Bioanalysis

MACHEREY-NAGEL

User manual

PCR clean-up and Gel extraction

- NucleoSpin® Gel and PCR Clean-up Midi
- NucleoSpin® Gel and PCR Clean-up Maxi

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1 Components

1.1 Kit contents

	NucleoSpin® Gel and PCR Clean-up Midi	NucleoSpin® Gel and PCR Clean-up Maxi
REF	**20 preps 740986.20**	**20 preps 740610.20**
Binding Buffer NTI	200 mL	450 mL
Wash Buffer NT3 (Concentrate)*	50 mL	100 mL
Elution Buffer NE**	30 mL	60 mL
NucleoSpin® Gel and PCR Clean-up Midi Columns	20	–
NucleoSpin® Gel and PCR Clean-up Maxi Columns	–	20
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* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer NE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol

Consumables

- 15 mL centrifuge tubes (NucleoSpin® Gel and PCR Clean-up Midi)
- 50 mL centrifuge tubes (NucleoSpin® Gel and PCR Clean-up Maxi)
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for 15 mL tubes (NucleoSpin® Gel and PCR Clean-up Midi)
- Centrifuge for 50 mL tubes (NucleoSpin® Gel and PCR Clean-up Maxi)
- Heating block, water bath, or thermomixer for gel extraction
- Scalpel to cut agarose gels
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)

2 Product description

2.1 The basic principle

NucleoSpin® Gel and PCR Clean-up Midi and **NucleoSpin® Gel and PCR Clean-up Maxi** are developed as 2 in 1 kits. DNA fragments can be purified from enzymatic reactions such as PCR as well as from agarose gels.

The sample is mixed with Binding Buffer NTI and, in case of a cut out gel band, heated to dissolve the agarose. In the presence of chaotropic salt the DNA is bound to the silica membrane of a NucleoSpin® Gel and PCR Clean-up Column. Contaminations are removed by simple washing steps with ethanolic Wash Buffer NT3. Pure DNA is finally eluted under low salt conditions with slightly alkaline Elution Buffer NE (5 mM Tris/HCl, pH 8.5).

2.2 Kit specifications

- **NucleoSpin® Gel and PCR Clean-up kits** are designed for fast purification of PCR products or DNA from enzymatic reactions, and the extraction of DNA fragments from TAE or TBE agarose gels.
- The **NucleoSpin® Gel and PCR Clean-up Midi** allows the processing of up to 4 mL of PCR / enzymatic reaction or 4 g of agarose gel.
- The **NucleoSpin® Gel and PCR Clean-up Maxi** allows the processing of up to 10 mL of PCR / enzymatic reaction or 10 g of agarose gel.
- The **NucleoSpin® Gel and PCR Clean-up** buffer formulations ensures complete removal of all kinds of contaminations like
  - nucleotides, primers
  - enzymes
  - mineral oil
  - PCR additives (e.g., salts, betaine, DMSO)
  - detergents (e.g., Tween 20, Triton X-100)
  - dyes (e.g., ethidiumbromide)
  - unbound labels and tags
- Primers from PCR reactions are quantitatively eliminated while small DNA fragments are still bound and purified with high recovery.
- **NucleoSpin® PCR and Gel Clean-up kits** can be used with all kinds of agarose gels (high or low melting) with 1 % to 5 % agarose and a variety of buffer systems like TAE or TBE (tips and tricks in section 2.4).
- Several support protocols extend the application range of **NucleoSpin® Gel and PCR Clean-up kits** to
  - Clean-up of DNA from reaction mixtures containing SDS (section 5.4, 6.4)
  - Clean-up of single stranded DNA (section 5.5, 6.5)
  - Extraction of RNA from agarose gels (section 5.3, 6.3)

- The purified and concentrated DNA can directly be used for hybridization, sequencing, PCR, restriction, ligation, in vitro transcription, labeling or any other kind of enzymatic reaction.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Gel and PCR Clean-up Midi	NucleoSpin® Gel and PCR Clean-up Maxi
Sample material	Up to 4 mL of PCR reaction or 4 g of gel	Up to 10 mL of PCR reaction or 10 g of gel
Binding capacity	75 µg	250 µg
Fragment length	50 bp– ~ 20 kbp	50 bp– ~ 20 kbp
Elution volume	200–400 µL	1000 µL

2.3 pH indicator

The optimal pH to bind even small DNA fragments to the silica membrane of the NucleoSpin® Gel and PCR Clean-up Column is approximately 5.0–6.0. The Binding Buffer NTI is sufficiently buffered to maintain this pH for all standard PCR reaction buffers or agarose gel buffer systems.

In addition, the colored binding buffer helps identify undissolved pieces of agarose during DNA gel extraction.

