MACHEREY-NAGEL

User manual

Bioanalysis

PCR clean up and Gel extraction

■ NucleoSpin® Gel and PCR Clean-up XS

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1 Components

1.1 Kit contents

	NucleoSpin® Gel and PCR Clean-up XS		
REF	**10 preps 740611.10**	**50 preps 740611.50**	**250 preps 740611.250**
Binding Buffer NTI	10 mL	40 mL	200 mL
Wash Buffer NT3 (Concentrate)*	6 mL	12 mL	50 mL
Elution Buffer NE**	13 mL	13 mL	13 mL
NucleoSpin® Gel and PCR Clean-up XS columns (yellow rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
Short protocol	1	1	1

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 XS** kit is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. 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.

* For preparation of working solutions and storage conditions see section 3.
** Composition of Elution Buffer NE: 5 mM Tris/HCl, pH 8.5

2 Product description

2.1 The basic principle

NucleoSpin® Gel and PCR Clean-up XS is designed as a 2-in-1 kit to isolate DNA from enzymatic reactions or agarose gels. Binding buffer NTI inactivates enzymes and promotes the binding of DNA to the silica layers inside the binding columns. Furthermore, Buffer NTI is used in combination with an incubation at 50 °C to melt agarose gels during DNA gel extraction. Bound DNA is stringently washed with buffer NT3 and eluted in elution volumes as low as 6 µL of Buffer NE, resulting in highly pure and concentrated DNA.

2.2 Kit specifications

- The NucleoSpin® Gel and PCR Clean-up XS kit is designed for research use only.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Gel and PCR Clean-up XS
Format	Minispin, XS format
Processing	Manual
Sample material	≤ 200 µL enzymatic reaction ≤ 200 mg agarose gel
Elution volume	≥ 6 µL
Preparation time	< 15 min / 12 preps (enzymatic reaction clean up) 30 min / 12 preps (gel extraction)
Binding capacity	5 µg
Use	For research use only

2.3 Elution procedure

The XS column format is designed for low elution volumes, resulting in high DNA concentrations. Nevertheless there are tips and tricks to enhance the DNA recovery, especially when working with elution volumes below 10 µL.

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.

Keep in mind that chaotropic salt carry-over will increase the absorption at 230 nm without negative effects on downstream applications. Even minute amounts of thiocyanate, well below an influential concentration, show a strong absorption at 230 nm. There is no effect on the absorption at 260 nm.

3 Storage conditions and preparation of working solutions

Attention: Buffer NTI contains guanidine thiocyanate! Wear gloves and goggles!

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

- All kit components can be stored at room temperature (18 – 25 °C) and are stable for at least one year.

Before starting the NucleoSpin® Gel and PCR Clean-up XS protocol prepare the following:

	NucleoSpin® Gel and PCR Clean-up XS		
REF	**10 preps** **740611.10**	**50 preps** **740611.50**	**250 preps** **740611.250**
Wash Buffer NT3 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol

4 Safety instructions

When working with the **NucleoSpin® Gel and PCR Clean-up XS** 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 ***http://www.mn-net.com/msds***).

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

The waste generated with the **NucleoSpin® Gel and PCR Clean-up XS** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

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

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.
- When performing a gel extraction, excise DNA band from agarose gel and determine the weight of the gel slice. Preheat a heating block, thermomixer or water bath to 50 °C.

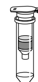
1 Adjust DNA binding condition

Mix **1 volume of sample** with **2 volumes** of **Buffer NTI** (e. g., mix 20 µL PCR reaction with 40 µL NTI or 150 mg agarose gel slice with 300 µL NTI).

Vortex

Agarose gel extraction only:

Incubate mixture at 50 °C with constant shaking or repeated vortexing every 2 – 3 min until the gel is completely dissolved.



