

MACHEREY-NAGEL

User manual



PCR clean-up and Gel extraction

- NucleoVerde® Gel and PCR Clean-up

December 2025 / Rev. 02

PCR clean-up, gel extraction

Protocol at a glance (Rev. 02)


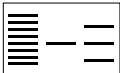

	PCR clean-up	Gel extraction
1 PCR clean-up, or DNA clean-up Adjust binding condition Gel extraction: Excise DNA fragment/ solubilize gel slice	 200 µL NTV / 100 µL PCR	  200 µL NTV / 100 mg gel 60 °C 5 – 10 min
2 Bind DNA	11,000 x g 30 s	11,000 x g 30 s
3 Wash silica membrane	600 µL NT3 11,000 x g 30 s	600 µL NT3 11,000 x g 30 s
4 Wash and Dry silica membrane	400 µL NT3 11,000 x g 1 min	400 µL NT3 11,000 x g 1 min
5 Elute DNA	15 – 50 µL water or elution buffer (not provided) 1 min RT 11,000 x g 1 min	15 – 50 µL water or elution buffer (not provided) 1 min RT 11,000 x g 1 min

Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	5
1.3	About this user manual	5
2	Product description	6
2.1	The basic principle	6
2.3	Kit specifications	7
2.4	Removal of small DNA fragments and primer-dimers	9
2.5	Tips and tricks for extractions from agarose gels	10
2.6	DNA recovery depends on fragment size and elution volume	11
2.7	Salt carry-over and low A_{260} / A_{230}	13
3	Storage conditions and preparation of working solutions	15
4	Safety instructions	16
4.1	Disposal	16
5	Protocols	17
5.1	PCR clean-up	17
5.2	DNA extraction from agarose gels	19
5.3	DNA extraction from polyacrylamide gels	21
5.4	RNA extraction from agarose gels (Buffer NTC)	23
5.5	DNA clean-up of samples containing SDS (Buffer NTB)	24
5.6	Single stranded DNA clean-up (Buffer NTC)	25
6	Appendix	26
6.1	Troubleshooting	26
6.2	Ordering information	29
6.3	Product use restriction / warranty	30

1 Components

1.1 Kit contents

NucleoVerde® Gel and PCR Clean-up		
REF	50 preps 740880.50	250 preps 740880.250
Binding Buffer NTV	30 mL	125 mL
Wash Buffer NT3 (Concentrate)*	12 mL	50 mL
NucleoVerde® Gel and PCR Clean-up Columns (yellow rings)	50	250
Collection Tubes (2 mL)	50	250
Leaflet	1	1

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

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

Reagents

- 96–100 % ethanol (for preparation of wash buffer NT3)
- Water or Elution Buffer NE (5 mM Tris/HCl, pH 8.5) for DNA elution

Consumables

- 2 mL microcentrifuge tubes for agarose gel melting
- 1.5 mL or 2 mL microcentrifuge tubes for elution
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Heating block, water bath, or thermomixer for gel extraction
- Tool to cut agarose gel pieces (e.g. scalpel or agarose gel band puncher)
- 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 **NucleoVerde® 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

NucleoVerde® Gel and PCR Clean-up is developed as a 2-in-1 kit allowing DNA fragments to be purified from agarose gels as well as from enzymatic reactions, such as PCR.

The sample is mixed with Binding Buffer NTV and in case of a cut-out gel band, it is heated to melt and dissolve the agarose. In the presence of the Binding Buffer NTV, the DNA is bound to the silica membrane of a NucleoVerde® 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 water. Optionally, DNA can be eluted with a slightly alkaline Elution Buffer NE (5 mM Tris/HCl, pH 8.5), which can be ordered separately.

2.2 Sustainable product development

Laboratory research continues to generate large amounts of waste, particularly plastics, chemicals, and packaging. Most of this waste is difficult to recycle due to contamination. As sustainability becomes increasingly important, life science companies face the challenge of reducing environmental impact while maintaining the high standards researchers rely on.

The **NucleoVerde® Gel and PCR Clean-up** kit was developed as a more environmentally responsible alternative to the trusted **NucleoSpin® Gel and PCR Clean-up** kit (740609.10/.50/.250). It offers the same high DNA recovery rates, purity, and reliability, however, with a significantly reduced ecological footprint.