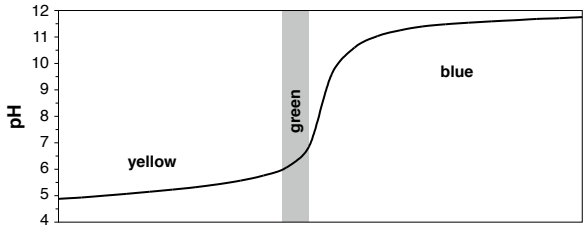


Figure 1 Titration curve of Binding Buffer NTI with pH indicator

A yellow color indicates the optimal pH < 6.0 (Figure 1). If the pH increases to around 7 after adding the sample, the solution will turn green. In case the pH is higher than 7 the solution turns blue. If a change in color is observed, the pH should be adjusted by adding more Buffer NTI or by titrating the pH to < 6.0 with 4 M sodium acetate pH 5.0 or small amounts of hydrochloric acid (HCl).

2.4 Tips and tricks for DNA extraction from agarose gels

Subject	Recommendation
Buffer system	TBE (Tris-Borate-EDTA) buffer has a higher buffering capacity than TAE (Tris-Acetate-EDTA) which is needed for runs overnight and offers a better resolution for small DNA fragments. TBE buffer can be used in combination with NucleoSpin® Gel and PCR Clean-up kits. However, **fresh TAE buffer should be preferred over TBE** for preparative agarose gels. TAE does not interact with agarose resulting in **higher DNA yields**. Additionally, linear DNA runs faster and the resolution of large DNA fragments is higher. Furthermore, supercoiled plasmid is separated better from linear and open circle DNA.
Running conditions	**The temperature during electrophoresis should be low** to increase the resolution of the DNA separation and to avoid melting of the gel and denaturation of DNA. Use fresh buffer, run the gel at low voltage (< 60 V), and as short as possible. As soon as the DNA band of interest is sufficiently separated from the rest, stop the gel and cut out the band.
Cutting out the band	**Expose the gel to UV light as short as possible.** Use the longest UV wave length that is allowed by your gel documentation system. Prolonged exposure and short wave lengths can damage the DNA. Wear gloves and a face mask to protect your skin and eyes from UV light. Make sure to cut through the gel vertically and remove all excess agarose. Use 0.7–1.0 % agarose gels rather than higher percentages.
Size of gel piece	Make sure to actually **weigh the gel** since its weight is easily underestimated. Up to 10 g of agarose gel can be dissolved with 10 mL of Buffer NTI and loaded onto the column in two steps. Please note that NucleoSpin® Gel and PCR Clean-up Midi is limited to 4g of agarose gel.

2.5 DNA recovery depends on fragment size and elution volume

After washing with Buffer NT3 the DNA is sticking to the silica membrane. The number of interactions with Si-OH groups of the silica increase with the size of the DNA fragment. Therefore, large DNA with several kilo base pairs binds much stronger and is much more difficult to elute than small DNA with just several hundred base pairs. **NucleoSpin® Gel and PCR Clean-up kits** are recommended for DNA up to 10–15 kbp. Longer fragments can be purified, but recovery might be low. Furthermore, fragments larger than 20 kbp might be mechanically damaged by the fast centrifugation through the membrane. For very large fragments using NucleoTrap® or NucleoTraP®CR should be considered (see ordering information, 7.2).

To elute the DNA, water and a pH > 7 is needed to reestablish the hydrate shell. It is highly recommended to **elute DNA with Elution Buffer NE** (5 mM Tris/HCl, pH 8.5) provided with the kit or standard TE buffer to ensure best elution efficiency. Note that EDTA in TE buffer may cause problems in subsequent enzymatic reactions. Do not use deionized water since its pH is usually too acidic. If even less salt than 5 mM Tris has to be used, dilute Elution Buffer NE with distilled water and make sure, the pH is still > 7. Unbuffered elution buffer should not be used.

The standard elution buffer volume is 2 x 200 µL (NucleoSpin® Gel and PCR Clean-up Midi) or **1000 µL** (NucleoSpin® Gel and PCR Clean-up Maxi) which is the best compromise for high DNA recovery and high DNA concentration for fragments < 1000 bp.

Elution after gel extraction is 10–20 % less efficient than elution of purified PCR products. Elution of several kbp long DNA fragments is 10–30 % less efficient than elution of 500 bp fragments. To improve the DNA recovery after gel extraction and/or for large DNA fragments, the following modifications can be applied to the standard elution procedure:

- Heat elution buffer to 70 °C and incubate elution buffer on the column at 70 °C for 5 minutes.
- Apply elution buffer to the column and centrifuge first at 30–50 x *g* for 1 min and then at 11.000 x *g* for 1 min.

The most relevant improvements in terms of nucleic acid recovery can be achieved by a two-fold elution. For a two-fold elution, the eluate of the elution step is reloaded onto the silica for a second elution procedure. Doing so, the total elution volume remains low while the recovery is enhanced.

