Bioanalysis

MACHEREY-NAGEL

User manual

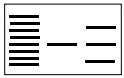
PCR clean-up and Gel extraction

- NucleoSpin® Gel and PCR Clean-up

April 2022 / Rev. 07

PCR clean-up, gel extraction

Protocol at a glance (Rev. 07)

	PCR clean-up	Gel extraction	DNA clean-up (with SDS)	Single stranded DNA clean-up
1 **PCR clean-up, DNA clean-up, or single stranded DNA clean-up:** **Adjust binding condition** **Gel extraction:** **Excise DNA fragment / solubilize gel slice**	 200 µL NTI/ 100 µL PCR	  200 µL NTI/ 100 mg gel 50 °C 5–10 min	 500 µL NTB/ 100 µL sample	 200 µL NTC/ 100 µL sample
2 **Bind DNA**			11,000 x *g* 30 s	
3 **Wash silica membrane**			700 µL NT3 11,000 x *g* 30 s Recommended: 2nd wash 700 µL NT3 11,000 x *g* 30 s	
4 **Dry silica membrane**			11,000 x *g* 1 min	
5 **Elute DNA**			15–30 µL NE RT 1 min 11,000 x *g* 1 min	

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1 Components

1.1 Kit contents

	NucleoSpin® Gel and PCR Clean-up		
	10 preps	**50 preps**	**250 preps**
REF	**740609.10**	**740609.50**	**740609.250**
Binding Buffer NTI	10 mL	40 mL	200 mL
Wash Buffer NT3 (Concentrate)*	6 mL	25 mL	2 x 50 mL
Elution Buffer NE**	13 mL	13 mL	30 mL
NucleoSpin® Gel and PCR Clean-up Columns (yellow rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
User manual	1	1	1

* 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

- 1.5 mL microcentrifuge tubes
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Heating block, water bath, or thermomixer for gel extraction
- Scalpel to cut agarose gels
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Gel and PCR Clean-up** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at www.mn-net.com.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

NucleoSpin® Gel and PCR Clean-up is developed as a 2-in-1 kit allowing DNA fragments to 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, it is 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. Finally, the pure DNA is 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** is designed for fast purification of PCR products, such as DNA from enzymatic reactions, as well as the extraction of DNA fragments from TAE or TBE agarose gels.
- Only two volumes of binding buffer per volume of sample are needed to process up to 200 µL of PCR / enzymatic reaction, or 200 mg of agarose gel, with only one loading step. By adding additional Binding Buffer NTI (see ordering information) it is possible to load an unlimited amount of sample volumes onto a single column (tips and tricks in section 2.5).
- Up to ~ 15 µg DNA from 50 bp to at least ~ 20 kbp can be purified efficiently in 10–20 min with average recoveries from ~ 60 to ~ 90 % depending on the fragment size and elution procedure (details in section 2.6).
- The **NucleoSpin® Gel and PCR Clean-up** buffer formulation ensures complete removal of all kinds of contaminations such as
  - nucleotides, primers
  - enzymes
  - mineral oil
  - PCR additives (e.g., salts, betaine, DMSO)
  - detergents (e.g., Tween 20, Triton X-100)
  - dyes (e.g., ethidiumbromide, crystal violet, Stain G, Midori Green, Roti®-Safe GelStain, DNA SafeStain)
  - unbound labels and tags
- Primers from PCR reactions are quantitatively eliminated while small DNA fragments are still bound and purified with high recovery (details in section 2.6).

- The cut-off for small DNA fragments can be shifted from < 50 bp to several hundred bp by diluting Binding Buffer NTI to remove primer-dimers from target PCR products (details in section 2.3).
- The pH-indicator in Binding Buffer NTI ensures optimal binding conditions with pH < 7.0 (details in section 2.4). The yellow color facilitates to identify undissolved agarose during DNA gel extraction.
- **NucleoSpin® Gel and PCR Clean-up** 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 2.5). The kit also works with low conductivity borate electrophoresis systems.
- Concentrated elution in down to 15 µL Elution Buffer NE (details in section 2.6).
- Several support protocols extend the application range of **NucleoSpin® Gel and PCR Clean-up** to

  - Clean-up of DNA from reaction mixtures containing SDS (section 5.5)

