrate)**	100 ml	2 x 100 ml	200 ml	6 x 200 ml
Buffer NE	25 ml	50 ml	125 ml	6 x 125 ml
NucleoSpin® Extract Binding Plate (yellow)	1	2	4	24
MN Wash Plate (including six paper sheets)	1	2	4	24
Elution Plate, u-bottom (including one adhesive cover foil)	1	2	4	24
Protocol	1	1	1	6

* The kit for 24 x 96 preparations (Cat. No. 740 618.24) consists of 6 x Cat. No. 740 618.4

** For preparation of working solution and storage conditions see section 3.

2 Product description

2.1 The basic principle

Within the **NucleoSpin® 96 Extract II** procedure the addition of chaotropic salt (buffer NT) leads to reversible adsorption of the PCR products to the silica membrane of the **NucleoSpin® 96 Extract 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 Extract II** is designed for the fast 96-well purification of PCR products in the microtiter plate format.
- If using less than 96 samples the rubber pad or self-adhering PE foil (see ordering information) should be used in order to cover non used wells to maintain sufficient vacuum.
- The kit is for use with the NucleoVac 96 vacuum manifold (see ordering information) or similar suitable vacuum manifolds (see section 2.4).
- The 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 100–10,000 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 $A_{260/280}$ ratio is > 1.8.
- Eluted PCR products are ready-to-use for e.g. automated fluorescent sequencing (e.g. ABI 3700, 3100, 377, 373, LICOR, MegaBace, ALF), cloning or microarray technology.
- The **NucleoSpin® 96 Extract II** kit allows for the simultaneous processing of up to 96 samples typically within 45 minutes.

Kit specifications at a glance	
Parameters	NucleoSpin® 96 Extract II
Desalination, removal of enzymes, nucleotides and /or labeling reagents like biotin or radioactive ATP etc.	++
Direct purification of amplified DNA	++
Elution volume	75-150 µl
Binding capacity	20 µg
Time/prep	45 min/96 preps

2.3 General comments

- **Membrane capacity:** The silica membrane allows purification of up to 15 µg DNA. The adsorption of DNA to the membrane is pH dependent. High DNA yields are achieved for reaction mixtures with pH < 8.0.
- **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 1). An elution volume of 75-125 µl buffer NE (included in the kit), as well as a 3-5 min incubation at room temperature of the elution buffer on the silica membrane are recommended.

