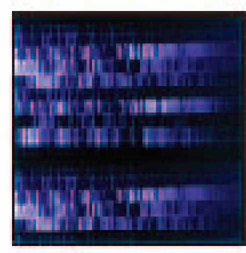

# PCR clean-up Gel extraction

## User manual

NucleoSpin® Extract II

September 2005/Rev. 02

MACHEREY-NAGEL

# Protocol at a glance (Rev. 02)

## NucleoSpin® Extract II

| | Gel extraction | | PCR clean-up |
|---|---|---|---|
| 1 Excise DNA fragment | | | |
| 2 Gel lysis / Adjust binding conditions | 200 µl NT / 100 mg<br>50°C<br>5-10 min | | 200 µl NT / 100 µl |
| 3 Bind DNA | 1 min<br>11,000 x g | | |
| 4 Wash silica membrane | 600 µl NT3<br>1 min<br>11,000 x g | | |
| 5 Dry silica membrane | 2 min<br>11,000 x g | | |
| 6 Elute DNA | 15-50 µl NE<br>1 min RT<br>1 min<br>11,000 x g | | |

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# 1 Kit contents

| | NucleoSpin® Extract II | | |
|---|---|---|---|
| | **10 preps** | **50 preps** | **250 preps** |
| **Cat. No.** | **740609.10** | **740609.50** | **740609.250** |
| Buffer NT | 6 ml | 30 ml | 2 x 75 ml |
| Buffer NT3 (concentrate) | 7 ml | 2 x 7 ml | 40 ml |
| Buffer NE | 5 ml | 15 ml | 50 ml |
| NucleoSpin® Extract II columns (yellow) | 10 | 50 | 250 |
| NucleoSpin® collecting tubes (2 ml) | 10 | 50 | 250 |
| Protocol | 1 | 1 | 1 |

# 2 Product description

## 2.1 The basic principle

With the **NucleoSpin® Extract II** method, DNA binds to a silica membrane in the presence of chaotropic salt added by binding buffer NT. The binding mixture is loaded directly onto **NucleoSpin® Extract II** columns. Contaminations like salts and soluble macromolecular components are removed by a simple washing step with ethanolic buffer NT3. Pure DNA is finally eluted under low ionic strength conditions with slightly alkaline buffer NE (5 mM Tris/HCl, pH 8.5).

## 2.2 About this user manual

Experienced users who are performing the purification of PCR* products or DNA fragments from agarose gels using a **NucleoSpin® Extract II** isolation kit may refer to the Protocol-at-a-glance instead of this user manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read the entire user manual.

## 2.3 Kit specifications

- The **NucleoSpin® Extract II** kit is designed for the purification of DNA from TAE/TBE agarose gels and for direct purification of PCR* products (two applications in one kit).
- The **NucleoSpin® Extract II** buffer formulation ensures complete removal of primers from PCR* reactions while small DNA fragments are still bound and purified with high recovery.
- With **NucleoSpin® Extract II** even DNA fragments from PCR* reaction buffers rich in various detergents can be purified with high recovery.
- The adsorption of DNA to the **NucleoSpin® Extract II** membrane is pH-dependent. Optimal recovery is achieved by using TAE standard gels or reaction mixtures with pH 6-8.
- Standard as well as low melting agarose gels can be used.
- The prepared DNA fragments can be used directly in applications like automated fluorescent DNA sequencing, PCR*, or any kind of enzymatic manipulation.

* PCR is patented by Roche Diagnostics

| Kit specification at a glance | |
|---|---|
| **Parameters** | **NucleoSpin® Extract II** |
| DNA fragments from agarose gels | ✓ |
| Concentration, removal of salts, enzymes, nucleotides a/o labeling reagents like biotin or radioactive ATP etc. | ✓ |
| Direct purification of amplified DNA | ✓ |
| Elution volume | 15-50 µl |
| Binding capacity | 15 µg |
| Time/prep | 10 min for 6 preps |
| Purification of reaction mixtures **without SDS** | see section 7.1 |
| Purification of reaction mixtures **containing SD** | see section 7.2 |
| Purification of **single stranded DNA** | see section 7.3 |
| Removal of small DNA fragments and **primer-dimers** | see section 2.4 |

## 2.4 Removal of small DNA fragments and primer-dimers

NucleoSpin® Extract II is designed to remove even traces of unused primers and at the same time to purify PCR products down to 65 bp. However, in some cases it is necessary to exclude these small fragments, e.g. primer-dimers or side products resulting from unspecific annealing, since they might interfere with your downstream sequencing or cloning applications.