  - Clean-up of single stranded DNA (section 5.6)

  - Extraction of RNA from agarose gels (section 5.4)

  - Extraction of DNA from polyacrylamide gels (section 5.3)
- 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
Sample material	Up to 200 µL of PCR reaction or 200 mg of gel (more sample with additional Binding Buffer NTI and multiple loading steps)
Binding capacity	25 µg
Fragment length	50 bp – ~ 20 kbp
Elution volume	15–30 µL
Optimal recovery	< 15 µg, 100–500 bp, 30 µL
Preparation time	10 min for 6 PCR purifications 20 min for 6 gel extractions

2.3 Removal of small DNA fragments and primer-dimers

NucleoSpin® Gel and PCR Clean-up is designed to remove even traces of unused primer while purifying PCR products down to 50 bp at the same time. However, in some cases it is necessary to exclude these small fragments. For example, primer dimers, or side products resulting from unspecific annealing, may interfere with downstream sequencing or cloning applications.

Removal of double stranded DNA > 50 bp can be achieved by diluting an aliquot of Buffer NTI with sterile water in an appropriate ratio and then proceeding with the standard protocol (see section 5.1). Diluting Buffer NTI in a certain range lowers the binding efficiency for small fragments without compromising the recovery of larger PCR products. However, the dilution ratio will highly depend on the fragment. Therefore, for each size of small fragments > 50 bp that has to be removed, as well as for each PCR system, the appropriate ratio of Buffer NTI dilution can be determined in advance.

Influence of fragment size: ***The smaller the fragment in question, the less you have to dilute Buffer NTI.***

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 NTI. ***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 NTI will lead to removal of small fragments up to 100 bp. Otherwise adding 1 to 3 volumes of water to 1 volume of Buffer NTI will be sufficient.***

Therefore, for each size of small fragments > 50 bp that has to be removed, and for each PCR system, you can determine the appropriate ratio of Buffer NTI dilution, in advance.

Figure 1 shows a purification result with a Buffer NTI dilution series. Pure Buffer NTI (lane 3), as well as Buffer NTI plus one volume of water (lane 4), lead to 100 % recovery of a PCR fragment ladder (lane 2). Increasing dilution of buffer NTI leads to a shift of the size cut-off towards larger fragments. 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 %.

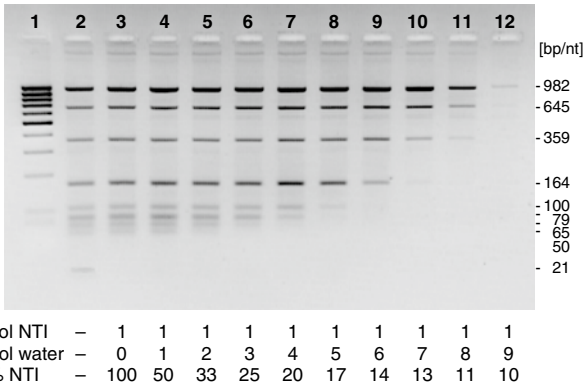


Figure 1 Purification of PCR reactions using Buffer NTI dilutions

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

2.4 pH indicator

The optimal pH to bind even small DNA fragments to the silica membrane of the NucleoSpin® Gel and PCR Clean-up Columns 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.

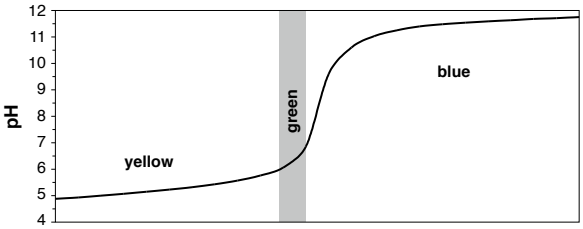


Figure 2 Titration curve of Binding Buffer NTI with pH indicator

A yellow color indicates the optimal pH < 6.0 (Figure 2). 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).

