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# PCR Clean-up

## User Manual

NucleoSpin® 8 Extract II

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MACHEREY-NAGEL

# Table of contents

# 1 Components

## 1.1 Kit contents

| | NucleoSpin® 8 Extract II | |
|---|---|---|
| | **12 x 8 preps** | **60 x 8 preps** |
| **Cat. No.** | **740668** | **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® Extract Binding Strips (yellow rings) | 12 | 60 |
| MN Wash Plates (including 6 Paper Sheets) | 1 | 5 |
| Tube Strips (with Cap Strips)[3] | 1 | 5 |
| Elution Plates, U-bottom (including one Self-adhering PE Foil) | 1 | 5 |
| User Manual | 1 | 1 |

## 1.2 Reagents and equipment to be supplied by user

Reagents: 96-100% Ethanol

The **NucleoSpin® 8 Extract II** kit can be used manually with the NucleoVac 96 Vacuum Manifold (Cat. No. 740681) by using the Starter Set A containing Column Holder A and NucleoSpin® Dummy Strips (see ordering information, section 6.1).

[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] Set of 1 rack with 12 8-well Tube Strips and 12 Cap Strips

# 2 Product description

## 2.1 Basic principle

The **NucleoSpin® 8 Extract II** kit is designed for vacuum based clean-up of DNA after amplification in PCR reactions or after enzymatic treatment. Within the **NucleoSpin® 8 Extract II** procedure the addition of chaotropic salt (Buffer NT) leads to a reversible adsorption of the DNA to the silica membrane of the **NucleoSpin® Extract 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 Extract II** is designed for DNA clean-up e.g. purification of PCR products in the convenient 8-well strip format. The 8-well strip format allows for 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 64-10,000 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 e.g. automated fluorescent sequencing, cloning or microarray technology.

- Using the **NucleoSpin® 8 Extract II** kit allows simultaneous processing of up to 48 samples typically within 30 minutes.

| Kit specification at a glance | |
|---|---|
| **Parameters** | **NucleoSpin® 8 Extract II** |
| Direct purification of amplified DNA | ++ |
| Desalination, removal of enzymes, nucleotides and /or labeling reagents like biotin or radioactive ATP etc. | ++ |
| Elution volume | 75-175 µl |
| DNA binding capacity | 15 µg |
| Time/prep | 30 min/48 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. A lower pH-value 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, as well as a 1-3 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 |

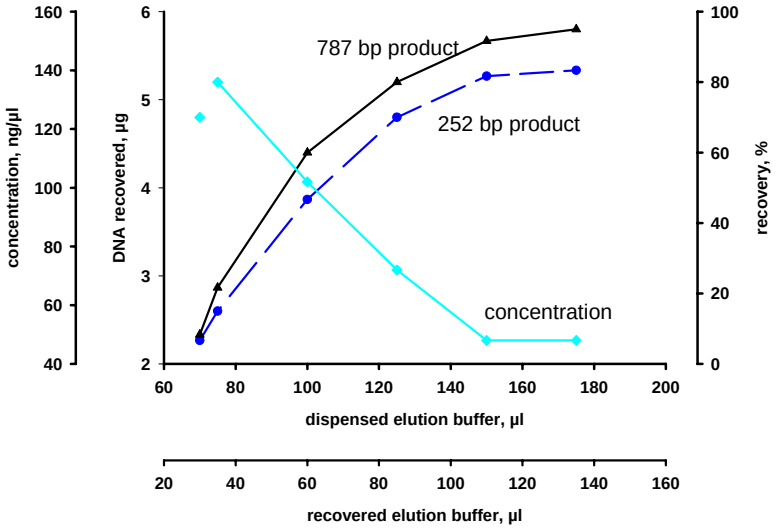



Fig. 1: Recovery rate and concentration depend on elution volume. Two different PCR products (252 bp, 787 bp) have been purified with NucleoSpin® 8 Extract II kit.

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 Setting up vacuum

Use suitable house vacuum, a membrane pump or a water aspirator. Adjust vacuum as indicated in the individual steps of the protocol. See ordering information, section 6.2, for vacuum regulator.

- 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 -0.2 to -0.4 bar (pressure difference). Alternatively adjust vacuum that during the purification the sample flows through the column with a rate of 1-2 drops per second.