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

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

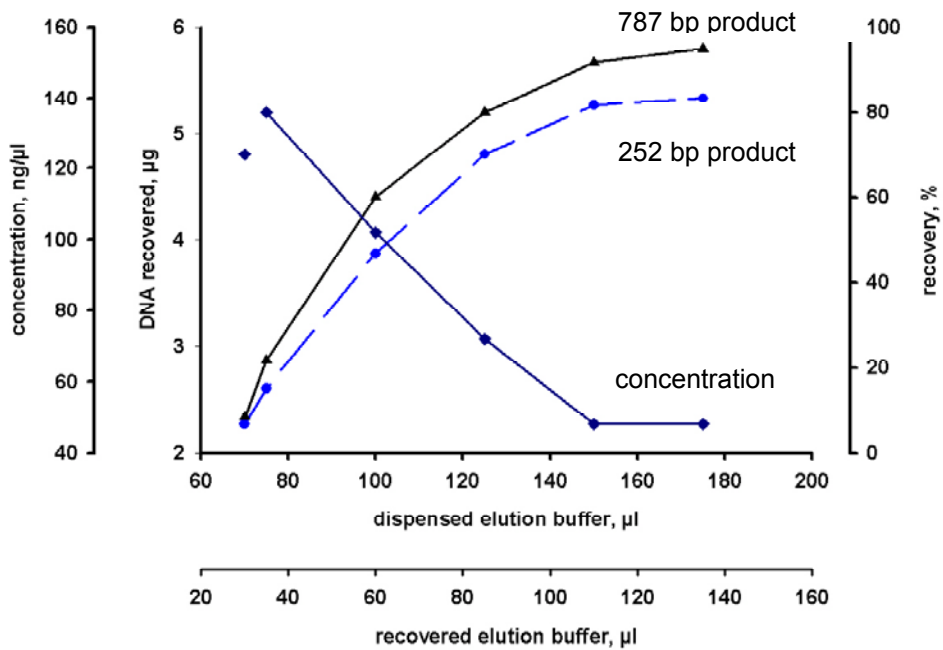


Fig. 1: Recovery rate and concentration depend on elution volume. Two different PCR products (252 bp and 787 bp) have been purified with the NucleoSpin® 96 Extract II kit.

Table 1: Average DNA recovery rate depends on the size of PCR product	
Size of PCR product [bp]	Average DNA recovery rate [%]
64	60– 80
164	70 – 85
200	70 – 85
490	85 – 95
982	85 – 95
1500	80
2000	75
4000	50 - 60

2.4 Suitability for other common vacuum manifolds

The **NucleoSpin® 96 Extract II** kit can be used with other common vacuum manifolds. For further details see list below.

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

*In general the QIAvac 96 is suitable for the use with the NucleoSpin® Extract II Binding Plate. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate and Elution Plate, u-bottom in order to ensure best performance.

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

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

Attention:

Buffer NT contains chaotropic salt. Wear gloves and goggles

- All components of the **NucleoSpin® 96 Extract II** kit should be stored at room temperature and are stable up to one year.

Before starting any **NucleoSpin® 96 Extract II** protocol prepare the following:

- Add the indicated volume of 96 – 100 % ethanol to buffer NT3 concentrate.


	NucleoSpin® 96 Extract II			
	1 x 96 preps	2 x 96 preps	4 x 96 preps	24 x 96 preps
Cat. No.	740 658.1	740 658.2	740 658.4	740 658.24
Buffer NT3 (concentrate)	100 ml	2 x 100 ml	200 ml	6 x 200 ml
	add 400 ml ethanol	add 400 ml ethanol to each bottle	add 800 ml ethanol	add 800 ml ethanol to each bottle

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

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 96 Extract II** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section

Contents	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
NT	guanidine thiocyanate	 Xn*	Harmful by inhalation, in contact with skin and if swallowed	R 20/21/22	S 13

Risk Phrases

R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed

Safety Phrases

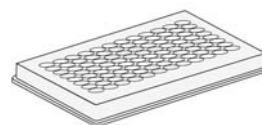
S 13 Keep away from food, drink and animal feedstuffs

* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 General Procedure

1 Perform PCR reaction

up to 100 µl reaction volume

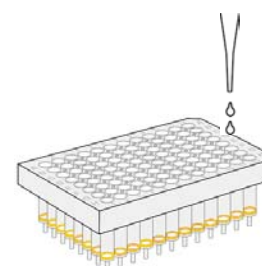


2 **Adjust** the volume of the reaction mix to 100 µl using Tris buffer (pH 7.0 – 7.5), nuclease-free water (pH 7.0 - 7.5), or use elution buffer NE.

for PCR samples < 100 µl

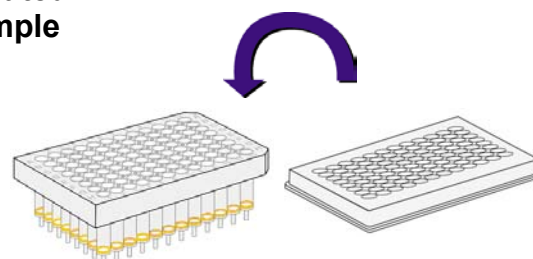
3 **Dispense** binding buffer to NucleoSpin® Extract Binding Plate (yellow)

200 µl NT



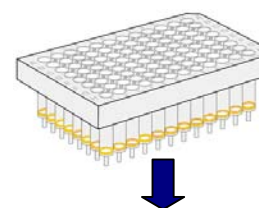
4 **Transfer** PCR samples to NucleoSpin® Extract Binding Plate and mix

100 µl diluted PCR sample



5 **Bind** DNA to silica membrane of the NucleoSpin® Extract Binding Plate by applying vacuum.