NucleoVerde® features a compact kit design to reduce material usage, shipping volume and mass. Buffer bottles are smaller to minimize plastic waste and avoid discarding excess buffer. All collection tubes are made from at least 70 % post-consumer recycled plastic and deliver the same reliable performance. We have also introduced plastic bags made with 80 % biobased material and implemented more sustainable product labels as they are produced with recycled material.

We reviewed every buffer and additive with a focus on necessity and sustainability. **NucleoVerde®** uses less buffers and bottles. Binding and wash buffer volumes are reduced, and the elution step is performed with water instead of separate buffer, eliminating the need for Buffer NE. These changes reduce both chemical usage and packaging.

The pH indicator, commonly used in binding buffers, has been removed as every reduction in chemical load counts.

While sustainability was the main goal, performance was never compromised. **NucleoVerde®** uses the same silica membrane technology as the NucleoSpin® Gel and PCR Clean-up kit and has undergone extensive validation to ensure equivalent results for gel extraction and PCR clean-up.

By choosing **NucleoVerde®**, laboratories can maintain scientific excellence while reducing their environmental impact. It's a step toward more responsible research, without compromise.

2.3 Kit specifications

- **NucleoVerde® Gel and PCR Clean-up** is designed for fast purification of DNA fragments from TAE agarose gels as well as the extraction of PCR products, such as DNA from enzymatic reactions.
- Two volumes of binding buffer per volume of sample are needed to process up to 200 mg of agarose gel or 200 µL of PCR / enzymatic reaction, with only one loading step. By adding additional Binding Buffer NTV (see ordering information) it is possible to load an increased amount of sample volumes onto a single column (tips and tricks in section 2.5).
- Up to 25 µg DNA from 50 bp to ~ 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 **NucleoVerde® 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)
 - dyes (e.g., 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 NTV to remove dsDNA > 50 bp from target PCR products (details in section 2.3).
- **NucleoVerde® Gel and PCR Clean-up** can be used with different kinds of agarose gels (high or low melting) with 1 % to 5 % agarose and buffer systems like TAE (tips and tricks in section 2.5).
- Concentrated elution in down to 15 µL water (details in section 2.6).

- Several support protocols extend the application range of **NucleoVerde® Gel and PCR Clean-up** to
 - Clean-up of DNA from reaction mixtures containing SDS (section 5.5, Buffer NTB required which has to be ordered separately: 150 mL Buffer NTB, REF 740595.150, see ordering information)
 - Clean-up of single stranded DNA (section 5.6, Buffer NTC required which has to be ordered separately: 125 mL Buffer NTC, REF 740654.100, see ordering information)
 - Extraction of RNA from agarose gels (section 5.4, Buffer NTC required which has to be ordered separately: 125 mL Buffer NTC, REF 740654.100, see ordering information)
 - Extraction of DNA from polyacrylamide gels (section 5.3, 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)
- 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	NucleoVerde® Gel and PCR Clean-up
Sample material	Up to 200 mg of gel or 200 µL of PCR reaction (more sample with additional Binding Buffer NTV and multiple loading steps)
Theoretical Binding capacity	25 µg
Fragment length	50 bp – ~ 20 kbp
Elution volume	15–50 µL
Optimal recovery	< 15 µg, 100–10,000 bp with 30–50 µL elution volume
Preparation time	10 min for 6 PCR purifications 20 min for 6 gel extractions
Use	For research use only

2.4 Removal of small DNA fragments and primer-dimers

NucleoVerde® 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 (primer dimers), may interfere with downstream sequencing or cloning applications.

Removal of double stranded DNA > 50 bp can be achieved by diluting an aliquot of Buffer NTV with sterile water in an appropriate ratio and then proceeding with the standard protocol (see section 5.1). Diluting Binding Buffer NTV 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 NTV dilution can be determined in advance.

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

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 NTV.

Adding up to 1 volume of water to 1 volume of Buffer NTV can remove small DNA fragments up to 500 bp.

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 NTV dilution, in advance.

