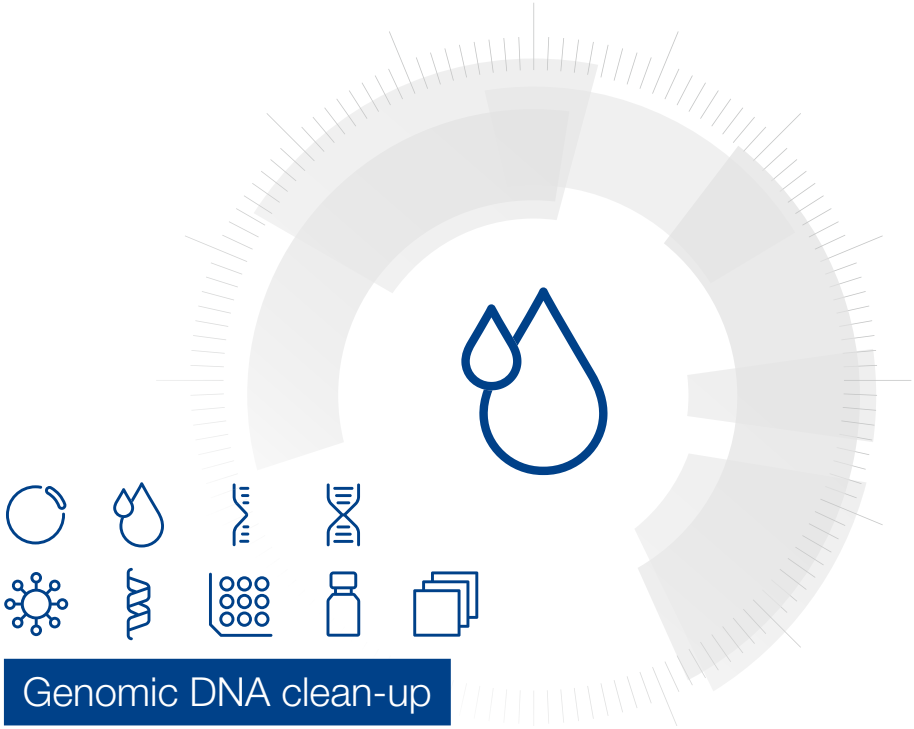
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA clean-up

- NucleoSpin® gDNA Clean-up

September 2023 / Rev. 05

gDNA clean-up

Protocol-at-a-glance (Rev. 05)

		NucleoSpin® gDNA Clean-Up
1 Adjust DNA binding conditions		150 µL sample + 450 µL DB Vortex 5 s (For smaller sample volumes adjust to 150 µL with water, for larger sample volumes increase binding buffer proportionally.)
2 Bind DNA		Load sample on NucleoSpin® gDNA Clean-up Column 11,000 x *g* 30 s
3 Wash silica membrane	 	**1st wash** + 700 µL DW Vortex 2 s 11,000 x *g* 30 s
	 	**2nd wash** + 700 µL DW Vortex 2 s 11,000 x *g* 30 s
4 Dry silica membrane	 	11,000 x *g* 1 min
5 Elute DNA	 	50 µL DE RT 1 min 11,000 x *g* 30 s (Optional: Repeat elution with first eluate or another 50 µL of fresh Buffer DE. Heating elution buffer to 70 °C might further promote elution.)

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1 Components

1.1 Kit contents

	NucleoSpin® gDNA Clean-up		
REF	**10 preps 740230.10**	**50 preps 740230.50**	**250 preps 740230.250**
Binding Buffer DB	25 mL	25 mL	125 mL
Wash Buffer DW (Concentrate)*	6 mL	25 mL	3 x 50 mL
Elution Buffer DE**	13 mL	13 mL	30 mL
NucleoSpin® gDNA Clean-up Columns (light green rings)	10	50	250
Collection Tubes (2 mL)	10	50	250
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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
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® gDNA Clean-up** 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***.

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

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

2 Product description

2.1 Basic principle

Prepurified and especially high molecular weight genomic DNA dissolved in water, elution buffer, or any reaction buffer is mixed with Binding Buffer DB and loaded onto a NucleoSpin® gDNA Clean-up Column.

All kinds of contaminants are removed by two washing steps with Wash Buffer DW.

After a drying step, pure and concentrated DNA can be eluted with Elution Buffer DE (5 mM Tris/HCl, pH 8.5).

