



# **PCR clean-up**

## **User manual**

NucleoSpin<sup>®</sup> Multi-8 Extract

November 2003/Rev. 02

**MACHERY-NAGEL**



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# 1 Kit contents

	<b>NucleoSpin® Multi-8 Extract</b>	<b>NucleoSpin® Multi-8 Extract</b>
	<b>12 x 8 preps</b>	<b>60 x 8 preps</b>
<b>Cat. No.</b>	<b>740622</b>	<b>740622.5</b>
Buffer NTB	60 ml	3 x 100 ml
Buffer NT3 (concentrate)*	50 ml	200 ml
Buffer NE	20 ml	100 ml
NucleoSpin® Extract Binding Strips	12	60
MN Wash Plate (including 6 paper sheets)	1	5
MN Tube Strips	12	60
Cap Strips	12	60
Protocol	1	1

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

## 2 Product description

The **NucleoSpin® Multi-8 Extract** kit is recommended for purification of PCR products. The purified DNA is of high quality and can be used directly e.g. for sequencing. Recoveries between 75 – 90 % are obtained for DNA fragments in a size range of 100 – 10,000 bp. Primers, nucleotides, salts and polymerase are removed effectively. The kit is designed for use with the NucleoVac 96 vacuum manifold (Cat. No. 740 681).

### 2.1 Basic principle

The addition of chaotropic salt leads to a reversible adsorption of the DNA to the silica membrane of the NucleoSpin® Extract Strips. High purity of the PCR products eluted is achieved by removal of primers, salts, nucleotides, and proteins (polymerases, BSA) in subsequent washing steps. Pure DNA is finally eluted in elution buffer NE (5 mM Tris/HCl, pH 8.5) or water and can be used directly for further applications.

### 2.2 Kit specifications

**NucleoSpin® Multi-8 Extract** is designed for the flexible purification of PCR products in the convenient 8-well strip format.

- The kit is for use with the NucleoVac 96 vacuum manifold (see ordering information) or similar suitable vacuum manifolds (see section 2.4).
- 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 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.
- Using the **NucleoSpin® Multi-8 Extract** kit allows simultaneous processing of up to 48 samples typically within 30 minutes.

Kit specification at a glance	
Parameters	NucleoSpin® Multi-8 Extract
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	30 min/48 preps

## 2.3 Elution Procedure

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. 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.

The default volume of dispensed elution buffer in the available programs is 125 µl.

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

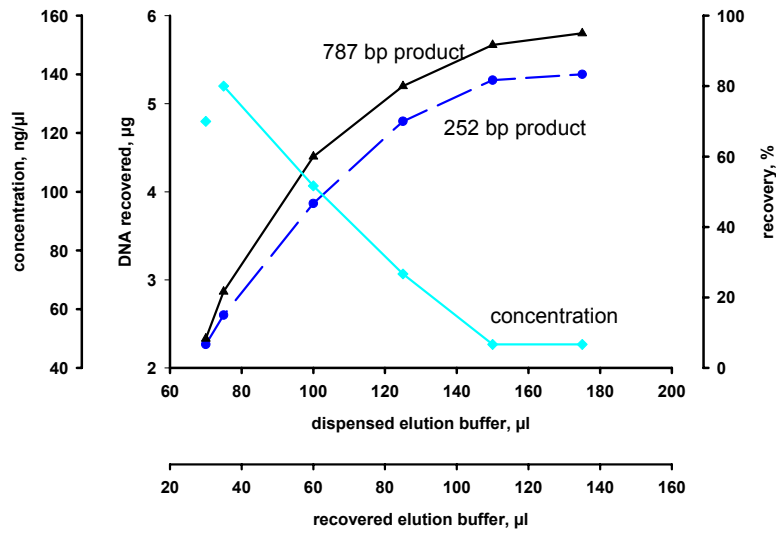


Fig. 1: Recovery rate and concentration depend on elution volume. Two different PCR products (252, 787 bp) have been purified with NucleoSpin® Robot-96 Extract.

Table 1: Average DNA recovery rate depends on the size of PCR product	
Size of PCR product [bp]	Average DNA recovery rate [%]
94	30 – 50
164	70 – 85
200	70 – 85
490	85 – 95
982	85 – 95
1500	80
2000	75
4000	50 - 60

## 2.4 Required Hardware

The **NucleoSpin® Multi-8 Extract** kit can be used manually with the NucleoVac 96 vacuum manifold (Cat. No. 740681) by using the Starter Set A containing Column Holders A and Dummy Strips (see ordering information).

Processing of the **NucleoSpin® Multi-8 Extract** kit under centrifugation is possible by using the Starter Set C (see ordering information), containing Column Holders, Dummy Strips, MN Square-well Blocks, MN Tube Strips. For detailed information refer to the Starter Set C manual.