This is especially true for gel extraction procedures. DNA tends to get stuck to the silica matrix and shows a delayed elution profile. Multiple rounds of elution can increase the DNA recovery significiantly.

2.6 Salt carry-over and low A_{260} / A_{230}

The silica membrane technology to purify RNA or DNA is based on the ability of chaotropic salts to destroy the water shell around nucleic acids. Two commonly used chaotropic salts are guanidine hydrochloride (GuHCl) and guanidinium thiocyanate (GuSCN). In solution they both have the same guanidinium cation but different anions. These anions are not only responsible for their different behavior towards nucleic acids but also for their different UV absorption spectra. GuHCl exhibits only minimal absorption < 220 nm even at a concentration of 1 M, whereas GuSCN already shows significant absorption < 240 nm (1 mM, Figure 1) and even < 260 nm (1 M).

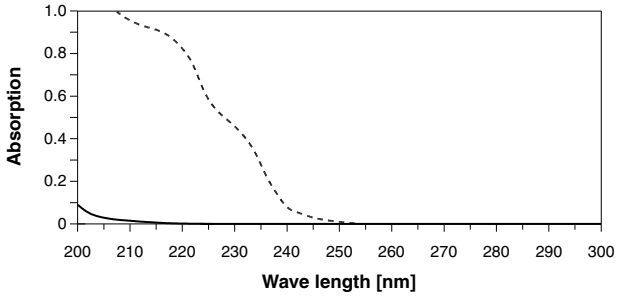


Figure 2 UV absorption spectra of 1 mM GuHCl (solid line) and 1 mM GuSCN (dotted line)

Especially the difference in absorption at 230 nm can have a huge impact on the purity ratio A_{260} / A_{230} if DNA is contaminated with chaotropic salts. Carry over of GuSCN can lower the ratio from its ideal value of > 2.0 to below 1.5 or even 1.0. GuHCl on the other hand is invisible at this wave length and does not alter the ratio at all. This effect, however, is only detectable with very small amounts of DNA like typical yields of PCR reactions or gel extractions. Technical advances in UV-VIS spectrometry now allow measuring these small amounts of DNA in small volumes thereby raising concerns that the DNA might be too “dirty”. This problem does usually not occur with larger amounts of DNA since its own absorption at 230 nm masks the small contribution of any contamination.

The concentration of contaminating chaotropic salt is usually in the range of 100 µM to 1 mM and does not have any negative influence on enzymatic downstream applications like, e.g., PCR, restriction, or ligation. Figure 2 shows qPCR inhibition by GuSCN and GuHCl. It demonstrates that PCR only starts to be inhibited by chaotropic salts with about 100-fold higher concentration (40 mM). Furthermore it shows clearly, that not only GuSCN can cause inhibition but also the photometrically invisible GuHCl.

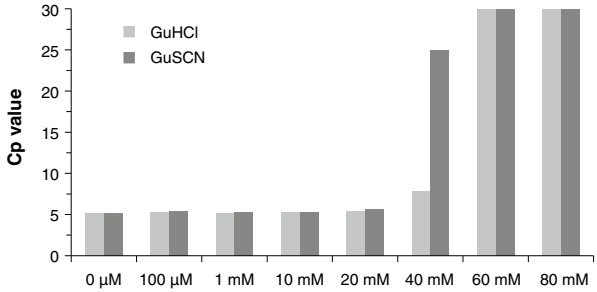


Figure 3 qPCR inhibition by GuHCl (light gray) and GuSCN (dark gray)
A 164 bp DNA fragment was amplified from 5 ng pBS template with DyNAmo Capillary Master Mix (NEB) in a Lighcycler real-time PCR machine (Roche) in the presence of 0–80 mM GuHCl or GuSCN.

Salt carry over always happens – with both GuSCN and GuHCl – and could only be minimized by most extensive washing. This, however, is unnecessary, since the final concentration of chaotropic salt in eluates is much too small to have any negative effect. Thus, non-ideal A_{260}/A_{230} can simply be ignored.

3 Storage conditions and preparation of working solutions

Attention: *Buffer NTI contains chaotropic salt. Wear gloves and goggles!*

Storage conditions:

- All kit components can be stored at room temperature and are stable for at least one year.

Before starting any **NucleoSpin® Gel and PCR Clean-up** protocol 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 at least one year.

	NucleoSpin® Gel and PCR Clean-up Midi	NucleoSpin® Gel and PCR Clean-up Maxi
REF	**20 preps 740986.20**	**20 preps 740610.20**
Wash Buffer NT3 (Concentrate)	50 mL Add 200 mL ethanol	100 mL Add 400 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® Gel and PCR Clean-up Midi** and the **NucleoSpin® Gel and PCR Clean-up Maxi** kit contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section.*

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS-Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
NTI	Guanidinium thiocyanate 30–60 % *Guanidinthiocyanat 30–45 %* CAS 593-84-0	 WARNING *ACHTUNG*	302, 412	264W, 273, 301+312, 330

Hazard phrases

H 302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.