2.5 Tips and tricks for extractions 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 electrophoreses overnight and offers a better resolution for small DNA fragments. TBE buffer can be used in combination with NucleoSpin® Gel and PCR Clean-up. However, it is preferred to use **fresh TAE buffer 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 more efficiently from linear and open circle DNA.
Running conditions	**The temperature during electrophoresis should be low** in order to increase the resolution of the DNA separation and avoid melting of the gel, thus causing denaturation of the DNA. Use fresh buffer and run the gel at low voltage (< 60 V), for as short as possible. As soon as the DNA band of interest is sufficiently separated, stop the gel and cut out the fragment.
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 200 mg of agarose gel can be dissolved with 400 µL of Buffer NTI and loaded onto the column in one step. However, virtually unlimited amounts of gel can be loaded without clogging the column by increasing Buffer NTI proportionally (for separate Buffer NTI, see ordering information) and adding multiple loading steps.

2.6 DNA recovery depends on fragment size and elution volume

Upon completion of the wash steps with Buffer NT3, the DNA will adhere to the silica membrane. The number of interactions with Si-OH groups of the silica increase with the size of the DNA fragment. As a result, 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** is recommended for DNA up 10–15 kbp. Longer fragments can be purified but recovery may be low. In addition, fragments larger than 20 kbp may be mechanically damaged by the fast centrifugation through the membrane. For very large fragments, consider using NucleoTrap® or NucleoTraP®CR (see ordering information).

To elute the DNA, water with 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) which is provided with the kit. However, a standard TE buffer may also be used to ensure best elution efficiency. Please 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. Buffer NE can be diluted with distilled water in case a lower salt concentration is required. Please make sure that the pH remains > 7.

The **standard elution buffer volume is 15–30 µL** which is the best compromise for high DNA recovery and high DNA concentration for fragments < 1000 bp (Figure 3).

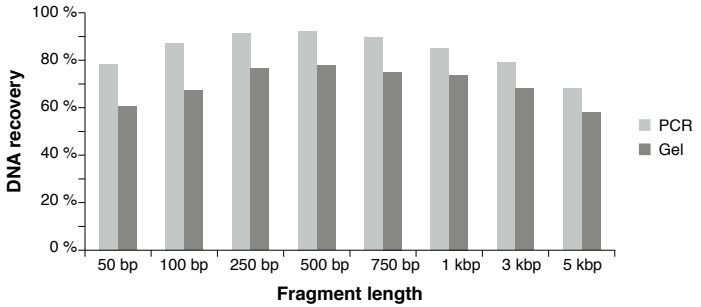


Figure 3 Fragment length dependent DNA recovery
2 µg of 100 bp DNA ladder (Fermentas) or 2 µg of linearized vectors of 3 and 5 kbp were purified from standard PCR buffer or 200 mg 1 % TAE agarose gel. DNA was eluted in 30 µL Elution Buffer NE.

Elution after gel extraction is 10–20 % less efficient than elution of purified PCR products. In addition, 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

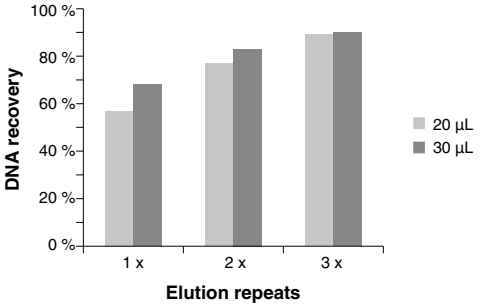


Figure 4 Multiple elution steps increase recovery
3 µg of a 5 kbp fragment were purified from standard PCR buffer and eluted 1, 2 or 3 times with 20 or 30 µL of fresh elution buffer.

If higher DNA concentrations are required, elution volumes < 30 µL can be used. Keep in mind that although the concentration can be more than doubled (Figure 5 A), total DNA recovery will be significantly reduced for volumes < 15 µL (Figure 5 B). For large DNA fragments and gel extractions, the losses may be even more pronounced.

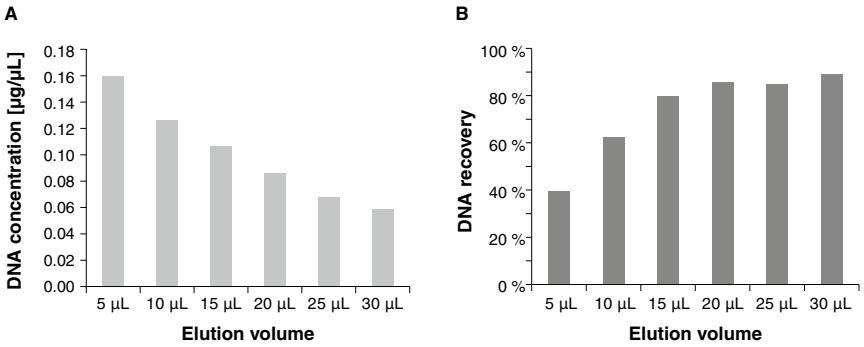


Figure 5 Elution volume dependent DNA recovery and concentration
2 µg of 100 bp DNA ladder (Fermentas) were purified from standard PCR buffer and eluted with increasing volumes.