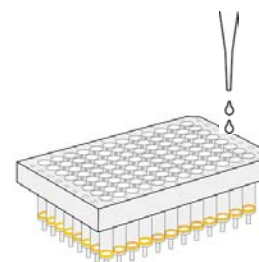
approx. – 0.2 bar* (1 min)



6 **Wash** silica membrane.

2 x 900 µl NT3

approx. – 0.2 bar* (1 min)

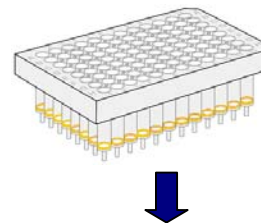


7 Remove MN Wash Plate.

8 Dry NucleoSpin[®] Extract Binding Plate by applying vacuum.

optional: before applying vacuum dry the outlets of the NucleoSpin[®] Extract Binding Plate by placing it on a paper sheet

**10 min – 15 min
maximum vacuum***

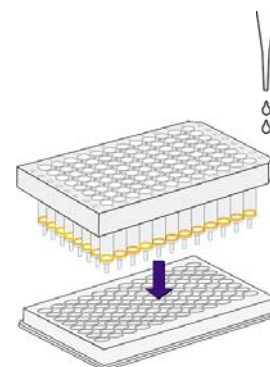


9 Insert the Elution Plate, u-bottom

10 Elute highly pure PCR product DNA.

optional: incubate at room temperature for 1 – 3 min

**75-150 µl NE
approx. – 0.4 bar*
(1 min)**



**) reduction of atmospheric pressure*

5.1 Standard protocol for PCR clean-up

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

1 Perform PCR reaction.

2 Adjust the volume of reaction mix.

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.

Prepare the NucleoVac 96 vacuum manifold:

Insert spacers *MTP/Multi-96 plate*, notched side up, into the grooves located on the short sides of the manifold. Insert waste container into manifold base. Rest the MN Wash Plate inside the manifold base. Insert the NucleoSpin[®] Extract Binding Plate into the manifold lid. Close manifold base with the manifold lid. Close the vacuum manifold's valve, check and adjust the vacuum (pressure difference -200 mbar).

3 Dispense binding buffer NT to the NucleoSpin[®] Extract Binding Plate

Add 2 volumes of buffer NT for one volume of the PCR reaction mix (e.g. for 100 µl reaction add 200 µl buffer NT).

4 Transfer PCR samples onto the NucleoSpin[®] Extract Binding Plate (yellow) and mix.

Mix by pipetting up and down 5 times.

5 Bind DNA to silica membrane.

Apply vacuum by opening the valve and press down the plate slightly until flow-through starts. Allow the samples to pass the columns and release vacuum by closing the valve.

6 Wash silica membrane.

Add 900 µl buffer NT3 (with ethanol added) to each well of the NucleoSpin® Extract Binding Plate.

Apply vacuum by opening the valve. Press down the NucleoSpin® Extract Binding Plate slightly until flow-through starts. Allow the buffer to pass the columns. Release vacuum.

Repeat this washing step once.

7 Remove MN Wash Plate.

After the final washing step close the valve, release the vacuum and remove the NucleoSpin® Extract Binding Plate. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

8 Dry NucleoSpin® Extract Binding Plate.

Remove any residual washing buffer from the NucleoSpin® Extract Binding Plate. If necessary, tap the outlets of the NucleoSpin® Extract Binding Plate onto a clean paper sheets (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the NucleoSpin® Extract Binding Plate into the lid and close the manifold. Apply vacuum of 300 - 400 mbar (pressure difference) 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 removed completely before eluting DNA.