H 412 Harmful to aquatic life with long lasting effects.
Schädlich für Wasserorganismen, mit langfristiger Wirkung.

Precaution phrases

P 264W Wash with water thoroughly after handling.
Nach Gebrauch mit Wasser gründlich waschen.

P 273 Avoid release to the environment.
Freisetzung in die Umwelt vermeiden.

P 301+312 IF SWALLOWED: Call a POISON CENTER / doctor / … / if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt / … anrufen.

P 330 Rinse mouth.
Mund ausspülen.

For further information please see Material Safety Data Sheets (***www.mn-net.com***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**www.mn-net.com**).*

 The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

5 NucleoSpin® Gel and PCR Clean-up Midi protocols

5.1 PCR clean-up

The following protocol is suitable for PCR clean-up as well as DNA concentration and removal of salts, enzymes, etc. from enzymatic reactions (SDS < 0.1 %).

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding condition

For sample volumes < 1 mL adjust the volume of the reaction mixture to 1 mL with water. It is not necessary to remove mineral oil.

Mix 1 **volume of sample** with **2 volumes** of **Buffer NTI** (e.g., mix 1 mL PCR reaction and 2 mL Buffer NTI).

+ 2 vol NTI per 1 vol sample

2 Bind DNA

Place a **NucleoSpin® Gel and PCR Clean-up Midi Column** into a Collection Tube (15 mL) and load the sample.

Load sample

Centrifuge for **1 min at ≥ 3,000 x *g***. Discard flow-through and place the column back into the collection tube.

≥ 3,000 x *g* 1 min

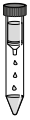
3 Wash silica membrane

Add **4 mL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Midi Column. Centrifuge for **1 min** at **≥ 3,000 x *g***. Discard flow-through and place the column back into the collection tube.

+ 4 mL NT3

≥ 3,000 x *g* 1 min

Repeat the washing step.



+ 4 mL NT3

≥ 3,000 x *g* 1 min

4 Dry silica membrane

Centrifuge for **10 min** at **≥ 3,000 x *g*** to remove **Buffer NT3** completely. Make sure the spin column does not come in contact with the flow through while removing it from the centrifuge and the collection tube.

<u>Note:</u> Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

≥ 3,000 x *g*
10 min

5 Elute DNA

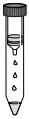
Place the NucleoSpin® Gel and PCR Clean-up Midi Column into a **new** 15 mL centrifuge tube (not provided). Add **200 µL Buffer NE** and incubate at **70 °C** for **5 min**. Centrifuge for **2 min** at ≥ **3,000 x *g***.

+ 200 µL NE

70 °C
5 min

≥ 3,000 x *g*
2 min

Repeat elution with fresh elution buffer.



+ 200 µL NE

70 °C
5 min

≥ 3,000 x *g*
2 min

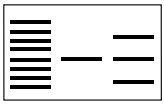
5.2 DNA extraction from agarose gels

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Excise DNA fragment / solubilize gel slice

Note: Minimize UV exposure time to avoid damaging the DNA. Refer to section 2.4 for more tips on agarose gel extraction.



Take a clean scalpel to excise the DNA fragment from an agarose gel. Remove all excess agarose.

Determine the weight of the gel slice and transfer it to a clean tube.

+ 1 mL NTI per 1 g gel

For each **1 g of agarose gel < 2 %** add **1 mL Buffer NTI**.

For gels containing > 2 % agarose, double the volume of Buffer NTI.

Incubate sample for **10–20 min** at **50 °C**. Vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

50 °C
10–20 min

2 Bind DNA

Load sample

Place a **NucleoSpin® Gel and PCR Clean-up Midi Column** into a Collection Tube (15 mL) and load the sample.

Centrifuge for **1 min** at **≥ 3,000 x *g***. Discard flow through and place the column back into the collection tube.

≥ 3,000 x *g*
1 min

Repeat this step to load more sample material.

3 Wash silica membrane

Add **4 mL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Midi Column. Centrifuge for **1 min** at **≥ 3,000 x *g***. Discard flow through and place the column back into the collection tube.

+ 4 mL NT3

≥ 3,000 x *g*
1 min

Repeat this washing step.

+ 4 mL NT3

≥ 3,000 x *g*
1 min

4 Dry silica membrane

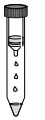
Centrifuge for **10 min** at **≥ 3,000** x ***g*** to remove **Buffer NT3** completely. Make sure the spin column does not come in contact with the flow through while removing it from the centrifuge and the collection tube.