2.7 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 6) and even < 260 nm (1 M).

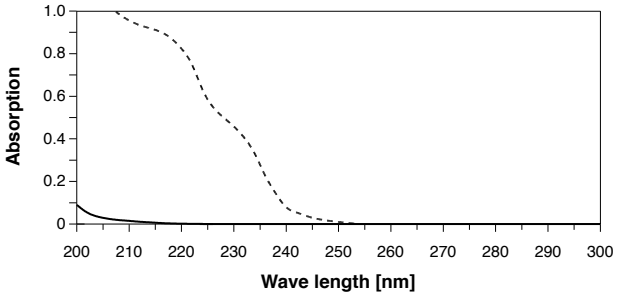


Figure 6 UV absorption spectra of 1 mM GuHCl (solid line) and 1 mM GuSCN (dotted line)

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 can not be detected at this wave length and does not influence the ratio. However, the effect of GuSCN is only detectable with very small amounts of DNA such as typical yields of PCR reactions or gel extractions. Measuring small amounts of DNA in small volumes might lead to absorption ratios that rise concerns about DNA purity. However, this effect

does not usually occur with larger amounts of DNA since its own absorption at 230 nm masks small contributions 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, for example, PCR, restriction or ligation. Figure 7 shows qPCR inhibition by GuSCN and GuHCl, demonstrating that PCR only starts to be inhibited by chaotropic salts with approximately a100-fold higher concentration (40 mM). Moreover, it shows that both chaotropic salts lead to PCR inhibition at elevated concentrations.

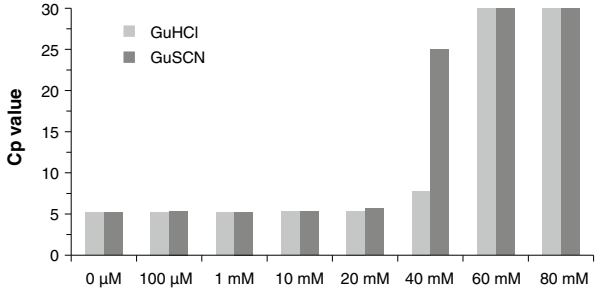


Figure 7 qPCR inhibition by GuHCl (light gray) and GuSCN (dark gray)
A 164 bp DNA fragment was amplified from 5 ng pBS template with DyNAmo Capillary Master Mix (NEB) in a Lightcycler real-time PCR machine (Roche) in the presence of 0–80 mM GuHCl or GuSCN.

The final concentration of chaotropic salt in eluates is too low to have negative effects on downstream applications. Therefore, low A_{260}/A_{230} ratio can be neglected when using this product.

3 Storage conditions and preparation of working solutions

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

CAUTION: Buffer NTI contains guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Storage conditions:

- The **NucleoSpin® Gel and PCR Clean-up** kit should be stored at room temperature and is stable until: see package label.

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		
	10 preps	**50 preps**	**250 preps**
REF	**740609.10**	**740609.50**	**740609.250**
Wash Buffer NT3 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol	2 x 50 mL Add 200 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® Gel and PCR Clean up** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

Caution: Guanidinium thiocyanate in NTI buffer can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 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 very small sample volumes < 30 µL adjust the volume of the reaction mixture to 50–100 µL with water.

It is not necessary to remove mineral oil.

Mix **1 volume of sample** with **2 volumes** of **Buffer NTI** (e.g., mix 100 µL PCR reaction and 200 µL Buffer NTI).

Note: For removal of small fragments like primer dimers dilutions of Buffer NTI can be used instead of 100 % Buffer NTI. Please refer to section 2.3.