2.5 Tips and tricks for extractions from agarose gels

Subject	Recommendation
Running conditions	<p>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 for as short as possible. As soon as the DNA band of interest is sufficiently separated, stop the gel and cut out the fragment.</p>
Cutting out the band	<p>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.</p>
Size of gel piece	<p>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 Binding Buffer NTV and loaded onto the column in one step. However, increased amounts of gel can be loaded without clogging the column by increasing Buffer NTV proportionally (for separate Buffer NTV, see ordering information) and adding multiple loading steps.</p>
Recovery increase of 50 – 100 bp fragments	<p>Recovery of dsDNA with approximately 50 bp can be increased by adding 0.2 volumes of ethanol to the NTV e.g 100 mg agarose + 200 µL NTV + 30 µL ethanol).</p>

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. **NucleoVerde® Gel and PCR Clean-up** is recommended for DNA up to 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 recommended to reestablish the hydrate shell. Alternatively, it is recommended to elute DNA with slightly alkaline buffer (e.g. Elution Buffer NE, 5 mM Tris/HCl, pH 8.5; see ordering information). However, a standard TE buffer may also be used to ensure best elution efficiency. Please note that EDTA in TE buffer may cause issues in subsequent enzymatic reactions. The use of deionized water with acidic pH might result in somewhat lower DNA yield compared to slightly alkaline buffer NE. Buffer NE can be diluted with distilled water in case a lower salt concentration is required. A pH of > 7 is recommended.

The **standard elution buffer volume is 15–50 µL** which is the best compromise for high DNA recovery and high DNA concentration for fragments < 1000 bp (Figure 1).

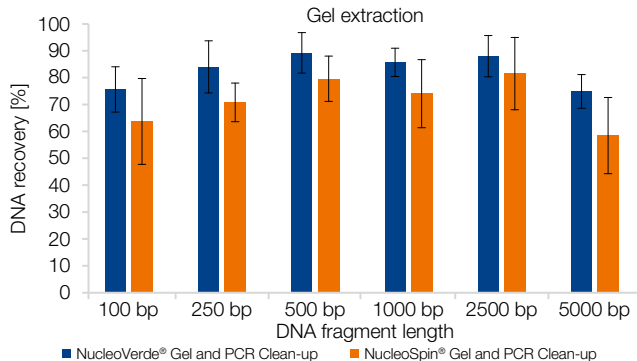


Figure 1 Fragment length dependent DNA recovery

Six distinct DNA fragments (125 ng per fragment) in the range 100 bp - 5000 bp were purified from 150 mg of a 2% agarose gel using the NucleoVerde® Gel and PCR Clean-up kit or the NucleoSpin® Gel and PCR Clean-up kit with 30 µL of elution volume. Eluates were separated via TAE gel electrophoresis and the band intensity was compared to the original input. DNA recovery was calculated utilizing ImageJ.

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 solution (water or buffer) to 70 °C and incubate elution buffer on the column at 70 °C for 5 minutes.
- Apply water or elution buffer to the column and prolong the incubation to 3 min. Centrifuge 11,000 x g for 1 min

The most relevant improvements in terms of nucleic acid recovery can be achieved by elution with 50 µL or a two-fold elution of a smaller volume like 15 µL. 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 significantly.

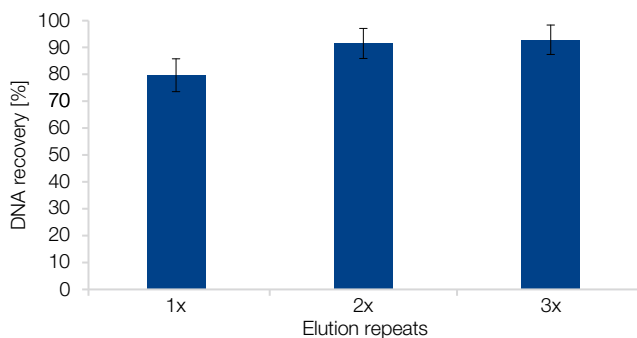


Figure 2 Multiple elution steps increase recovery

1 µg of a DNA ladder with a size range from 250 bp to 10,000 bp was purified from 150 mg of 2% agarose gel. The DNA was then eluted on three occasions with 20 µL of water, showing that a second elution step can result in a notable enhancement of the DNA recovery. DNA quantities were measured fluorometrically.

If higher DNA concentrations are required, elution volumes < 30 µL can be used. Keep in mind that although the concentration can be increased by decreasing elution volume, total DNA recovery will be significantly reduced for volumes < 15 µL (Figure 3). For large DNA fragments and gel extractions, the loss may be even more pronounced.