## 2.5 Suitable vacuum manifolds

Besides the NucleoVac 96 Vacuum Manifold the NucleoSpin® 8 Extract kit can be used with other common vacuum manifolds. For further details see list below:

| Vacuum manifold | Compatibility | Additional equipment |
|---|---|---|
| Qiagen/ QIAvac 96* | yes | MN Frame (see ordering information) |
| Promega Vac-Man 96** | yes | - |

As for the NucleoVac 96 Vacuum Manifold the Starter Set A is required if other vacuum manifolds are used, too.

*In general the QIAvac 96 is suitable for the use with the Column Holder A and NucleoSpin® Extract 8 Binding Strips. 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.

**MN Wash Plate can not be used

# 3 Storage conditions and preparation of working solutions

**Attention**

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

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

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

- Add the indicated volume of 96-100 % ethanol to Buffer NT3 Concentrate.

| | **NucleoSpin® 8 Extract II** | |
|---|---|---|
| | **12 x 8 preps** | **60 x 8 preps** |
| **Cat. No.** | **740 668** | **740 668.5** |
| Buffer NT3 (Concentrate) | 100 ml<br>add 400 ml ethanol | 200 ml<br>add 800 ml ethanol |

# 4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 8 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

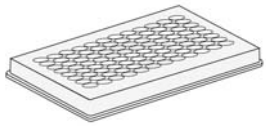
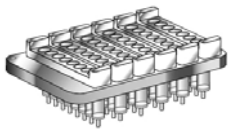
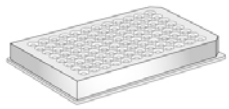
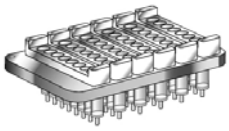
S 13 Keep away from food, drink and animal feedstuffs

* 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)

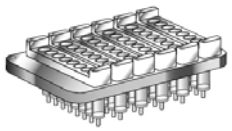
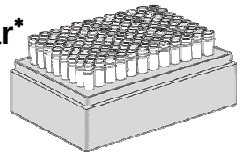
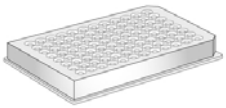
# 5 Protocol

## 5.1 General procedure

For detailed information on each step see section 5.2.

| | | | |
|---|---|---|---|
| **1** | **Adjust** 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 Buffer NE | **100 µl reaction volume** |  |
| **2** | **Prepare** the NucleoVac 96 Vacuum Manifold | | |
| **3** | **Dispense** Binding Buffer NT to NucleoSpin® Extract Binding strips | **200 µl NT** |   Column Holder A with NucleoSpin® Extract Binding Strips and MN Wash Plate |
| **4** | **Transfer** PCR samples to NucleoSpin® Extract Binding Strips and mix | **100 µl diluted sample, mix** | |
| **5** | **Bind** DNA to silica membrane of the NucleoSpin® Extract Binding Strips by applying vacuum | **-0.2 to -0.4 bar*<br>1 min** | |
| **6** | **Wash** silica membrane | **2 x 900 µl NT3**<br><br>**-0.2 to -0.4 bar<br>1 min** | |
| **7** | **Remove** MN Wash Plate | | |
| **8** | **Dry** silica membrane of the NucleoSpin® Extract Binding Strips<br><br>*Optional: Before applying vacuum dry the outlets of the NucleoSpin® Extract Binding Strips by placing them on a Paper Sheet.* | **10-15 min, max. vacuum** |  Column Holder A with NucleoSpin® Extract Binding Strips |

* Reduction of atmospheric pressure

| | | | |
|---|---|---|---|
| **9** | **Insert** rack withTube Strips or Elution Plate, U-bottom | |  |
| **10** | **Elute** DNA | **75-150 µl NE**<br>**-0.2 to -0.4 bar***   <br>**1 - 2 min** | Column Holder A with NucleoSpin® Extract Binding Strips and Tube Strips or Elution Plate, U-bottom |

* Reduction of atmospheric pressure

## 5.2 Standard protocol for DNA clean-up

**1 Adjust volume of sample reaction mix to 100 µl**

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

*Note: Adjusting the reaction volume to 100 µl allows easier and more convenient transfer of the samples with a multichannel pipette.*

**2 Prepare the NucleoVac 96 Vacuum Manifold**

Insert an appropriate number of NucleoSpin® Extract 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® 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. Place 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. Check and adjust the vacuum (pressure difference -0.2 bar). Release the vacuum.