+ 2 vol NTI per 1 vol sample

2 Bind DNA

Place a **NucleoSpin® Gel and PCR Clean-up Column** into a Collection Tube (2 mL) and load up to 700 µL sample.

Load sample

Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the collection tube.

11,000 x *g* 30 s

Load remaining sample if necessary and repeat the centrifugation step.

3 Wash silica membrane

Add **700 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the collection tube.

+ 700 µL NT3

11,000 x *g* 30 s

Recommended: Repeat previous washing step to minimize chaotropic salt carry-over and improve A_{260}/A_{230} values (see section 2.7 for detailed information).

+ 700 µL NT3

11,000 x *g* 30 s

4 Dry silica membrane

Centrifuge for **1 min** at **11,000 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.

11,000 x *g*

1 min

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.

5 Elute DNA

+ 15–30 µL NE

RT
1 min

Place the NucleoSpin® Gel and PCR Clean-up Column into a new 1.5 mL microcentrifuge tube (not provided). Add **15–30 µL Buffer NE** and incubate at **room temperature** (15–25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 x *g***.

11,000 x *g*
1 min

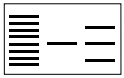
Note: DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with fresh buffer, heating to 70 °C and incubation for 5 min. See section 2.6 for detailed information.

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 2.5 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.

+ 200 µL NTI per 100 mg gel

For each **100 mg of agarose gel < 2 %** add **200 µL Buffer NTI**.

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

Incubate sample for **5–10 min** at **50 °C**. Vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

50 °C
5–10 min

2 Bind DNA

Load sample

Place a **NucleoSpin® Gel and PCR Clean-up Column** into a Collection Tube (2 mL) and load up to 700 µL sample.

11,000 x *g*
30 s

Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the collection tube.

Load remaining sample if necessary and repeat the centrifugation step.

3 Wash silica membrane

Add **700 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the collection tube.

+ 700 µL NT3

11,000 x *g*
30 s

Recommended: Repeat previous washing step to minimize chaotropic salt carry-over and low A_{260}/A_{230} (see section 2.7 for detailed information).

+ 700 µL NT3

11,000 x *g*
30 s

4 Dry silica membrane

Centrifuge for **1 min** at **11,000 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.

11,000 x *g*

1 min

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.

5 Elute DNA

Place the NucleoSpin® Gel and PCR Clean-up Column into a **new** 1.5 mL microcentrifuge tube (not provided). Add **15–30 µL Buffer NE** and incubate at **room temperature** (15–25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 x *g***.

+ 15–30 µL NE

RT
1 min

11,000 x *g*
1 min

Note: DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with fresh buffer, heating to 70 °C and incubation for 5 min. See section 2.6 for detailed information.

5.3 DNA extraction from polyacrylamide gels

In polyacrylamide gels, the acrylamide monomers are covalently linked in a chemical reaction. Therefore, the gel cannot be dissolved like agarose gels to extract the trapped DNA.

Polyacrylamide gels are usually extracted by the “crush and soak” method where a small piece of gel is crushed and incubated in a diffusion buffer. The DNA is then allowed to passively diffuse out of the gel and is then purified from the diffusion buffer. The diffusion buffer (500 mM ammonium acetate, pH 8.0, 0.1 % SDS, 1 mM EDTA, 10 mM magnesium acetate) is not provided with the kit.

1 Prepare sample

Excise the DNA fragment with a scalpel or razor blade in a minimal amount of polyacrylamide. Weigh the gel slice and transfer it to a 1.5 ml microcentrifuge tube (not provided).

Excise DNA fragment

2 Crush gel

Crush the gel slice using a disposable pipette tip with a melted end to resemble a pestle for the microcentrifuge tube “mortar”. The smaller the pieces, the better the DNA recovery.

Crush gel slice

3 Extract DNA

Add **200 µL of diffusion buffer** to each **100 mg** of crushed gel. Make sure that all gel pieces are submerged in diffusion buffer.

Incubate for **30–60 min** at **50 °C** or **over night** at **37 °C**.

50 °C
30–60 min

or

37 °C
over night

4 Remove polyacrylamide

Centrifuge for **1 min** at **11,000 x *g*** to pellet the polyacrylamide and transfer the supernatant to a new microcentrifuge tube (not provided).