Removal of double stranded DNA <65 bp can be achieved by diluting an aliquot of buffer NT with sterile water in an appropriate ratio and then proceed with the standard protocol (see section 6). Diluting buffer NT in a certain range lowers the binding efficiency for small fragments without compromising the recovery of larger PCR products. Which dilution ratio to choose depends on the fragment size that is to be purified as well as on the PCR buffer system that is used.

**Influence of fragment size:** ***The smaller the fragment in question is, the less you have to dilute buffer NT.***

**Influence of PCR buffer system:** The influence of the PCR buffer system on the removal of small fragments is more complex. Some reaction buffers contain detergents like Tween or high concentrations of additives like betaine to lower the melting temperature of the DNA template. These substances can usually be found in PCR buffers for high fidelity or long range PCR. They tend to lower the binding efficiency of DNA to the silica membrane and therefore have to be considered when choosing a dilution ratio of buffer NT. ***As a rule of thumb if a PCR buffer system without special additives is used, adding 3 to 5 volumes of water to 1 volume of buffer NT will lead to removal of small fragments up to 100 bp. Otherwise adding 1 to 3 volumes of water to 1 volume of buffer NT will be sufficient.***

So for each size of small fragments <65 bp that has to be removed and for each PCR system there may be checked the appropriate ratio of buffer NT dilution in advance.

Figure 1 shows a purification result with an NT dilution series. Pure NT (lane 3) as well as NT plus one volume of water (lane 4) lead to 100% recovery of a PCR fragment ladder (lane 2). Use of more diluted buffer NT cuts off more and more of the low molecular mass bands. Usually a dilution with 5 volumes of water should be sufficient to eliminate even larger unwanted primer-dimer fragments while purifying the 164 bp fragment with >90%.

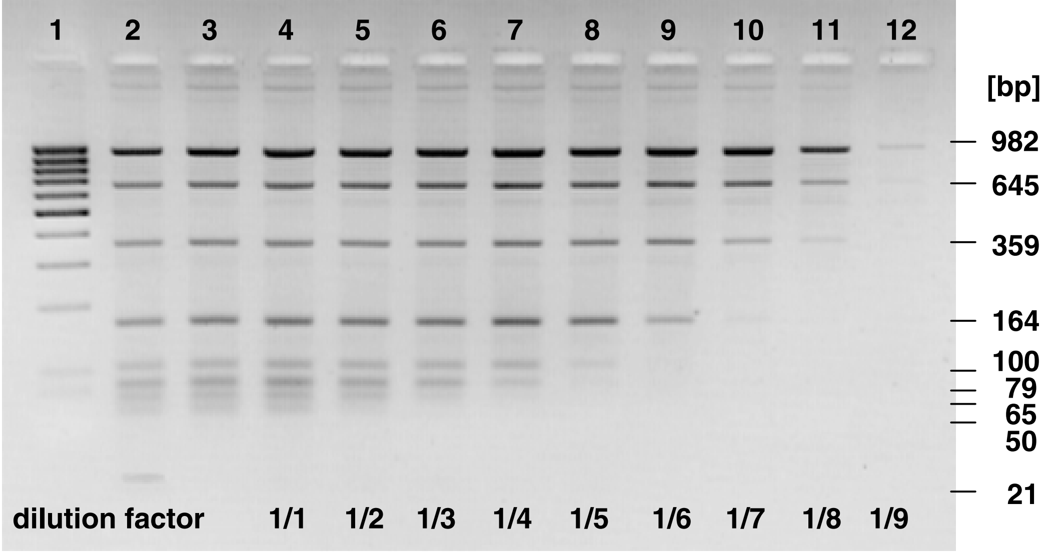



**Figure 1 Purification of PCR reactions using buffer NT dilutions**

Lane 1: GeneRuler 100 bp DNA Ladder (MBI Fermentas)
Lane 2: DNA ladder input (21 b primer, 50, 65, 79, 100, 164, 359, 645 and 982 bp fragment) amplified using Biotaq DNA Polymerase (Bioline)
Lane 3: Purification with 100% NT
Lane 4-12: Purification with NT diluted with 1-9 volumes of water

## 2.5 Elution procedures

- For elution of DNA one of the following solutions can be used: Buffer NE (supplied, 5 mM Tris/HCl, pH 8.5) / TE buffer, pH 8.5 / distilled water, pH 8.5.