<u>Note:</u> Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

≥ 3,000 x *g*
10 min

5 Elute DNA

Place the NucleoSpin® Gel and PCR Clean-up Midi Column into a **new** 15 mL centrifuge tube (not provided). Add **200 µL Buffer NE** and incubate at **70 °C** for **5 min**. Centrifuge for **2 min** at **≥ 3,000 x *g***.

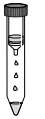


+ 200 µL NE

70 °C
5 min

≥ 3,000 x *g*
2 min

Repeat elution with fresh elution buffer.



+ 200 µL NE

70 °C
5 min

≥ 3,000 x *g*
2 min

5.3 RNA extraction from agarose gels (Buffer NTC)

Not only DNA but also RNA can be extracted from agarose gels. To efficiently bind especially the small, single stranded RNA **Binding Buffer NTC** has to be used instead of standard Binding Buffer NTI.

To fractionate RNA run a standard RNA gel with denaturing RNA loading buffer but **do not use formaldehyde or glyoxal**. These compounds not only inactivate RNases and denature RNA but also modify RNA. As a result the RNA yield is significantly reduced and more important the RNA might not work properly in enzymatic downstream applications like RT-PCR or *in vitro* transcriptions.

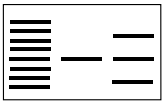
Without formaldehyde the RNA is very sensitive to contaminating RNases. Use gloves and make sure all equipment is RNase-free, especially the agarose, and the running buffers. Run the gel as short and as cold (low voltage, cold room) as possible. Note that the RNA might form secondary structures and might run differently from denaturing agarose gels.

Note: Buffer NTC has to be ordered separately (125 mL Buffer NTC, REF 740654.100, see ordering information, 7.2)

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Excise DNA fragment / solubilize gel slice



Note: Minimize UV exposure time to avoid damaging the DNA. Refer to section 2.4 for more tips on agarose gel extraction.

Take a clean scalpel to excise the DNA fragment from an agarose gel. Remove all excess agarose.

Determine the weight of the gel slice and transfer it to a clean tube.

+ 1 vol NTC per 1 vol sample

For each **1 g of agarose gel < 2 %** add **1 mL Buffer NTC**.

For gels containing > 2 % agarose, double the volume of Buffer NTC.

Incubate sample for **10–20 min** at **50 °C**. Vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

50 °C
10–20 min

2 Bind RNA

Continue with **step 2** of the protocol for DNA extraction from agarose gels (section 5.2).

5.4 DNA clean-up of samples containing SDS (Buffer NTB)

Buffer NTI from the NucleoSpin® Gel and PCR Clean-up kit is compatible with most commonly used detergents except sodium dodecyl sulfate (SDS). For purification of DNA from samples without SDS the standard protocol for PCR clean-up can be used (see section 5.1). For purification of DNA from SDS containing buffers, for example 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 Buffer NTB, REF 740595.150, see ordering information, section 7.2).**

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1	**Adjust DNA binding condition** Mix **1 volume of sample** with **5 volumes of Buffer NTB** (e.g., 1 mL reaction mix with 5 mL Buffer NTB). *Note: If SDS starts to precipitate add 1 volume of isopropanol or warm sample to 20–30 °C.*	**+ 5 vol NTB per 1 vol sample**
2	**Bind DNA** Continue with **step 2** of the protocol for PCR clean up (section 5.1).	

5.5 Single stranded DNA clean-up (Buffer NTC)

Buffer NTI from the NucleoSpin® Gel and PCR Clean-up kit 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 4).

***Note:* Buffer NTC has to be ordered separately (125 mL Buffer NTC, REF 740654.100, see ordering information, section 7.2).**

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1	**Adjust DNA binding condition** Mix **1 volume of sample** with **2 volumes** of **Buffer NTC** (e.g., 1 mL PCR reaction mix and 2 mL Buffer NTC). *If your sample contains large amounts of detergents or other critical substances, double the volume of Buffer NTC.*	**+ 2 vol NTC per 1 vol sample**
2	**Bind DNA** Continue with **step 2** of the protocol for PCR clean up (section 5.1).	

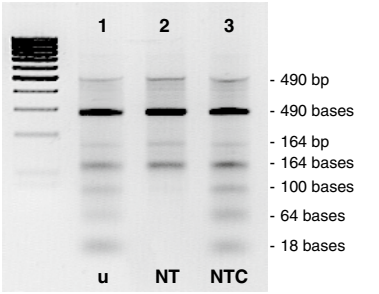


Figure 4 Purification of dsDNA and ssDNA using buffers NTI and NTC
PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with λ-Exonuclease to yield single stranded DNA. Samples were purified using Binding Buffer NTI and NTC and run on a 1 % TAE agarose 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), Buffer NTI removes ssDNA < 150 bases (NTI, lane 2), whereas Buffer NTC leads to full recovery of even primer oligonucleotides (NTC, lane 3).