11,000 x *g*

1 min

Transfer supernatant

Alternatively, transfer the mixture to a **NucleoSpin® Gel and PCR Clean-up Column** and centrifuge **1 min** at **11,000 x *g*** to retain the gel on the column. Keep the flow-through which contains the DNA!

or

11,000 x *g*

1 min

<u>Optional:</u> To increase the final yield, repeat step 3 and 4 and combine both supernatants or flow-throughs.

Keep flow-through

5 Adjust DNA binding condition

+ 2 vol NTI per 1 vol sample

Mix **1 volume** of sample with **2 volumes** of **Buffer NTI**. (e.g., 200 µL diffusion buffer and 400 µL of Buffer NTI).

Small amounts of precipitating SDS do not influence the purification. Do not remove the precipitate.

Optional:
+ 2 vol ethanol

or

+ 2 vol NTC per 1 vol sample

Note: To obtain higher yields for small fragments < 50 bp add two volumes of ethanol or use Buffer NTC instead of Buffer NTI. Buffer NTC is not provided with the kit but can be ordered separately (see ordering information).

6 Bind DNA

Continue with **step 2** of the protocol for PCR clean-up (section 5.1).

5.4 RNA extraction from agarose gels (Buffer NTC)

Not only DNA but also RNA can be extracted from agarose gels. To efficiently bind 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 may not work properly in enzymatic downstream applications, such as 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 may form secondary structures and may run differently from denaturing agarose gels.

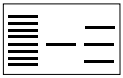
Note: Buffer NTC has to be ordered separately (125 mL Buffer NTC, REF 740654.100, see ordering information)

Before starting the preparation:

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

1 Excise RNA fragment / solubilize gel slice

Note: Minimize UV exposure time to avoid damaging the RNA. Refer to section 2.5 for more tips on agarose gel extraction.



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

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

+ 200 µL NTC per 100 mg gel

For each **100 mg of agarose gel < 2 %** add **200 µL Buffer NTC**.

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

50 °C
5–10 min

Incubate sample for **5–10 min** at **50 °C**. Vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

2 Bind RNA

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

5.5 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 with the exception 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).**

Before starting the preparation:

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

1 Adjust DNA binding condition

+ 5 vol NTB per 1 vol sample

Mix **1 volume of sample** with **5 volumes** of **Buffer NTB** (e.g., 100 µL reaction mix with 500 µL Buffer NTB).

Note: If SDS starts to precipitate add 1 volume of isopropanol or warm sample to 20–30 °C.

2 Bind DNA

Continue with **step 2** of the protocol for PCR clean-up (section 5.1).

5.6 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 8).

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

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., 100 µL PCR reaction mix and 200 µL Buffer NTC).

+ 2 vol NTC per 1 vol sample

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

2 Bind DNA

Continue with **step 2** of the protocol for PCR clean-up (section 5.1).

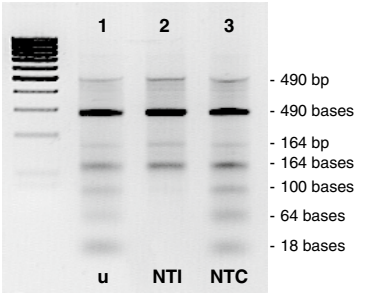


Figure 8 Purification of dsDNA and ssDNA using buffers NTI and NTC
PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with λ-Exonuclease to yield single stranded DNA. Samples were purified using Binding Buffer NTI and NTC and run on a 1 % TAE agarose 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).

5.7 Processing NucleoSpin® Gel and PCR Clean-up kit using a vacuum manifold

We recommend the NucleoVac 24 Vacuum Manifold for processing NucleoSpin® columns under vacuum (see ordering information). However, other vacuum manifold for processing mini spin columns are suitable as well.

1 Adjust DNA binding conditions or excise DNA fragment/solubilize gel slice

Please follow step 1 of the standard protocol for the chosen starting material.

2 Bind DNA

Place a **NucleoSpin® Gel and PCR Clean-up Column** onto a suitable vacuum manifold with Luer-connections like the NucleoVac 24 Vacuum Manifold and load up to 700 µL sample. Do not close the lid!