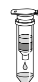
+ 2 vol NTI per 1 vol sample

2 Bind DNA

Place a **NucleoSpin® Gel and PCR Clean-up XS Column** into a Collection Tube (2 mL) and load up to 500 µL sample.

Centrifuge for **30 s** at **11,000 x *g***. Discard flowthrough if it exceeds 100 µL and place the column back into the collection tube.

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

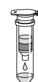


Load sample

11,000 x *g*
30 s

3 Wash silica

Add **500 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up XS Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flowthrough and place the column into a 1.5 mL microcentrifuge tube (not supplied)



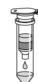
+ 500 µL NT3

11,000 x *g*
30 sec

4 Wash and dry silica

Add **300 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up XS column.

Centrifuge for **1 min at 11,000 x *g***. Discard flowthrough and place the column into a 1.5 mL microcentrifuge tube (not supplied).



+ 300 µL NT3

11,000 x *g*
1 min

5 Elute DNA

Add **6 – 12 µL Buffer NE** and incubate at room temperature (18 – 25 °C) for **1 min**.

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

Reload the eluates onto the column and repeat the elution step.

+ 6 – 12 µL NE

RT
1 min

11,000 x *g*
1 min

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete dissolving of gel slice	*Time and temperature* • Increase incubation time and vortex mixture repeatedly every 2 – 3 min until the gel slice is completely dissolved. Do not increase the incubation temperature to prevent loss of thermally denatured single stranded DNA
Low DNA yield	*Reagents prepared or stored improperly* • Add the indicated volume of 96 – 100 % ethanol to Buffer NT3. Mix well and keep bottle closed tightly. Prevent conditions where ethanol might evaporate (e. g., high temperatures, open bottles). *Incompletely dissolved gel slice* • Weigh agarose block and add 2 volumes of Buffer NTI. Vortex mixture continuously or every 2 – 3 min while melting the agarose gel. If necessary, increase incubation times, but do not increase incubation temperature. *Ethanol carry-over* • Take care that the columns' outlets or walls do not come in contact with the NT3 flowthrough after the second washing step. If in doubt, place the column back into the empty collection tube and re-centrifuge.

Problem	Possible cause and suggestions
Low DNA yield	*Incomplete elution* • If DNA recovery is low, the remaining DNA is presumably still sticking to the silica. Losses during the binding or washing step are unlikely if the buffers are used, stored and prepared according to the protocol. To enhance the DNA recovery, it is recommended to either increase the total elution volume or to use repeated elution steps with the same aliquot, especially when using low elution volumes below 10 μL. Simply reload the eluate onto the silica and repeat the centrifugation. *Input quantification overestimated* • Keep in mind, that photometry will sum up the absorption of any molecule which absorbs at a certain wavelength, e. g., 260 nm. A PCR reaction will contain not only the desired DNA amplificates, but also residual primers, unused dNTPs, enzymes, certain detergents or buffer substances, which all absorb at 260 nm and increase the measured “DNA” concentration. • *A photometric determination of the product* recovery is not possible. To assess the product recovery, alternative techniques like gel electrophoresis, capillary electrophoresis or dsDNA specific fluorometry must be used. De-pending on the PCR mix, 80 – 90 % of the initial absorption at 260 nm will be removed by the purification process.
Low DNA yield	*Smeared bands* • Agarose gels might show artifacts when the agarose mesh is irregular due to insufficient homogenization of agarose in TAE / TBE buffer, differences in solidification, local thickness or other influences which alter the migration speed. The DNA itself might also be able to migrate in different conformations, depending on sequence and buffer temperature. While the main band might show a strong signal it is still possible that some fragments will migrate slightly faster or slower in an insufficient amount to give a detectable signal or show a smear. Excising the band closely will remove those fragments but is recommended nevertheless to reduce the amount of agarose.