- Note: EDTA in TE buffer may cause problems in subsequent reactions, and the pH of distilled water should be checked before use to avoid lower recovery yields. See Table 1 for the correlation between dispensed elution buffer volumes and typical recoveries for purification of 1-5 µg of PCR* fragments (for gel extraction, recovery is approx. 10 % lower).

- With an elution volume of 15 µl of buffer NE a typical recovery of 70-95 % is usually obtained for DNA fragments between 50-10,000 bp resulting in highly concentrated eluates (see Table 1, Figure 2). If larger amounts (5-15 µg) of DNA have to be purified (e.g. from PCR* reactions > 100 µl or gel slices > 200 mg) elution with at least 50 µl of buffer NE is recommended. Primers are not bound.

- Yields of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer NE and incubate for 1-2 min before collecting eluate by centrifugation. For fragments >10 kb the use of our **NucleoTraP® CR** kit is recommended.

Table 1: DNA Recovery with NucleoSpin® Extract II

| Fragment length | Elution volume | Recovery |
|---|---|---|
| 65 bp | 15 µl | 85 % |
| | 25 µl | 90 % |
| | 50 µl | 95 % |
| | 100 µl | 95 % |
| 400 bp | 15 µl | 85 % |
| | 25 µl | 95 % |
| | 50 µl | 100 % |
| | 100 µl | 100 % |
| 700 bp | 15 µl | 85 % |
| | 25 µl | 90 % |
| | 50 µl | 95 % |
| | 100 µl | 95 % |
| 1500 bp | 15 µl | 85 % |
| | 25 µl | 85 % |
| | 50 µl | 90 % |
| | 100 µl | 95 % |

* PCR is patented by Roche Diagnostics

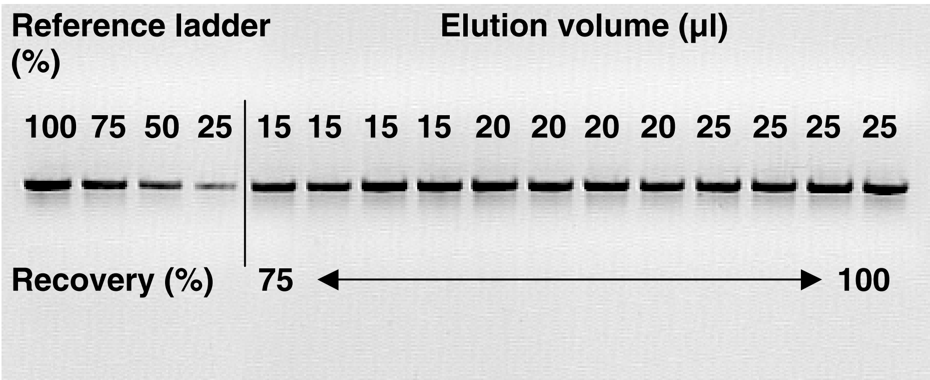



**Figure 2 DNA recovery with different elution volumes**

A PCR sample with a fragment size of 782 bp was purified from a 1 % agarose gel according to the standard protocol of NucleoSpin® Extract II using different elution volumes as shown. All elution volumes were adjusted to 25 µl plus 4.5 µl loading dye. For analysis the mixture was loaded on a 1 % TAE gel. The recovery was estimated by comparison with a fragment ladder.

# 3 Storage conditions and preparation of working solutions

**Attention:**

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

- The **NucleoSpin® Extract II** kit should be stored at room temperature and is stable for up to one year.