2.2 Kit specifications

- The NucleoSpin® gDNA Clean-up kit is designed for the rapid purification of previously isolated small and especially high molecular weight genomic DNA. It is used to clean-up and concentrate genomic DNA after crude extraction methods, for example using Trizol, or after enzymatic, or chemical reactions.
- No need for organic denaturants or chloroform extractions.
- Any impurities like phenol, enzymes, salts, dyes, labels, nucleotides, small oligonucleotides, and even up to 5 % detergents (e.g., SDS, Triton, Tween, Lauroylsarcosin) are removed completely.
- Binding Buffer DB and Wash Buffer DW are specifically developed to allow a very gentle binding and washing to ensure the highest possible DNA recovery for high molecular weight DNA as well as for DNA fragments down to 100 bp.
- The eluted DNA is ready-to-use for all standard downstream applications such as PCR, endonuclease restriction, Southern Blotting and labeling.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® gDNA Clean-up
Typical sample size	150 µL DNA solution
Typical amount of DNA	< 25 µg
Typical recovery	80 – 90 %
Fragment size	100 bp – approx. 50 kbp
Binding capacity	50 µg
Elution volume	50 – 100 µL
Preparation time	< 15 min/10 preps
Format	Mini spin column
Use	For research use only

2.3 Removal of RNA

Nucleotides and small oligonucleotides are removed by the gentle binding conditions and the stringent washing steps. To remove contamination of RNA completely, it is recommend to add 1 µg of RNase A (see ordering information) to 150 µL of sample and to incubate at room temperature for 5 – 15 min.

2.4 How to interpret yield and purity from UV-VIS

The most common method to determine the DNA yield is UV-VIS spectroscopy. The DNA concentration in the final eluate can be calculated from its absorption maximum at 260 nm (A_{260}) based on the fact that an absorption of A_{260} = 1 corresponds to 50 µg/mL double stranded DNA. However, this calculation assumes the absence of any other compound that absorbs UV light at 260 nm. Any contamination with phenol, RNA, protein, or detergents, etc. significantly contributes to the total absorption at 260 nm, thus leading to an overestimation of the real DNA concentration.

Purity ratio A_{260}/A_{230}

To facilitate the decision whether the yield as determined from A_{260} readings can be trusted or not, the ratio of the absorption at 260 nm and 230 nm can be used. The ratio A_{260}/A_{230} should be higher than 2.0 for pure DNA and is acceptable down to ratios of about 1.5. Smaller values around or even below 1.0 indicate significant amounts of impurities and the real DNA concentration is far below its calculated value.

Purity ratio A_{260}/A_{280}

Another indicator of DNA purity is the ratio A_{260}/A_{280}, which should be between 1.8 and 1.9. Values below 1.8 indicate protein contamination, whereas higher values indicate RNA contamination. However, this ratio should be treated with caution, since contamination with protein and RNA at the same time can compensate each other and result in a perfect A_{260}/A_{280}.

Agarose gel electrophoresis

As a consequence, the DNA should always be run on an agarose gel to evaluate the DNA quality in terms of size distribution and to verify the UV-VIS quantification especially if A_{260}/A_{230} and A_{260}/A_{280} are beyond the acceptable range.

3 Storage conditions and preparation of working solutions

Attention: Buffer DB contains guanidine hydrochloride. Wear gloves and goggles!

Storage conditions:

- All kit components should be stored at 15 – 25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts. If precipitation occurs, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is dissolved.

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

- **Wash Buffer DW**: Add the indicated volume of ethanol (96 – 100 %) to **Buffer DW Concentrate**. Mark the label of the bottle to indicate that ethanol has been added. Buffer DW is stable at room temperature (15 – 25 °C) for at least one year.

	NucleoSpin® gDNA Clean-up		
REF	**10 preps 740230.10**	**50 preps 740230.50**	**250 preps 740230.250**
Wash Buffer DW (Concentrate)	6 mL Add 14 mL ethanol	25 mL Add 60 mL ethanol	3 x 50 mL Add 110 mL ethanol to each bottle

4 Safety instructions

When working with the **NucleoSpin® gDNA Clean-up** kits 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: Guanidine hydrochloride in Buffer DB 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® gDNA Clean-up** 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 treatment 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 NucleoSpin® gDNA Clean-up protocol

Before starting the preparation:

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

1 Adjust DNA binding conditions

Add **450 µL** Binding Buffer DB to **150 µL** DNA solution.