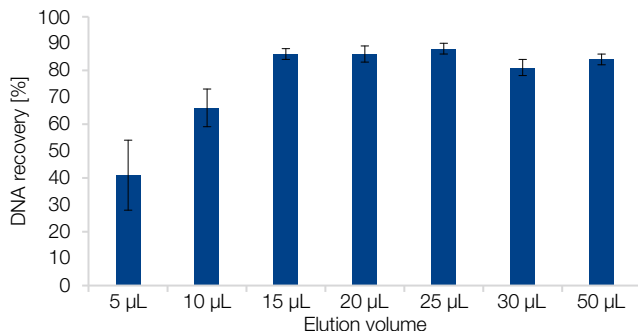


Figure 3 Elution volume dependent DNA recovery and concentration

A quantity of 1.4 µg of a DNA ladder with a size range from 250 bp to 10,000 bp was purified from 100 mg of a 2 % agarose gel, and subsequently eluted with increasing volumes. DNA quantities were analyzed via UV-spectrometry. The NucleoVerde® Gel and PCR Clean-up kit has been demonstrated to facilitate efficient elution in as little as 15 µL of water.

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 salts and thiocyanate salts. These ions are not only responsible for their different behavior towards nucleic acids but also for their different UV absorption spectra. Guanidine exhibits only minimal absorption < 220 nm even at a concentration of 1 M, whereas thiocyanate already shows significant absorption < 240 nm (1 mM, Figure 6) and even < 260 nm (1 M).

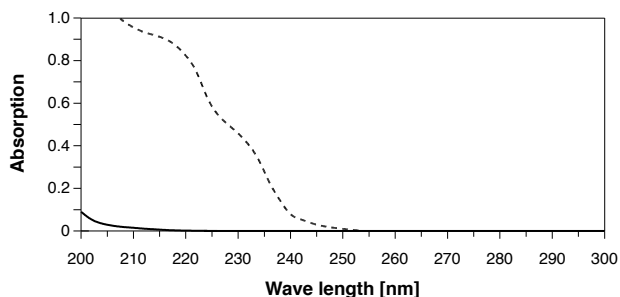


Figure 4 UV absorption spectra of 1 mM GuHCl (solid line) and 1 mM GuSCN (dotted line)

Especially the difference in absorption at 230 nm may have a huge impact on the purity ratio A_{260}/A_{230} if DNA is contaminated with thiocyanate salts. Carry-over of thiocyanate can lower the ratio from its ideal value of > 2.0 to below 1.0. GuHCl can not be detected at this wave length and does not influence the ratio. However, the effect of thiocyanate is

only detectable with 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 raise 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 μ M to 1 mM and does not have any negative influence on enzymatic downstream applications, for example, PCR, restriction or ligation. The following figure shows qPCR inhibition by GuSCN and GuHCl, demonstrating that PCR only starts to be inhibited by chaotropic salts with approximately a 100-fold higher concentration (40 mM). Moreover, it shows that both chaotropic salts lead to PCR inhibition at elevated concentrations.

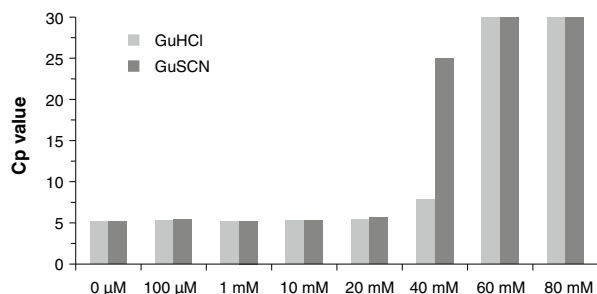


Figure 5 qPCR inhibition by GuHCl (light gray) and GuSCN (dark gray)

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 typically too low to have negative effects on downstream applications. Therefore, low A_{260}/A_{230} ratio can be neglected when using this product. For applications where a good A_{260}/A_{230} ratio is essential, an extra wash step is recommended.

3 Storage conditions and preparation of working solutions

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

CAUTION: Buffer NTV contains 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 **NucleoVerde® Gel and PCR Clean-up** kit should be stored at room temperature and is stable until: see package label.

Before starting any **NucleoVerde® Gel and PCR Clean-up** protocol prepare the following:

- **Wash Buffer NT3:** Add the indicated volume of ethanol (96–100 %) to **Buffer NT3 Concentrate – nondenatured ethanol is recommended**. 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.

NucleoVerde® Gel and PCR Clean-up		
REF	50 preps 740880.50	250 preps 740880.250
Wash Buffer NT3 (Concentrate)	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol

4 Safety instructions

When working with the **NucleoVerde® 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: Thiocyanate in NTV 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.

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.
- Check if water or elution buffer is available.

1 Adjust DNA binding condition

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



**+ 2 vol NTV
per
1 vol sample**

To 1 volume of (adjusted) sample add 2 volumes of Binding Buffer NTV and mix well (e.g. adjust 20 µL of PCR Mix with 80 µL water and add 200 µL Binding Buffer).