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

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

| | **NucleoSpin® Extract II** | | |
|---|---|---|---|
| | **10 preps** | **50 preps** | **250 preps** |
| **Cat. No.** | **740609.10** | **740609.50** | **740609.250** |
| Buffer NT3 | 7 ml<br>add 28 ml ethanol | 2 x 7 ml<br>add 28 ml ethanol | 40 ml<br>add 160 ml ethanol |

# 4 Safety instructions – risk and safety phrases

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

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

| Buffer | 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

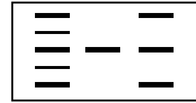
**Safety Phrases**

S 13 Keep away from food, drink and animal feedstuffs

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

# 5 Protocol for DNA extraction from agarose gels

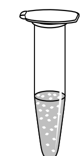
## 1 Excise DNA fragment



Take a clean scalpel to excise the DNA fragment from an agarose gel. Excise gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer it to a clean tube.

## 2 Gel lysis

For each **100 mg** of agarose gel add **200 µl buffer NT**.



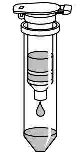
**+ 200 µl NT per 100 mg gel**

*For gels containing > 2 % agarose, double the volume of buffer NT. The maximum amount of gel slice per NucleoSpin® Extract II column is 400 mg or 200 mg of a high percentage gel > 2 %. In this case 2 loading steps are required (step 3).*

Incubate sample at **50°C** until the gel slices are dissolved **(5-10 min)**. Vortex the sample briefly every 2-3 min until the gel slices are dissolved **completely**!

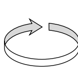
**50°C, 5-10 min**

## 3 Bind DNA



Place a NucleoSpin® Extract II column into a 2 ml collecting tube and load the sample.

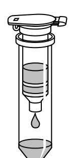
**load sample**



Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

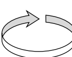
**1 min, 11,000 x g**

## 4 Wash silica membrane



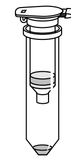
Add **600 µl buffer NT3**. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

**+ 600 µl NT3**



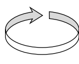
**1 min, 11,000 x g**

**5 Dry silica membrane**



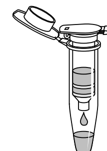
Centrifuge for **2 min** at **11,000 x g** to remove **buffer NT3** quantitatively. Make sure the spin column doesn't come in contact with the flow-through while removing it from the centrifuge and the collecting tube.

**2 min,**
**11,000 x g**



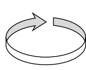
*Residual ethanol from buffer NT3 might inhibit subsequent reactions and has to be removed in this step. In addition to centrifugation, total removal can be achieved by incubation of NucleoSpin® Extract II columns for 2-5 min at 70°C prior to elution.*

**6 Elute DNA**



Place the NucleoSpin® Extract II column into a **clean** 1.5 ml microcentrifuge tube. Add **15-50 µl elution buffer NE** and incubate at **room temperature** for **1 min** to increase the yield of eluted DNA. Centrifuge for **1min** at **11,000 x g**.

**+ 15-50 µl NE**



*Yield of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature for 1 min before collecting eluate by centrifugation.*

**1 min,**
**11,000 x g**

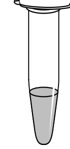
# 6 Protocol for direct purification of PCR products

### 1 Adjust DNA binding conditions

Mix **1 volume** of **sample** with **2 volumes** of **buffer NT** (e.g. mix 100 µl PCR reaction and 200 µl NT).

*For sample volumes < 50 µl adjust the volume of the reaction mix to 50 µl using TE buffer (pH 7.5).*

*For removal of DNA fragments < 65 bp, dilutions of buffer NT can be used instead of 100 % NT. Please refer to section 2.4.*



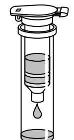
**+ 2 vol NT**

**per**

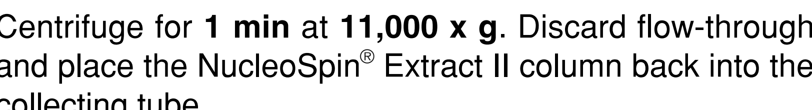
**1 vol sample**

### 2 Bind DNA

Place a NucleoSpin® Extract II column into a 2 ml collecting tube and load the sample.



**load sample**

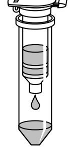


Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

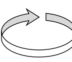
**1 min, 11,000 x g**

### 3 Wash silica membrane

Add **600 µl buffer NT3**. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.



**+ 600 µl NT3**

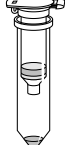


**1 min, 11,000 x g**

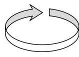
### 4 Dry silica membrane

Centrifuge for **2 min** at **11,000 x g** to remove **buffer NT3** quantitatively. Make sure the spin column doesn't come in contact with the flow-through while removing it from the centrifuge and the collecting tube.

