Genomic DNA clean-up

User manual

NucleoSpin® gDNA Clean-up XS

August 2011 / Rev. 02

MACHEREY-NAGEL

Genomic DNA clean-up

Protocol-at-a-glance (Rev. 02)

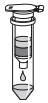
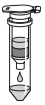
		XS NucleoSpin® gDNA Clean-up XS
1	**Prepare sample**	 Adjust up to 400 µL aqueous sample with buffer TE to 800 µL
2	**Adjust DNA binding conditions**	 200 µL NT
3	**Bind DNA**	  Load 500 µL diluted sample 11,000 x *g* 30 s Load remaining sample 11,000 x *g* 30 s
4	**Wash silica membrane**	  Turn spin cup inside the centrifuge by 180° compared to the loading position 100 µL B5 11,000 x *g* 2 min
5	**Elute DNA**	  **1.** 6–10 µL BE 11,000 x *g* 1 min **2.** 6–10 µL BE 11,000 x *g* 1 min
6	**Removal of residual ethanol and concentration**	 90 °C 8 min (2 x 10 µL elution) or 5 min (10 µL elution)

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Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® gDNA Clean-up XS		
	10 preps	**50 preps**	**250 preps**
REF	**740904.10**	**740904.50**	**740904.250**
Binding Buffer NT	10 mL	25 mL	2 x 25 mL 1 x 10 mL
Wash Buffer B5 (Concentrate)*	6 mL	6 mL	6 mL
Elution Buffer BE**	5 mL	5 mL	15 mL
NucleoSpin® gDNA Clean-up XS Columns (light green rings)	10	50	250
Collection Tubes (2 mL)	3 x 10	3 x 50	3 x 250
User manual	1	1	1

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

** Composition of Elution Buffer BE: 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 (for sample lysis and DNA elution)
- Disposable pipette tips

Equipment

- Manual pipettors
- Thermal heating block
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® gDNA Clean-up XS** kit read the detailed protocol sections of this user manual. 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***.

2 Product description

2.1 Basic principle

The **NucleoSpin® gDNA Clean-up XS** kit is designed for a fast and convenient purification of genomic DNA from aqueous samples (e.g., phenol / chloroform extracts). PCR inhibitors (e.g., indigo) are efficiently removed and DNA is concentrated with high recovery. Due to its high sensitivity the kit is particularly well suited for trace levels of DNA from forensic samples. The optimized protocol allows for up to 400 µL of aqueous sample to be processed without the need for error-prone repeated loading steps. However, multiple loading steps can be used without difficulty to process larger sample volumes. The special funnel design of the thrust rings inside the **NucleoSpin® gDNA Clean-up XS Column** in combination with the very small membrane allows for high recovery with very small elution volumes (5–30 µL) which results in highly concentrated DNA.

Appropriate conditions under which DNA binds to the silica membrane are created by addition of Binding Buffer NT. The mixture is then applied to the **NucleoSpin® gDNA Clean-up XS Column** and the DNA binds to a silica membrane. A subsequent washing step efficiently removes contaminations and highly pure DNA is finally eluted with 5–30 µL of a slightly alkaline elution buffer of low ionic strength (5 mM Tris/HCl pH 8.5).

2.2 Kit specifications

- The **NucleoSpin® gDNA Clean-up XS** kit is recommended for the purification and concentration of genomic DNA from very dilute aqueous samples. Typical sample materials comprise for example PCR inhibitor containing solutions, Proteinase K reaction mixtures, or the aqueous phase of phenol / chloroform extractions.
- The robust membrane allows for multiple loading steps to process even large sample volumes.
- The special column design and the very small membrane lead to a significantly reduced dead volume which allows for high recovery of small amounts of DNA with as little as 5–30 µL elution buffer.
- DNA is ready-to-use for all common downstream applications like (e.g., real-time PCR).
- The preparation time is approximately 20 min for 6–12 samples.

Table 1: Kit specifications at a glance	
Parameter	**NucleoSpin® gDNA Clean-up XS**
Sample material	< 400 µL solution containing < 2 µg DNA
Typical recovery	60–70 %
Fragment size	100 bp–approx. 50 kbp
A_{260}/A_{280}	1.8–1.9
Elution volume	6–10 µL
Preparation time	20 min/6 preps (exclusive preceding extraction or lysis)
Format	Mini spin column – XS design

2.3 Handling of sample material

The **NucleoSpin® gDNA Clean-up XS** procedure is designed for very small amounts of genomic DNA and the typical downstream applications are thus very sensitive. It is consequently highly recommended to perform sampling and DNA purification with special care, in order to avoid a contamination of the sample or the purified DNA with unwanted DNA-containing material (e.g., fingerprints, hair particles, aerosol, dust).

Moreover, a cross-contamination between samples has to be excluded. The following precautions are recommended:

- Wear personal protection equipment (lab coat, gloves, goggles).
- Use aerosol resistant pipette tips.
- Always change pipette tips between liquid transfers.
- Briefly centrifuge after mixing steps in order to remove droplets from tube lid.