+ Load sample

-0.2 to -0.4 bar*

1 min

Apply vacuum of **-0.2 to -0.4 bar* (1 min).**

When the sample has passed the NucleoSpin® Gel and PCR Clean-up Column, release the vacuum.

If necessary, load remaining sample and repeat the step.

3 Wash silica membrane

Add **700 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Column. Do not close the lid!

+ 700 µL NT3–0.2 to -0.4 bar*

1 min

Apply vacuum of **-0.2 to -0.4 bar* (1 min)**.

When the sample has passed the NucleoSpin® Gel and PCR Clean-up Column, release the vacuum.

4 *Optional:* *Repeat washing step*

Repeat previous washing step to minimize chaotropic salt carry-over and to improve A_{260}/A_{230} values (see section 2.7 of the NucleoSpin® Gel and PCR Clean-up manual).

+ 700 µL NT3–0.2 to -0.4 bar*

1 min

* Reduction of atmospheric pressure

5 Dry silica membrane

-0.4 to -0.6 bar*

5 min

Option 1: Drying by vacuum

Apply vacuum of **-0.4 to -0.6 bar*** for **5 min** to remove **Buffer NT3** completely. Run the vacuum pump continuously. Achieving and keeping a continuous air flow is of more importance than reaching the precise mentioned reduction of atmospheric pressure. Do not close the lid!

Release the vacuum.

Option 2: Drying by centrifugation

11,000 x *g*

1 min

Place the NucleoSpin® Gel and PCR Clean-up Column into a Collection Tube (2 mL). Centrifuge for **1 min** at **11,000 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

6 Elute DNA

+ 15–30 µL NE

RT

1 min

11,000 x *g*

1 min

Place the NucleoSpin® Gel and PCR Clean-up Column into a new 1.5 mL microcentrifuge tube (not provided). Add **15–30 µL Buffer NE** and incubate at room temperature (18–25 °C) for **1 min**. Centrifuge for **1 min** at **11,000 x *g***.

Note: DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with either fresh Buffer NE (reducing concentration), or by reloading the eluates for a second elution step as well as by increased incubation times at elevated temperatures (e.g., 70 °C).

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete dissolving of gel slice	*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 silica membrane* 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 the centrifuge and collection tube and avoid contact of spin cup with flow-through. *Incomplete elution* Especially for larger amounts of DNA (> 5 µg), 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 2.6 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 where water is used for elution or 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* Before elution, centrifuge 5 min at 11,000 x *g* or incubate column for 5–10 min at 70 °C 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 without having the spin cup make contact with the flow-through. Use either a different brand of ethanol to reconstitute Buffer NT3 or ethanol that is not denatured. The denaturing components may not evaporate as fast as ethanol and end up concentrated in the eluate, inhibiting enzymes like ligase. *Carry-over of chaotropic salts* Perform the optional washing step. Additionally, 250 µL 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.) *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 2.7 for detailed information.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Gel and PCR Clean-up	740609.10/.50/.250	10/50/250
NucleoSpin® Gel and PCR Clean-up XS	740611.10/.50/.250	10/50/250
Buffer NTI	740305.120	200 mL
Buffer NTB	740595.150	150 mL
Buffer NTC	740654.100	100 mL
Buffer NT3 (Concentrate) (for 125 mL Buffer NT3)	740598	25 mL
Collection Tubes (2 mL)	740600	1000
NucleoVac 24 Vacuum Manifold	740299	1
NucleoVac Mini Adapter	740297.100	100
NucleoVac Valves	740298.24	24
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.

6.3 References

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

6.4 Product use restriction / warranty

NucleoSpin® Gel and PCR Clean-up 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.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

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

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

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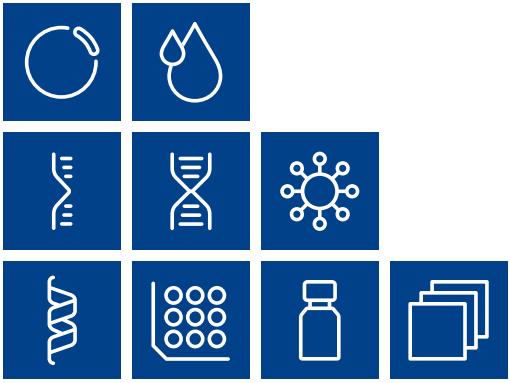

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