*Residual ethanol from buffer NT3 might inhibit subsequent reactions and has to be removed in this step. In addition to centrifugation, total removal can be achieved by incubation of NucleoSpin® Extract II columns for 2-5 min at 70°C prior to elution.*

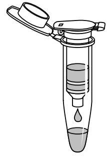


**2 min, 11,000 x g**



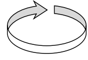
## 5 Elute DNA

Place the NucleoSpin® Extract II column into a **clean** 1.5 ml microcentrifuge tube. Add **15-50 µl elution buffer NE** and incubate at **room temperature** for **1 min** to increase the yield of eluted DNA. Centrifuge for **1min** at **11,000 x g**.



**+ 15-50 µl NE**

*Yield of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature for 1 min before collecting eluate by centrifugation.*



**1 min, 11,000 x g**

# 7 Support protocols

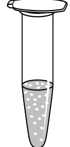
## 7.1 Concentration and removal of salts, enzymes, etc. Purification of samples without SDS (buffer NT)

***Note: Buffer NT is provided with the NucleoSpin® Extract II kits.***

**1 Adjust DNA binding conditions**

Mix **1 volume** of sample with **2 volumes** of **buffer NT** (e.g. 200 µl NT and 100 µl reaction mixture).

*If your sample contains large amounts of detergents or other critical substances, double the volume of NT.*



**+ 2 vol NT per 1 vol sample**

**2 Bind DNA**

Continue with **step 2** of the protocol for direct purification of PCR products (section 6).

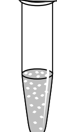
## 7.2 Concentration and removal of salts, enzymes, etc. Purification of samples containing SDS (buffer NTB)

The NucleoSpin® Extract II buffer NT is compatible with most commonly used detergents except sodium dodecyl sulfate (SDS). For purification of DNA from SDS containing buffers, e.g. in applications like "Chromatin Immunoprecipitation" (CHiP), the SDS compatible binding buffer NTB can be used.

***Note: Buffer NTB has to be ordered separately (150 ml NTB, Ref. 740595.150, see Ordering information).***

**1 Adjust DNA binding conditions**

Mix **1 volume** of **sample** with **5 volumes** of **buffer NTB** (e.g. 100 µl reaction with 500 µl NTB).



**+ 5 vol NTB per 1 vol sample**

**2 Bind DNA**

Continue with **step 2** of the protocol for direct purification of PCR products (section 6).

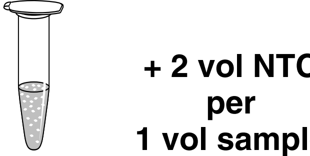
## 7.3 Purification of single stranded DNA (buffer NTC)

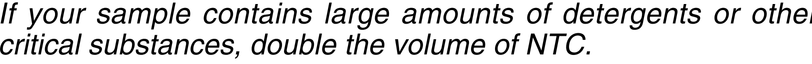
The NucleoSpin® Extract II buffer NT is able to bind single stranded DNA (ssDNA) > 150 bases. Shorter oligonucleotides, especially primers, are completely removed. If you need to purify short ssDNA, the additional binding buffer NTC can be used (see Figure 3).

***Note: Buffer NTC has to be ordered separately (100 ml NTC, Ref. 740654.100, see Ordering information).***

**1 Adjust DNA binding conditions**

Mix **1 volume** of sample with **2 volumes** of **buffer NTC** (e.g. 100 µl PCR reaction mix and 200 µl NTC).



*If your sample contains large amounts of detergents or other critical substances, double the volume of NTC.*

**2 Bind DNA**

Continue with **step 2** of the protocol for direct purification of PCR products (section 6).