Vortex for **5 s**.

Note: If sample volume is less than 150 µL, fill up with water to 150 µL. If more than 150 µL of sample has to be processed, increase Binding Buffer DB proportionally. Multiple loading steps might be necessary in step 2.

150 µL sample

+ 450 µL DB

Vortex 5 s

2 Bind DNA

Place a **NucleoSpin® gDNA Clean-up Column** in a Collection Tube (2 mL).

Load **up to 700 µL sample solution** onto the column.

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

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

Load sample

11,000 x *g*
30 s

3 Wash silica membrane

1st wash

Add **700 µL Buffer DW** to the NucleoSpin® gDNA-Clean-up Column.

Close the lid, vortex for 2 s, and centrifuge for **30 s** at **11,000 x *g***.

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

+ 700 µL DW

Vortex 2 s

11,000 x *g*
30 s

2nd wash

Add **700 µL** Buffer DW to the NucleoSpin® gDNA-Clean-up Column.

Close the lid, vortex for **2 s**, and centrifuge for **30 s** at **11,000 x *g***.

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

+ 700 µL DW

Vortex 2 s

11,000 x *g*
30 s

4 Dry silica membrane

Centrifuge for **1 min** at **11,000 x *g*** and discard the collection tube.

11,000 x *g*
1 min

<u>Note:</u> Residual ethanolic wash buffer might inhibit enzymatic reactions.

5 Elute DNA

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

+ 50 µL DE
RT
1 min

Add **50 µL Buffer DE** to the column.

Do not close the lid and incubate for **1 min** at **room temperature**.

Close the lid and centrifuge for **30 s** at **11,000 x *g***.

11,000 x *g*
1 min

<u>Note:</u> DNA yield can be increased by eluting a second time. Either re-apply the first eluate to the column or use 50 µL of fresh Elution Buffer DE.

Heating the elution buffer to 70 °C can further increase the elution efficiency.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no DNA yield	*Reagents not applied or restored properly* • Always dispense exactly the buffer volumes given in the protocol! • Always follow the given instructions closely with regard to order and mode of mixing (shaking, vortexing etc.). • Add the indicated volume of ethanol (96 – 100 %) to Wash Buffer DW Concentrate and mix thoroughly (see section 5 for more information). • Keep bottles tightly closed in order to prevent evaporation or contamination.
Suboptimal performance of DNA in downstream experiments	*Carry-over of ethanol or salt* • Make sure to dry the silica membrane and the NucleoSpin® gDNA Clean-up Column completely before elution to avoid carry-over of ethanolic Wash Buffer DW. • Check if Buffer DW has been equilibrated to room temperature before use. Washing at lower temperatures decreases the efficiency of salt removal.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® gDNA Clean-up	740230.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® gDNA Clean-up XS	740904.10 / .50 / .250	10 / 50 / 250 preps
RNase A (lyophilized)	740505.50 740505	50 mg 100 mg
Collection Tubes (2 mL)	740600	1000

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.

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.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

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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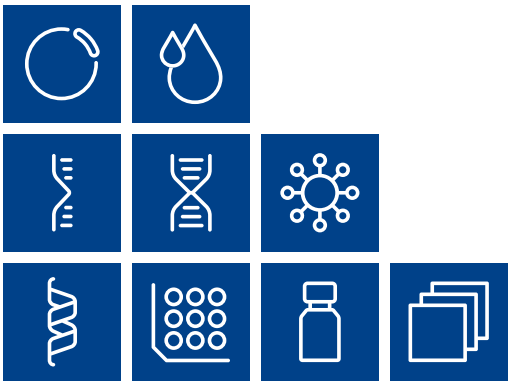
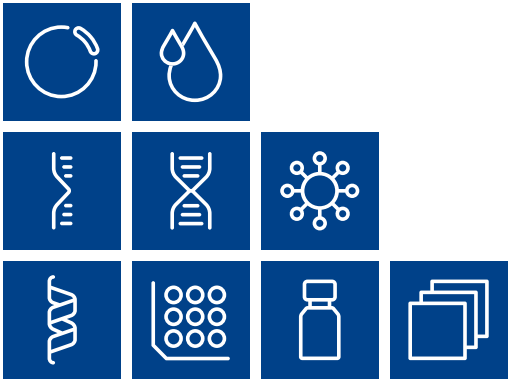


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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