6 NucleoSpin® Gel and PCR Clean-up Maxi protocols

6.1 PCR clean-up

The following protocol is suitable for PCR clean-up as well as DNA concentration and removal of salts, enzymes, etc. from enzymatic reactions (SDS < 0.1 %).

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding condition

For sample volumes < 1 mL adjust the volume of the reaction mixture to 1 mL with water. It is not necessary to remove mineral oil.

Mix 1 **volume of sample** with **2 volumes** of **Buffer NTI** (e.g., mix 1 mL PCR reaction and 2 mL Buffer NTI).

+ 2 vol NTI per 1 vol sample

2 Bind DNA

Place a **NucleoSpin® Gel and PCR Clean-up Maxi Column** into a Collection Tube (50 mL) and load the sample.

Load sample

Centrifuge for **1 min at ≥ 3,000 x *g***. Discard flow-through and place the column back into the collection tube.

≥ 3,000 x *g* 1 min

3 Wash silica membrane

Add **10 mL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Maxi Column. Centrifuge for **1 min** at **≥ 3,000 x *g***. Discard flow through and place the column back into the collection tube.

+ 10 mL NT3

≥ 3,000 x *g* 1 min

+ 10 mL NT3

Repeat the washing step.

≥ 3,000 x *g* 1 min

4 Dry silica membrane

Centrifuge for **10 min** at **≥ 3,000 x *g*** to remove **Buffer NT3** completely. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the collection tube.

Note: Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

≥ 3,000 x *g*
10 min

5 Elute DNA

Place the NucleoSpin® Gel and PCR Clean-up Midi Column into a **new** 50 mL centrifuge tube (not provided). Add **1000 µL Buffer NE** and incubate at **70 °C** for **5 min**. Centrifuge for **2 min** at ≥ **3,000 x *g***.

+ 1000 µL NE

70 °C
5 min

≥ 3,000 x *g*
2 min

Reload eluate into the column and repeat elution at least once.

Note: Repeated elution steps with the same volume of Buffer NE will increase the DNA recovery without reducing the DNA concentration.

Reload eluate into column

70 °C
5 min

≥ 3,000 x *g*
2 min

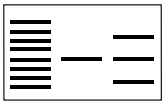
6.2 DNA extraction from agarose gels

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Excise DNA fragment / solubilize gel slice

Note: Minimize UV exposure time to avoid damaging the DNA. Refer to section 2.4 for more tips on agarose gel extraction.



Take a clean scalpel to excise the DNA fragment from an agarose gel. Remove all excess agarose.

Determine the weight of the gel slice and transfer it to a clean tube.

+ 1 mL NTI per 1 g gel

For each **1 g of agarose gel < 2 %** add **1 mL Buffer NTI**.

For gels containing > 2 % agarose, double the volume of Buffer NTI.

Incubate sample for **10–20 min** at **50 °C**. Vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

50 °C
10–20 min

2 Bind DNA

Load sample

Place a **NucleoSpin® Gel and PCR Clean-up Maxi Column** into a Collection Tube (50 mL) and load the sample.

Centrifuge for **1 min** at **≥ 3,000 x *g***. Discard flow through and place the column back into the collection tube.

≥ 3,000 x *g*
1 min

Repeat this step to load more sample material.

3 Wash silica membrane

+ 10 mL NT3

Add **10 mL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Maxi Column. Centrifuge for **1 min** at **≥ 3,000 x *g***. Discard flow through and place the column back into the collection tube.

≥ 3,000 x *g*
1 min

+ 10 mL NT3

Repeat this washing step.

≥ 3,000 x *g*
1 min

4 Dry silica membrane

Centrifuge for **10 min** at **≥ 3,000** x ***g*** to remove **Buffer NT3** completely. Make sure the spin column does not come in contact with the flow through while removing it from the centrifuge and the collection tube.

Note: Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

≥ 3,000 x *g*
10 min

5 Elute DNA

Place the NucleoSpin® Gel and PCR Clean-up Maxi Column into a **new** 50 mL centrifuge tube (not provided). Add **1000 µL Buffer NE** and incubate at **70 °C** for **5 min**. Centrifuge for **2 min** at **≥ 3,000 x *g***.

+ 1000 µL NE

70 °C
5 min

≥ 3,000 x *g*
2 min

Reload eluate into the column and repeat elution at least once.

Reload eluate into column

70 °C
5 min

Note: Repeated elution steps with the same volume of Buffer NE will increase the DNA recovery without reducing the DNA concentration.

≥ 3,000 x *g*
2 min

6.3 RNA extraction from agarose gels (Buffer NTC)

Not only DNA but also RNA can be extracted from agarose gels. To efficiently bind especially the small, single stranded RNA **Binding Buffer NTC** has to be used instead of standard Binding Buffer NTI.