2.4 Elution procedures

A high DNA concentration in the elution fraction is of importance and desirable for all typical downstream applications. This is of particular interest if the total volume of a reaction mixture is limited as this in turn limits the possible amount of DNA that can be added. Due to a high default elution volume, classical DNA clean-up kits often result in weakly concentrated DNA, if only small amounts of DNA are processed.

Such DNA may even require a subsequent concentration before it can be used for typical downstream applications.

In contrast to classical kits, **NucleoSpin® gDNA Clean-up XS** allows for efficient elution in a very small volume which results in highly concentrated DNA.

For forensic samples elution with 2 x 6 µL is recommended to maximize concentration and yield. A two-fold elution generally yields more DNA than just one elution with the same total buffer volume. Optionally, the second elution can be omitted to achieve the highest possible DNA concentration.

In general, larger volumes (10–30 µL) increase the overall DNA yield but naturally reduce the final DNA concentration. Elution buffer volumes > 30 µL will only slightly increase total DNA yield.

2.5 Concentration and removal of residual ethanol

For most applications removal of trace levels of ethanol is not required. However, if a large volume of eluate has to be used as PCR template a heat incubation of the eluate is recommended. An incubation of for example 8 min at 90 °C for a 20 µL eluate removes residual ethanol in the eluate and concentrates the DNA to approximately 11 µL resulting in a significantly increased sensitivity in downstream applications. The template may then represent up to 40% of the total PCR reaction volume. The necessity of this step may be individually tested.

An incubation at 90 °C, however, will denature DNA. If non-denatured DNA is required for downstream applications other than PCR (e.g., ligation or cloning) we recommend an incubation of 17 min at 75 °C to remove ethanol from an eluate of 20 µL.

Even if ethanol is of no concern for the downstream application the heat incubation is a useful means to concentrate an eluate. Use Figure 1 to estimate the necessary incubation time depending on your elution volume and the intended final volume. Take into consideration that incubation times may vary depending on the heating block or microcentrifuge tubes that are used. Shaking the tubes during incubation increases the evaporation rate even more.

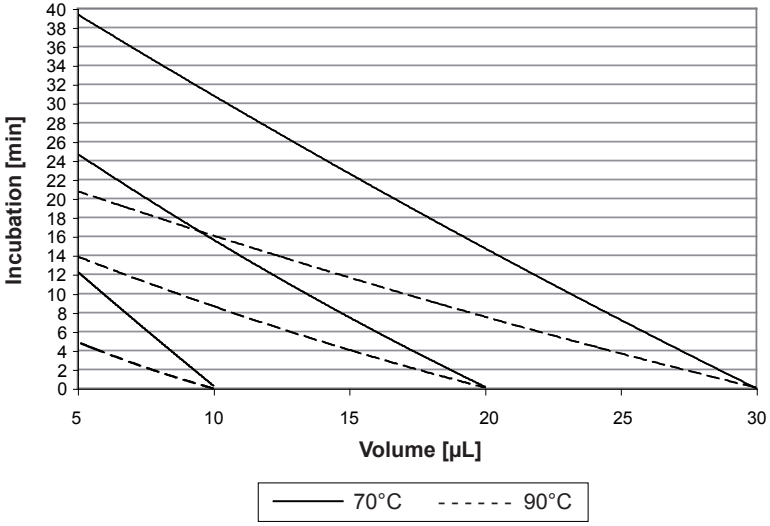


Figure 1: Concentration and removal of residual ethanol from eluates by heat treatment. Eluates of 10, 20, and 30 µL were incubated at 75 °C (non-denaturing) and 90 °C (denaturing) for 0–40 min without shaking. Choose your final volume and read the necessary incubation time from the appropriate curve. For other starting volumes just interpolate the array of curves.

3 Storage conditions and preparation of working solutions

Attention:

Buffer NT contains guanidinium thiocyanate. Wear gloves and goggles!

Storage conditions:

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

Before starting any **NucleoSpin® gDNA Clean-up XS** protocol prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96–100 %) to **Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer B5 can be stored at room temperature (18–25 °C) for at least one year.

	NucleoSpin® gDNA Clean-up XS		
	10 preps	**50 preps**	**250 preps**
REF	**740904.10**	**740904.50**	**740904.250**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	6 mL Add 24 mL ethanol	6 mL Add 24 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® gDNA Clean-up XS** kits contain hazardous contents.

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

4.1 Risk and safety phrases

Component *Inhalt*	Hazard contents *Gefahrstoff*	Hazard symbol *Gefahrstoffsymbol*	Risk phrases *R-Sätze*	Safety phrases *S-Sätze*
NT	Guanidinium thiocyanate *Guanidinthiocyanat*	✖ Xn*	R 20/21/22	S 13

Risk phrases

R 20/21/22 Harmful by inhalation, in contact with skin, and if swallowed.
Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.