**3 Dispense Binding Buffer NT to the NucleoSpin® Extract Binding Strips**

Add 2 volumes of Buffer NT for one volume of sample (e.g. 200 µl Buffer NT for 100 µl reaction mix).

**4 Transfer samples to the NucleoSpin® Extract Binding Strips and mix**

Mix sample and Buffer NT by pipetting up and down 5 times.

*Optional: Premix Buffer NT and sample in a suitable 96-well plate (e.g. Round-well Block). Transfer the mixture to the NucleoSpin® Extract Binding Strips.*

**5 Bind DNA to silica membrane of the NucleoSpin® Extract Binding Strips**

Apply vacuum by opening the valve and press down the Column Holder A slightly until flow-through starts. Allow the samples to pass the wells and release the vacuum.

## 6 Wash silica membrane of the NucleoSpin® Extract Binding Strips

Add **900 µl** of **Buffer NT3** (with ethanol added) to each well of the NucleoSpin® Extract Binding Strips. Apply vacuum and allow the buffer to pass the wells. Release the 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 Column Holder A with inserted NucleoSpin® Extract Binding Strips. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

## 8 Dry silica membrane of the NucleoSpin® Extract Binding Strips

Remove any residual washing buffer from the NucleoSpin® Extract Binding Strips. If necessary, dry the nozzles of the strips on a clean paper sheet (supplied with the kit) or soft tissue until no further drops come out. Insert the Column Holder A holding the NucleoSpin® Extract Binding Strips into the lid and close the manifold. Apply maximum vacuum for 10-15 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, release the vacuum.

## 9 Insert rack with Tube Strips for elution

Insert spacers (*Microtube Rack*), notched side up, into the grooves located at the short sides of the vacuum manifold. Place the Tube Strip rack on the spacers inside the manifold base and insert the appropriate number of Tube Strips. 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 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 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.

## 10 Elute DNA

Elute the DNA by adding 75-150 µl of Buffer NE (5 mM Tris-HCl, pH 8.5) or nuclease free water (pH 8.5) to each well of the NucleoSpin® Extract Binding Strips .

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 (-0.2 to -0.4 bar). Press down the column holder slightly and collect the eluted DNA. After the elution buffer has passed the wells release the vacuum.

Remove the Rack with Tube Strips or Elution Plate containing the eluted DNA.

# 6 Appendix

## 6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Poor DNA yield | *Buffer NT3 did not contain ethanol*<br>• Addition of the indicated volume of 96-100 % ethanol to the Buffer NT3 Concentrate is required before use.<br>*Inappropriate elution buffer*<br>• 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.<br>*Elution buffer volume insufficient*<br>• Optimal elution is achieved for an elution buffer volume of 100-150 µl. Do not use less than 75 µl of elution buffer. |
| Suboptimal performance of DNA in sequencing reactions | *Carryover of ethanol*<br>• 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.<br>*Elution of DNA with TE buffer*<br>• EDTA may inhibit sequencing reactions. Repurify or precipitate DNA by ethanol and elute/redissolve in Buffer NE or in water.<br>*Eluted DNA contains residual primers/primer dimers*<br>• 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® 8 Extract II | 740668 | 12 x 8 preps |
| NucleoSpin® 8 Extract II | 740668.5 | 60 x 8 preps |
| Round-well Block | 740671 | 20 |
| Elution Plate, U-bottom | 740672 | 20 |
| Self-adhering PE Foil | 740676 | 50 sheets |
| Tube Strips | 740637 | 5 racks |
| Cap Strips | 740638 | 30 |
| NucleoVac 96 Vacuum Manifold | 740681 | 1 |
| NucleoVac Vacuum Regulator | 740641 | 1 |
| Starter Set A<br>(for use of the NucleoSpin® 8-well strips on the NucleoVac 96 Vacuum Manifold) | 740684 | 1 set |

## 6.3 References

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

## 6.4 Product use restriction / warranty

**NucleoSpin® 8 Extract II** kit components were developed, designed, distributed, 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® 8 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

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