Finally, close the valve and ventilate the vacuum manifold.

9 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 MN Tube Strips (not provided with the kit, see ordering information):

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

10 Elute highly pure PCR-product DNA.

Add 75 - 150 µl of elution buffer NE (5 mM Tris-HCl, pH 8.5) or water (pH 8.5) to each well of the NucleoSpin[®] Extract Binding Plate.

The buffer should be dispensed onto the center of the silica membrane. Incubate for 1-3 min at room temperature, apply vacuum, and collect the eluted DNA. After the elution buffer has passed the columns, close the valve and release the vacuum.

Remove the Elution Plate, u-bottom (or MN Tube Strips) containing eluted DNA and seal them with the supplied adhesive cover foil (or Cap Strips for MN Tube Strips) for further storage.

5.2 Support protocol - elution of DNA using a centrifuge

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

- 1 Stop procedure after the final washing step with buffer NT3. Remove NucleoSpin[®] Extract Binding Plate from the manifold's top and tap on a paper sheet to remove residual wash buffer from the outlets.
-

- 2 Place the NucleoSpin[®] Extract Binding Plate on top of a MN Square-well Block or similar (not provided in the kit, see ordering information) and load the plate sandwich to a suitable centrifuge (see below). Centrifuge for 10 min at maximum speed (> 4,000 x g, optimal 5,800 x g) to dry the membrane and the outlets of the binding plate.

Note

We recommend to use a centrifuge (e.g. Hermle Z513, Qiagen/Sigma 4-15, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, Highplate[™] rotor, Beckman Coulter, Allegra R) with a swing-out rotor which is capable of accommodating the NucleoSpin[®] Extract Binding Plate/MN Square-well Block sandwich (bucket height: 85 mm). Do not use a microtiter plate as a support for the NucleoSpin[®] Extract Binding Plate. Microtiter plates like the provided Elution Plate, u-bottom may break when centrifuging at > 2,500 x g

- 3 Insert the NucleoSpin[®] Extract Binding Plate onto a new MN Square-well Block or Round-well Block (not provided in the kit, see ordering information). Remove the self-adhering PE foil and dispense elution buffer NE (50-75 μ l) directly onto the silica membrane. Incubate for 1-3 min.

Note:

Elution in 96-well PCR plates is possible. In order to stabilize the PCR plate during centrifugation use a suitable plate adapter. Alternatively, insert the PCR plate in a MN Square-well Block or Round-well Block. Place NucleoSpin[®] Extract Binding Plate on top and centrifuge the whole assembly for elution.

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

Remove the Square-well Block or Round-well Block containing eluted DNA and seal them with adhesive PE foil (supplied with the Elution Plate, u-bottom) or Cap Strips (suitable for Round-well Block only) 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 with buffers < pH 7.
Suboptimal performance of PCR product in sequencing reactions, problems with downstream applications	<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.
	<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[®] Extract Binding Plate 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.

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	Cat. No.	Pack of
NucleoSpin® 96 Extract II	740 658.1	1 x 96 preps
NucleoSpin® 96 Extract II	740 658.2	2 x 96 preps
NucleoSpin® 96 Extract II	740 658.4	4 x 96 preps
NucleoSpin® 96 Extract II	740 658.24	24 x 96 preps
MN Square-well Block	740 678	20
Square-well Block	740 670	20
Round-well Block	740 671	20
MN Tube Strips	740 637	5 sets
Cap Strips	740 638	30
Self-adhering PE Foil	740 676	50
MN Frame	740 680	1
NucleoVac 96 vacuum manifold	740 681	1
Vacuum Regulator	740 641	1

6.3 References

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

6.4 Product use restriction / warranty

NucleoSpin® 96 Extract II kits components were developed, designed and sold **for research purposes only**. They are suitable **for *in vitro* uses only**. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather in the responsibility of the user to verify the use of the **NucleoSpin® 96 Extract II** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL guarantees 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 an extra copy.

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