To fractionate RNA run a standard RNA gel with denaturing RNA loading buffer but **do not use formaldehyde or glyoxal**. These compounds not only inactivate RNases and denature RNA but also modify RNA. As a result the RNA yield is significantly reduced and more important the RNA might not work properly in enzymatic downstream applications like RT-PCR or *in-vitro* transcriptions.

Without formaldehyde the RNA is very sensitive to contaminating RNases. Use gloves and make sure all equipment is RNase-free, especially the agarose, and the running buffers. Run the gel as short and as cold (low voltage, cold room) as possible. Note that the RNA might form secondary structures and might run differently from denaturing agarose gels.

***Note:** Buffer NTC has to be ordered separately (125 mL Buffer NTC, REF 740654.100, see ordering information, section 7.3)*

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1	**Adjust RNA binding condition** Mix **1 volume** of sample with **1 volume** of **Buffer NTC** (e.g., 2 g gel and 2 mL Buffer NTC).	**+ 1 vol NTC per 1 vol sample**
2	**Bind RNA** Continue with **step 2** of the protocol for DNA extraction from agarose gels (section 6.2).	

6.4 DNA clean-up of samples containing SDS (Buffer NTB)

Buffer NTI from the NucleoSpin® Gel and PCR Clean-up kit is compatible with most commonly used detergents except sodium dodecyl sulfate (SDS). For purification of DNA from samples without SDS the standard protocol for PCR clean-up can be used (see section 6.1). For purification of DNA from SDS containing buffers, for example 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 Buffer NTB, REF 740595.150, see ordering information, section 7.3).**

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1	**Adjust DNA binding condition** Mix **1 volume of sample** with **5 volumes of Buffer NTB** (e.g., 1 mL reaction mix with 5 mL Buffer NTB). *Note: If SDS starts to precipitate add 1 volume of isopropanol or warm sample to 20–30 °C.*	**+ 5 vol NTB per 1 vol sample**
2	**Bind DNA** Continue with **step 2** of the protocol for PCR clean up (section 6.1).	

6.5 Single stranded DNA clean-up (Buffer NTC)

Buffer NTI from the NucleoSpin® Gel and PCR Clean-up Maxi kit 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 5).

***Note:* Buffer NTC has to be ordered separately (125 mL Buffer NTC, REF 740654.100, see ordering information, section 7.3).**

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1	**Adjust DNA binding condition** Mix **1 volume of sample** with **2 volumes** of **Buffer NTC** (e.g., 1 mL PCR reaction mix and 2 mL Buffer NTC). *If your sample contains large amounts of detergents or other critical substances, double the volume of Buffer NTC.*	**+ 2 vol NTC per 1 vol sample**
2	**Bind DNA** Continue with **step 2** of the protocol for PCR clean up (section 6.1).	

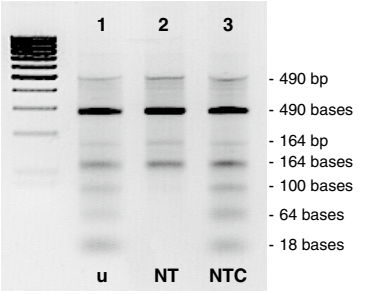


Figure 5 Purification of dsDNA and ssDNA using buffers NTI and NTC
PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with λ-Exonuclease to yield single stranded DNA. Samples were purified using Binding Buffer NTI and NTC and run on a 1 % TAE agarose 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), Buffer NTI removes ssDNA < 150 bases (NTI, lane 2), whereas Buffer NTC leads to full recovery of even primer oligonucleotides (NTC, lane 3).

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete melting of agarose slices	*Time and temperature* • Check incubation temperature and volume of Buffer NTI. Increase incubation time. Vortex every 2 min and check integrity of the gel slice. Very large gel slices can be crushed before addition of Buffer NTI to shorten the melting time.
Low DNA yield	*Reagents not prepared properly* • Add indicated volume of 96–100 % ethanol to Buffer NT3 Concentrate and mix well before use. *Incompletely dissolved gel slice* • Increase time or add another two volumes of Buffer NTI 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. *Insufficient drying of the **NucleoSpin® Gel and PCR Clean-up kit** silica membrane* • Centrifuge 10 min at ≥ 3,000 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 the centrifuge and collection tube and avoid contact of spin cup with flow-through. *Incomplete elution* • Especially for larger amounts of DNA, long DNA fragments (> 1000 bp), or after gel extraction, do multiple elution steps with fresh buffer, heat to 70 °C, and incubate for 5 min. See section 2.4 for detailed information.