Note: For removal of small fragments dilutions of Buffer NTV can be used instead of 100% Buffer NTV. Please refer to section 2.4

2 Bind DNA

Place a **NucleoVerde® Gel and PCR Clean-up Column** into a Collection Tube (2 mL, provided) and load up to 600 µL sample.



Load sample

Centrifuge for **30 s** at **11,000 x g**.



**11,000 x g
30 s**

Discard flow-through and place the column back into the collection tube.

If necessary, load remaining sample and repeat the centrifugation step.

3 Wash and dry silica membrane

Add **600 µL Buffer NT3** to the NucleoVerde® Gel and PCR Clean-up Column.



+ 600 µL NT3

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

Add **400 µL Buffer NT3** to the NucleoVerde® Gel and PCR Clean-up Column.



+ 400 µL NT3

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

Discard flow-through with collection tube.



11,000 x g
1 min

Note: Be careful handling the tube with column in order to avoid the flow-through to come into contact with the column outlet.

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

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.

4 Elute DNA

Place the **NucleoVerde® Gel and PCR Clean-up Column** into a new 1.5 mL microcentrifuge tube (not provided).



+ 15–50 µL
water

Add **15–50 µL** water or elution buffer (not provided in the kit) in the column onto the center of the silica membrane.

RT
1 min

Incubate at **room temperature** (15–25 °C) for **1 min**.

11,000 x g
1 min

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

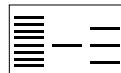
5.2 DNA extraction from agarose gels

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.
- Check if water or elution buffer is available.

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 or a DNA band punch out tool for agarose gels 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. Use of a 2 mL tube is recommended (not provided).



**+ 200 µL NTV
per
100 mg gel**

For each **100 mg of agarose gel ≤ 2 %** add **200 µL Buffer NTV**.

Incubate sample for **5–10 min** at **60 °C** in a thermoshake device mixing.

Alternatively incubate at 60 °C in a heat incubator and vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

**60 °C
5–10 min**

Note: Make sure the agarose gel piece is completely dissolved.

Note: If the liquid has contacted the lid due to mixing, spin briefly to clean the lid.

Note: If processing 5% agarose gel, a prolonged centrifugation might be required in the subsequent step.

2 Bind DNA

Place a **NucleoVerde® Gel and PCR Clean-up Column** into a Collection Tube (2 mL, provided) and load up to 600 µL sample.

**Load sample**

Centrifuge for **30 s** at **11,000 x g**.

**11,000 x g**
30 s

Discard flow-through and place the column back into the collection tube.

Load remaining sample if necessary and repeat the centrifugation step.

3 Wash and dry silica membrane

Add **600 µL Wash Buffer NT3** to the NucleoVerde® Gel and PCR Clean-up Column.

**+ 600 µL NT3**

Centrifuge for **30 s** at **11,000 x g**.

**11,000 x g**
30 s

Discard flow-through and place the column back into the collection tube.

Add **400 µL Wash Buffer NT3** to the NucleoVerde® Gel and PCR Clean-up Column.

**+ 400 µL NT3**

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

**11,000 x g**
1 min

Discard flow-through with collection tube.

Note: Be careful handling the tube with column in order to avoid the flow-through to come into contact with the column outlet.

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

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. If this drying step is performed, conduct an incubation step with elution buffer on the column for 5 min in the subsequent elution procedure.

4 Elute DNA

Place the NucleoVerde® Gel and PCR Clean-up Column into a **new** 1.5 mL microcentrifuge tube (not provided).



**+ 15–50 µL
water**

Add **15–50 µL** water or elution buffer (not provided in the kit) in the column onto the center of the silica membrane.

**RT
1 min**

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**

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.

**50 °C
30–60 min**

or

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

**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).

Alternatively, transfer the mixture to a **NucleoVerde® 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!

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



11,000 x g

1 min

Transfer supernatant

or



11,000 x g

1 min



Keep flow-through

5 Adjust DNA binding condition

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.

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).

**+ 2 vol NTI per
1 vol sample**

***Optional:*
+ 2 vol ethanol**

or

**+ 2 vol NTC
per 1 vol
sample**

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 NTV.

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.