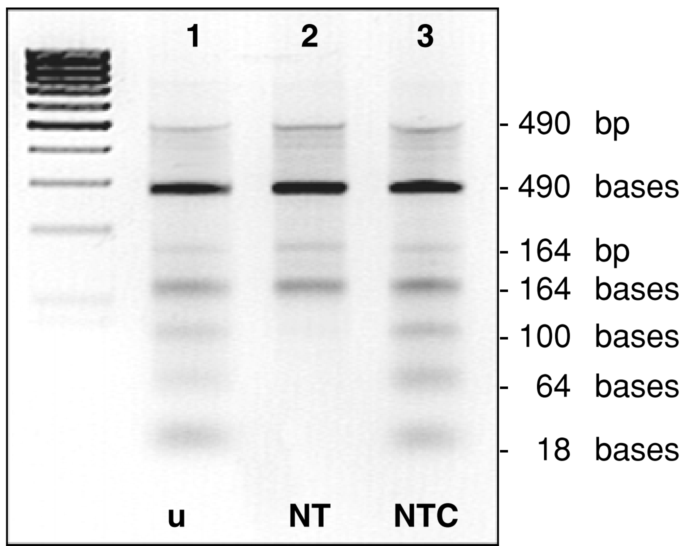



**Figure 3 Purification of dsDNA and ssDNA using buffers NT and NTC**

PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with I-Exonuclease. Samples were purified using binding buffers NT and NTC and run on a 1 % TAE agarose gel. Remaining double stranded DNA can be seen as faint bands. The corresponding single stranded DNA is running slightly faster due to secondary structure formation. Compared to the input DNA (u, lane 1), NT removes ssDNA < 150 bases (NT, lane 2), whereas NTC leads to full recovery of even primer oligonucleotides (NTC, lane 3).

# 8 Appendix

## 8.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Incomplete lysis of agarose slices | *High amount of agarose*<br>• Use doubled volumes of buffer NT for highly concentrated a/o LMP (low melting point) agarose gels.<br><br>*Time and temperature*<br>• Check incubation temperature. Depending on the weight of the gel slice, incubation (section 5, step 2) can be prolonged up to 20 min. Vortex every 2 min and check integrity of the gel slice. Very large gel slices can be quenched or crushed before addition of buffer NT. |
| Low DNA yield | *Reagents not applied properly*<br>• Add indicated volume of 96-100% ethanol to buffer NT3 concentrate and mix well before use.<br><br>*Incompletely dissolved gel slice*<br>• Increase time or add another two volumes of NT and vortex the tube every 2 minutes during incubation at 50°C. Small pieces of gel are hardly visible and contain DNA that will be lost for purification.<br><br>*Insufficient drying of the **NucleoSpin® Extract II** silica membrane*<br>• Centrifuge 5 min at 11,000 x g or incubate column for 2-5 min at 70°C before elution to remove ethanolic buffer NT3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Remove the spin cup carefully from centrifuge and collecting tube and avoid contact of spin cup with flow-through.<br><br>*Not enough elution buffer*<br>• Especially when larger amounts of DNA (> 5 µg) are bound, increase elution buffer volume up to 100 µl.<br><br>*Isolation of large DNA fragments*<br>• Preheat elution buffer NE to 70°C, and incubate on the silica membrane at room temperature for 2 min before centrifugation. |

| Problem | Possible cause and suggestions |
|---|---|
| Suboptimal performance of DNA in sequencing reactions | *Carry-over of ethanol/ethanolic buffer NT3*<br>• Centrifuge 5 min at 11,000 x g or incubate column for 2-5 min at 70°C before elution to remove ethanolic buffer NT3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Remove the spin cup carefully from centrifuge and collecting tube and avoid contact of spin cup with flow-through.<br><br>*Elution of DNA with buffers other than buffer NE e.g. TE buffer (Tris/EDTA)*<br>• EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in buffer NE or water.<br><br>*Not enough DNA used for sequencing reaction*<br>• Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions. |

## 8.2 Ordering information

| Product | Cat. No. | Pack of |
|---|---|---|
| NucleoSpin® Extract II | 740609.10 | 10 preps |
| NucleoSpin® Extract II | 740609.50 | 50 preps |
| NucleoSpin® Extract II | 740609.250 | 250 preps |
| Buffer NT | 740614.100 | 100 ml |
| Buffer NTB | 740595.150 | 150 ml |
| Buffer NTC | 740654.100 | 100 ml |
| Buffer NT3 concentrate (for 100 ml buffer) | 740598 | 20 ml |
| NucleoSpin® collecting tubes (2 ml) | 740600 | 1000 |

## 8.3 References

**Vogelstein B., and D. Gillespie.** 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76:** 615-619.

## 8.4 Product use restriction / warranty

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

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

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