Safety phrases

S 13 Keep away from food, drink, and animal feedstuffs.
Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.

4.2 GHS classification

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

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*		Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
NT	Guanidinium thiocyanate 30–60 % *Guanidinthiocyanat 30–60 %*		Warning *Achtung*		

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

* Hazard labeling not necessary if quantity per bottle below 125 g or mL (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 NucleoSpin® gDNA Clean-up XS protocol

Before starting the preparation:

- Check if Wash Buffer B5 was prepared according to section 3.

1 Prepare sample

Use up to **400 µL sample solution** and dilute with **buffer TE** (e.g., 10 mM Tris/HCl pH 7.5, 0.1 mM EDTA, not provided) to a **final volume of 800 µL**.

Note: For samples > 400 µL add 1 volume of TE buffer and increase Buffer NT proportionally in step 2.

+ TE to a final volume of 800 µL

Mix thoroughly by vortexing and spin down briefly to clear the lid.

Note: This dilution step might not be necessary for highly concentrated samples or uncomplicated pre-purified DNA. To proceed with up to 400 µL of undiluted sample adjust Buffer NT proportionally in step 2.

2 Adjust DNA binding conditions

Add **200 µL Buffer NT** to **800 µL diluted sample** and mix thoroughly by vortexing. Spin down briefly to clear the lid.

+ 200 µL NT per 800 µL of sample

Note: For undiluted samples or diluted sample volumes other than 800 µL adjust Buffer NT proportionally.

3 Bind DNA

Load 500 µL sample

For each sample, place one **NucleoSpin® gDNA Clean-up XS Column** into a **Collection Tube (2 mL)**.

11,000 x *g* 30 s

Add 500 µL of binding mixture to the column. Centrifuge for **30 s** at **11,000 x *g*** and discard the flow-through.

Load remaining sample

Add the remaining binding mixture to the column and centrifuge for **30 s** at **11,000 x *g***.

11,000 x *g* 30 s

Place the column into a new **Collection Tube (2 mL)**.

4 Wash silica membrane

+ 100 µL B5

11,000 x *g*
2 min

Turn the NucleoSpin® gDNA Clean-up XS Column inside the centrifuge by 180° compared to the loading position in order to allow optimal washing efficiency.

Add **100 µL Buffer B5**.

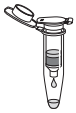
Centrifuge for **2 min** at **11,000 x *g***.

5 Elute DNA

Place the NucleoSpin® gDNA Clean-up XS Column into a 1.5 mL microcentrifuge tube (not supplied).

+ 6–10 µL BE

Add **6–10 µL Buffer BE** directly to the center of the membrane.



11,000 x *g*
1 min

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

Add another **6–10 µL Buffer BE** directly to the center of the membrane.

+ 6–10 µL BE

11,000 x *g*
1 min

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

<u>Note</u>: The elution volume can be varied from 5–30 µL. The second elution step can be omitted leading to higher concentration but lower yield. See section 2.4 for more information.

6 Removal of residual ethanol and concentration

90 °C, 8 min
(2 x 10 µL elution)

or

90 °C, 5 min
(10 µL elution)

Incubate eluate with open lid at **90 °C** for **8 min** (2 x 10 µL elution) or **5 min** (10 µL elution).

<u>Note</u>: For different elution volumes or incubation at 75 °C for non-denatured DNA see section 2.5 for detailed information.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA yield	*Low DNA content of the sample* • The content of DNA depends very much on sample type, amount and quality. *Reagents not prepared properly* • Add the indicated volume of 96–100 % ethanol to the Buffer B5 Concentrate and mix well before use.
No increase of PCR signal despite an increased volume of eluate used as template	*Residual ethanol in eluate* • Please see the detailed description of removal of residual traces of ethanol in section 2.5. *Carry-over of chaotropic salts* • Perform a second washing step with Buffer B5 to remove last traces of Buffer NT.
Discrepancy between A_{260} quantification values and PCR quantification values	*Silica abrasion from the membrane* • Due to the typically low DNA content in very small samples and the resulting low total amount of isolated DNA, DNA quantification via A_{260} absorption measurement is often hampered by the low sensitivity of this method. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect A_{260} quantification of small DNA amounts centrifuge the eluate for 30 s at > 11.000 x *g* and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen® fluorescent dye).
A_{260}/A_{280} ratio too high or too low	*Measurement not in the range of photometer detection limit* • In order to obtain a significant A_{260}/A_{280} ratio it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpected A_{260}/A_{280} ratios.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® gDNA Clean-up XS	740904.10 740904.50 740904.250	10 preps 50 preps 250 preps
Buffer NT	740614.100	100 mL
Buffer B5 (Concentrate) (for 100 mL Buffer B5)	740921	20 mL
Buffer BE	740306.100	100 mL
Collection Tubes (2 mL)	740600	1000

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

6.3 Product use restriction/warranty

NucleoSpin® gDNA Clean-up XS 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!

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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.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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