Problem	Possible cause and suggestions
Appearance of additional bands on agarose gel after gel extraction	*DNA was denatured during purification* • In case water is used for elution and agarose with a low ion content is used for agarose gel electrophoresis, the formation of denaturated (single-stranded) DNA might be promoted. To re-anneal the DNA, add all components of the subsequent enzymatic reaction omitting the enzyme. Incubate at 95 °C for 2 min and let the mixture cool slowly to room temperature (at this step the DNA re-anneals). Add the enzyme and continue with your downstream application. • Use fresh running buffer and run at low voltage to lower the temperature. High temperature might promote DNA denaturation during electrophoresis.
Suboptimal performance of DNA in sequencing, restriction, or ligation reactions	*Carry over of ethanol / ethanolic Buffer NT3* • Centrifuge 10 min at ≥ 3,000 x *g* or better incubate column for 5–10 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 the centrifuge and collection tube, and avoid contacting the spin cup with the flow-through. • Use a different brand of ethanol to reconstitute Buffer NT3 or ethanol that is not denatured. The denaturing components might not evaporate as fast as ethanol, end up concentrated in the eluate, and inhibit enzymes like ligase. *Carry over of chaotropic salts* • Perform the optional washing step. • Additionally, 1 mL NT3 can be loaded before the drying step. (Note: The volume of Buffer NT3 included in the kit is not sufficient for this modification for all preparations, but can be ordered separately, see ordering information, page 31.) *Elution of DNA with buffers other than Buffer NE, for example TE buffer (Tris/EDTA)* • EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Buffer NE or water. *Not enough DNA used for sequencing reaction* • Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions.

Problem	Possible cause and suggestions
Suboptimal performance of DNA in sequencing, restriction, or ligation reactions (continued)	*DNA was damaged by UV light* • Reduce UV exposure time to a minimum when excising a DNA fragment from an agarose gel.
Suboptimal performance of DNA in NanoDrop® Spectro-photometer Analysis or Agilent's Bioanalyzer	*Carry-over of traces of silica particles* • NanoDrop® Spectrophotometer technology is very sensitive to any particles included in the sample material. To pellet the silica particles centrifuge > 2 min at 11,000 x *g* and take the supernatant for further use.
Low ratio A_{260}/A_{230}	*Carry-over of chaotropic salts* • Refer to detailed troubleshooting "Suboptimal performance of DNA in sequencing, restriction, or ligation reactions - Carry-over of chaotropic salts" and see section 2.5 for detailed information.

7.2 Ordering information

Product	REF	Pack of
NucleoSpin® Gel and PCR Clean-up XS	740611.10/.50/.250	10/50/250
NucleoSpin® Gel and PCR Clean-up	740609.10/.50/.250	10/50/250
NucleoSpin® Gel and PCR Clean-up Midi	740986.20	20
NucleoSpin® Gel and PCR Clean-up Maxi	740610.20	20
Buffer NTI	740305.120	200 mL
Buffer NTB	740595.150	150 mL
Buffer NTC	740654.100	125 mL
Buffer NT3 Concentrate (for 125 mL Buffer NT3)	740598	25 mL
Collection Tubes (2 mL)	740600	1000
NucleoTrap®	740584.10/.50/.250	10/50/250
NucleoTrap®CR	740587.10/.50/.250	10/50/250

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

7.3 References

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

7.4 Product use restriction / warranty

NucleoSpin® Gel and PCR Clean-up Midi and **NucleoSpin® Gel and PCR Clean-up Maxi** 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.

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IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN-VITRO-DIAGNOSTIC USE!

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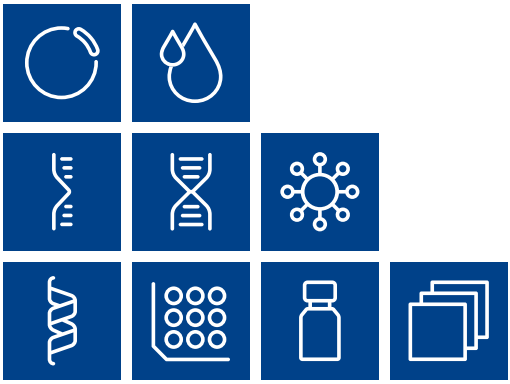

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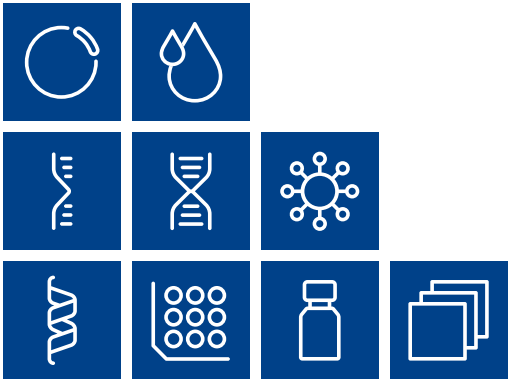
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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