## 2.5 Setting up vacuum

Use suitable house vacuum, membrane pump or water aspirator. Adjust vacuum as indicated in the individual steps of the protocol. See ordering information for vacuum regulator.

Establish a reliable vacuum source for the NucleoVac 96 manifold. The manifold may be used with vacuum pump, house vacuum or water aspirator. We recommend a vacuum of 200-400 mbar (pressure difference). Alternatively adjust vacuum that during the purification the sample flows through the column with a rate of 1-2 drops per second.

### 3 Storage conditions and preparation of working solutions

#### Attention

*Buffer NTB contains chaotropic salt! Wear gloves and goggles!*

- All components of the **NucleoSpin® Multi-8 Extract** kit should be stored at room temperature and are stable up to one year.

Before starting any **NucleoSpin® Multi-8 Extract** protocol prepare the following:

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

	<b>NucleoSpin® Multi-8 Extract</b>	<b>NucleoSpin® Multi-8 Extract</b>
	<b>12 x 8 preps</b>	<b>60 x 8 preps</b>
<b>Cat. No.</b>	<b>740 622</b>	<b>740 622.5</b>
Buffer NT3 (concentrate)	50 ml add 200 ml ethanol	200 ml add 800 ml ethanol

## 4 General Procedure

**1 Adjust** volume of PCR sample to 100 µl using Tris buffer (pH 7.0-7.5), nuclease-free water (pH 7.0-7.5), or buffer NE **up to 100 µl reaction volume**

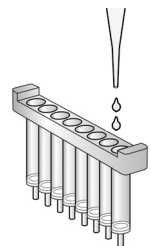
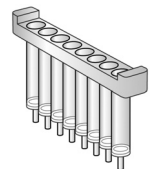
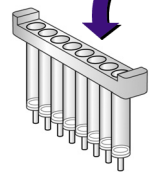
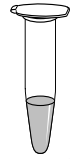
**2 Transfer** to appropriate vessel

**3 Add** binding buffer and mix **500 µl NTB mix**

**4 Transfer** sample to NucleoSpin® Extract Binding Strips

**5 Bind** DNA to silica membrane **1 min -200 to -400 mbar\***

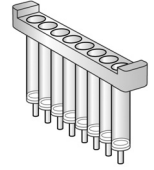
**6 Wash** silica membrane **2 x 900 µl NT3 1 min -200 to -400 mbar\***



\* Reduction of atmospheric pressure

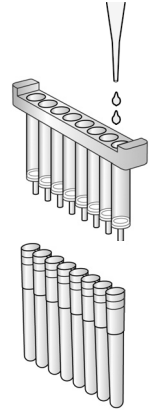
**7 Dry silica membrane**

**10 min,  
max. vacuum**



**8 Elute DNA**

**100-150 µl NE  
1 - 2 min  
-200 to -400 mbar\***



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\* Reduction of atmospheric pressure

## 4.1 Standard Protocol for PCR clean-up

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### 1 Adjust PCR reaction to 100 µl

For PCR reaction volumes less than 100 µl: Before starting the purification procedure, add Tris buffer (10 mM, pH 7.0-7.5), nuclease-free water (pH 7.0-7.5), or buffer NE to **adjust the reaction mixture to a final volume of 100 µl**. The amount of added mineral oil does not have to be considered

*Note:*

*Removal of mineral oil is not necessary.*

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### 2 For every PCR mix prepare one microcentrifuge tube and transfer sample

*Alternatively use a round-well block for mixing samples with buffer NTB (see ordering information).*

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### 3 Dispense 500 µl buffer NTB to each tube and mix well

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#### Prepare the NucleoVac 96 vacuum manifold

Insert appropriate number of NucleoSpin® Extract Binding Strips (yellow) into a Column Holder A. Close any unused openings of the Column Holder A with Dummy Strips.

*Note:*

*Make sure that the NucleoSpin® Extract 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. Rest the MN Wash Plate on top on the spacers. Insert Column Holder A with inserted NucleoSpin® Extract Binding Strips into the manifold lid and place lid on the manifold base.

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### 4 Transfer samples to the NucleoSpin® Extract Binding Strips

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### 5 Bind DNA to silica membrane

Close the valve of vacuum manifold. Adjust vacuum to –200 to –400 mbar. Open the valve and if necessary press down the column holder slightly until flow through starts. When the lysates have drained off, ventilate the manifold by closing the valve.

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## 6 Wash silica membrane

Add **900 µl** of **buffer NT3** (with ethanol) to each well of the NucleoSpin® Extract Binding Strips. Apply vacuum and allow the buffer to pass the columns. Ventilate the manifold.

Repeat this washing step once.