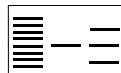
Note: Buffer NTC has to be ordered separately (125 mL Buffer NTC, REF 740654.100, see ordering information)

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 NTV from the NucleoVerde® 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

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



+ 5 vol NTB
per
1 vol sample

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)

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)

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete dissolving of gel slice	<i>Time and temperature</i>
	Check incubation temperature and volume of Buffer NTV. Increase incubation time. Preferentially, use a thermoshake device. Alternatively, vortex every 2 min and check integrity of the gel slice. Very large gel slices can be crushed before addition of Buffer NTV to shorten the melting time.
Low DNA yield	<i>Reagents not prepared properly</i>
	Add indicated volume of 96 – 100 % ethanol to Buffer NT3 Concentrate and mix well before use.
	<i>Incompletely dissolved gel slice</i>
Increase time until agarose gel piece is completely dissolved. Small pieces of gel are hardly visible and contain DNA that will be lost for purification.	<i>Insufficient drying of the NucleoVerde® Gel and PCR Clean-up silica membrane</i>
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.	<i>Incomplete elution</i>
Especially for larger amounts of DNA (> 5 µg), long DNA fragments (> 1000 bp), after gel extraction, or after prolonged column drying do multiple elution steps with fresh elution medium, 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	<p><i>DNA was denatured during purification</i></p>
	<p>In case where water is used for elution or agarose with a low ion content is used for agarose gel electrophoresis, the formation of denatured (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.</p> <p>Use fresh running buffer and run at low voltage to lower the temperature. High temperature might promote DNA denaturation during electrophoresis.</p>
Suboptimal performance of DNA in sequencing, restriction, or ligation reactions	<p><i>Carry-over of ethanol/ethanolic Buffer NT3</i></p>
	<p>Before elution, centrifuge 5 min at 11,000 xg 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.</p>
	<p>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.</p>
	<p><i>Carry-over of chaotropic salts</i></p>
	<p>Perform the optional washing step.</p>
	<p>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.)</p>
	<p><i>Elution of DNA with buffers other than Buffer NE, for example TE buffer (Tris/EDTA)</i></p>
	<p>EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Buffer NE or water.</p>
	<p><i>Not enough DNA used for sequencing reaction</i></p>
	<p>Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions.</p>

Problem	Possible cause and suggestions
Suboptimal performance of DNA in sequencing, restriction, or ligation reactions (continued)	<p><i>DNA was damaged by UV light</i></p> <p>Reduce UV exposure time to a minimum when excising a DNA fragment from an agarose gel.</p>
Suboptimal performance of DNA in NanoDrop® Spectrophotometer Analysis or Agilent's Bioanalyzer	<p><i>Carry-over of traces of silica particles</i></p> <p>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.</p>
Low ratio A_{260}/A_{230}	<p><i>Carry-over of chaotropic salts</i></p> <p>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.</p> <p><i>Carry-over of wash buffer</i></p> <p>Use of nondenatured ethanol is recommended to prepare the wash buffer because ethanol denaturants can negatively impact A_{260}/A_{230} ratios.</p>

6.2 Ordering information

Product	REF	Pack of
NucleoVerde® Gel and PCR Clean-up	740880.50/.250	50/250
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 NTV	740881.125	125 mL
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
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 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

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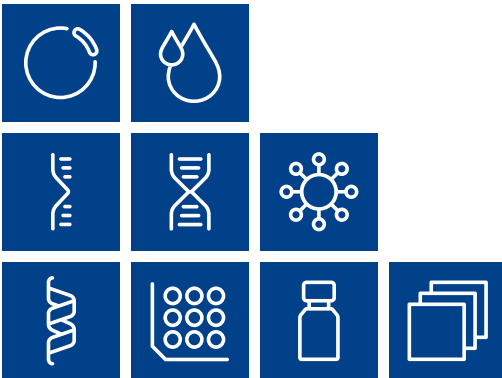
Last updated: 08/2022, Rev. 04

Please contact:
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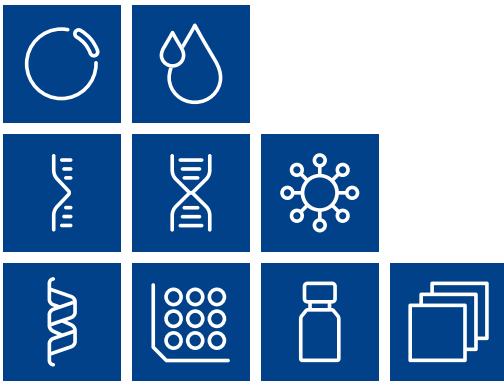
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Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
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High throughput
Accessories
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