Problem	Possible cause and suggestions
Low $A_{260/230}$	*Chaotropic salt carry-over* • To melt agarose gels and to bind nucleic acids to a silica surface, thiocyanate salts have proven effective and reliable. They have a long term track record showing successful purification of nucleic acids from various sample types and for various downstream applications. • Nevertheless, these chaotropic salts express a very high absorption at 230 nm even at negligible concentrations as low as the micro molar range, while the absorption at 260 nm is zero. Thiocyanate salts will therefore bias the A_{260}/A_{230} purity ratio but not the DNA quantification. A low A_{260}/A_{230} is no indicator for suboptimal performance in downstream applications in this case and can be ignored. • To improve the A_{260}/A_{230}, further washing steps with buffer NT3 can be performed. Additional Buffer NT3 will be necessary and can be ordered at MACHEREY-NAGEL (cat. no. 740598).
High A_{320}	*Silica abrasion* • In rare cases, sheared silica fibers might pass the filter frit of the columns and are eluted. When measured in a photometer, those fibers will scatter the light, resulting in an overall overestimation of the absorption values. A high A_{320} value above 0.05 will be an indicator for particles which scatter light. • Silica abrasion can be easily pelleted by centrifugation and is usually already spinned down by the elution. Vor-texing and mixing the eluates might resuspend these particles again. To prevent this it is recommended to transfer the clear eluate without the easily visible white pellet into a new centrifuge tube.

Problem	Possible cause and suggestions
Suboptimal performance in downstream applications	*DNA was damaged by UV light* • Reduce UV exposure time to a minimum when excising a DNA fragment from an agarose gel *Ethanol carry-over* • Due to the low elution volumes, ethanol carry-over from the second washing step can reach a high concentration in the eluates and might inactivate enzymes. Take care that the outlets or walls of the columns do not come in contact with the NT3 flowthrough from the second washing step. If in doubt, discard flowthrough and place the columns back into the empty collection tubes. Repeat the centrifugation step with empty columns. *Silica abrasion* • Silica debris might scatter or quench the signals during photometric or fluorescence based measurements. Furthermore, the channels of capillary electrophoresis might be blocked. If a white pellet is visible, spin down the eluates and transfer the clear supernatant without a white pellet into a new reaction tube.
Purification of RNA or single stranded DNA	*Buffer NTC* • Buffer NTI is used for double stranded DNA or long single stranded DNA above 500 bp in length. For shorter single stranded DNA or RNA, Buffer NTC is recommended. This buffer can be used for both, reaction clean up and gel extraction. • Buffer NTC can be ordered at MACHEREY-NAGEL (REF 740654.100)

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 / .100	10 / 100
NucleoTraP®CR	740587.10 / .100	10 / 100

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.

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.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

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Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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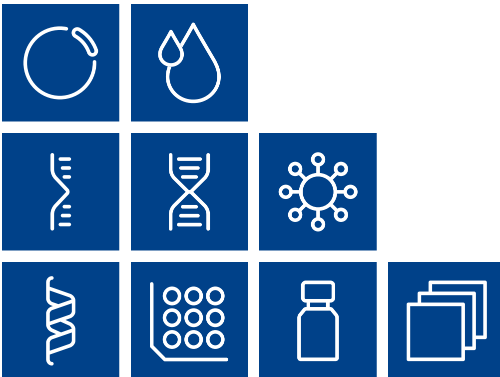

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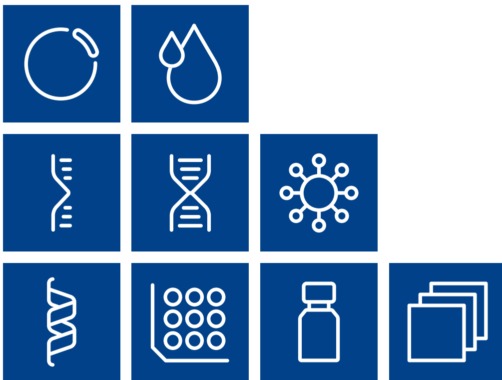

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