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## 7 Dry the membrane

After the final washing step close the valve, ventilate the vacuum manifold and remove the Column Holder A. Remove any residual washing buffer from the NucleoSpin® Extract Binding Strips. If necessary, dry the nozzles of the strips onto a clean blotting paper (supplied with the kit) or soft tissue until no drops come out. Remove manifold lid, MN Wash Plate, spacers, and waste reservoir from the vacuum manifold. Insert the Column Holder A holding the NucleoSpin® Extract Binding Strips into the lid and close the manifold. Apply maximum vacuum for 10 min to dry the membrane completely.

*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.

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## Prepare vacuum manifold for elution

Insert spacers (*Microtube Rack*), notched side up, into the grooves located at the short sides of the vacuum manifold. Rest the MN Tube Strip rack (supplied with the vacuum manifold) on the spacers inside the manifold base and insert the appropriate number of MN Tube Strips (supplied with the kit). Insert the column holder with the NucleoSpin® Extract Binding Strips in the manifold lid and place it on the manifold base.

Alternatively, elution can be performed in a microtiter plate. Insert spacers (*MTP/Multi-96 Plate*), notched side up, into the grooves located at the short sides of the vacuum manifold. Rest the microtiter plate (see ordering information) on the spacers inside the manifold base. Insert the column holder with the NucleoSpin® Extract Binding Strips in the manifold lid and place it on the manifold base.

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## **8 Elute DNA**

Elute the DNA by adding 100 - 150 µl of buffer NE (5 mM Tris-HCl, pH 8.5) or sterile dist. water (pH 8.5) to each well.

The elution buffer should be dispensed carefully onto the center of the silica membrane. Incubate the buffer on the membrane for 1- 3 minutes. Apply vacuum (-200 to -400 mbar) by opening the valve. Press down the column holder slightly and collect the eluted DNA. After the elution buffer has passed the columns, close the valve and ventilate the vacuum manifold.

Remove the collection tubes or microtiter plate containing the eluted DNA for further investigations.

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## 5 Appendix

### 5.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	<p><i>Buffer NT3 did not contain ethanol</i></p> <ul data-bbox="501 472 1414 539" style="list-style-type: none"> <li>• Addition of the indicated volume of 96 - 100 % ethanol to the buffer NT3 concentrate is required before use.</li> </ul>
	<p><i>Inappropriate elution buffer</i></p> <ul data-bbox="501 629 1445 741" style="list-style-type: none"> <li>• Elution is most effective at pH 8.5. If using water, check the pH value before use. Always use low salt buffer (NE) or water for elution.</li> </ul>
	<p><i>Elution buffer volume insufficient</i></p> <ul data-bbox="501 831 1374 898" style="list-style-type: none"> <li>• Optimal elution is achieved for an elution buffer volume of 100 - 150 µl. Do not use less than 100 µl of elution buffer.</li> </ul>
Suboptimal performance of DNA in sequencing reactions	<p><i>Carryover of ethanol</i></p> <ul data-bbox="501 1010 1469 1111" style="list-style-type: none"> <li>• Be sure to remove all of ethanolic buffer NT3 after the final wash step. Dry NucleoSpin® Extract Binding Strips for at least 10 min with maximum airflow.</li> </ul>
	<p><i>Elution of DNA with TE buffer</i></p> <ul data-bbox="501 1200 1445 1267" style="list-style-type: none"> <li>• EDTA may inhibit sequencing reactions. Repurify or precipitate DNA by ethanol and elute/redissolve in buffer NE or in water.</li> </ul>
	<p><i>Eluted DNA contains residual primers/primer dimers</i></p> <ul data-bbox="501 1357 1469 1435" style="list-style-type: none"> <li>• Minimize amount of primers in PCR reaction mixture. Make sure that the ratio of binding buffer NTB : PCR reaction mixture is 5:1.</li> </ul>

## 5.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Multi-8 Extract	740622	12 x 8 preps
NucleoSpin® Multi-8 Extract	740622.5	60 x 8 preps
Round-well Blocks	740671	20
Elution Plate, U-Bottom	740672	20
Self-adhering PE Foil	740676	50 sheets
MN Tube Strips (1 rack, 12 strips/8 tubes each)	740637	5 racks
Cap Strips (for one 8-well tube strip)	740638	30
NucleoVac 96 vacuum manifold	740681	1
Starter Set A for use of the NucleoSpin® 8 kits on the NucleoVac 96 vacuum manifold	740684	1 Set
Starter Set C for use of the NucleoSpin® 8 kits under centrifugation	740683	1 Set

## 5.3 References

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

## 5.4 Product Use Restriction / Warranty

**NucleoSpin® Mult-8 Extract** 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® Multi-8 Extract** 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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