



# Genomic DNA clean-up

## User manual

### NucleoSpin<sup>®</sup> gDNA Clean-up

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# gDNA clean-up

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

### NucleoSpin® gDNA Clean-up

<b>1 Adjust DNA binding conditions</b>		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.)
<b>2 Bind DNA</b>		Load sample on NucleoSpin® gDNA Clean-up Column 11,000 x g 30 s
<b>3 Wash silica membrane</b>	   	<b>1<sup>st</sup></b> + 700 µL DW Vortex 2 s 11,000 x g 30 s  <b>2<sup>nd</sup></b> + 700 µL DW Vortex 2 s 11,000 x g 30 s
<b>4 Dry silica membrane</b>	 	11,000 x g 1 min
<b>5 Elute DNA</b>	 	50 µL DE RT 1 min 11,000 x g 30 s  <i>(Optional: Repeat elution with first eluate or another 50 µL of fresh Buffer DE. Heating elution buffer to 70°C might further promote elution.)</i>

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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
User manual	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
- Personal protection equipment (e.g., lab coat, gloves, goggles)

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

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

### 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***.

## 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 DB.

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

## 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 (18–25 °C) 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 room temperature (18–25 °C) and are stable for at least one year. 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 (18–25 °C) for at least one year.

NucleoSpin® gDNA Clean-up			
	10 preps	50 preps	250 preps
REF	740230.10	740230.50	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

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

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

### GHS classification

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

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
DB	Guanidine hydrochloride 1–10% + ethanol 55–75 % <i>Guanidinhydrochlorid 1–10 % + Ethanol 55–75 %</i>		Danger  <i>Gefahr</i>	H 225  P 210, 233, 403+235

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

### Hazard phrases

H 225 Highly flammable liquid and vapour  
*Flüssigkeit und Dampf leicht entzündbar.*

### Precaution phrases

P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*

P 233 Keep container tightly closed.  
*Behälter dicht verschlossen halten.*

P 403+235 Store in a well ventilated place. Keep cool.  
*Kühl an einem gut belüfteten Ort aufbewahren.*

## 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**

#### 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

##### 1<sup>st</sup> 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**

**2<sup>nd</sup> 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.

*Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.*



**11,000 x g  
1 min**

**5 Elute DNA**

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

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

Do not close the lid and incubate for **1 min** at **room temperature (18–25 °C)**.

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

*Note: 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.*



**+ 50 µL DE**

**RT  
1 min**

**11,000 x g  
1 min**

## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor or no DNA yield	<p><i>Reagents not applied or restored properly</i></p> <ul style="list-style-type: none"> <li>• Always dispense exactly the buffer volumes given in the protocol!</li> <li>• Always follow the given instructions closely with regard to order and mode of mixing (shaking, vortexing etc.).</li> <li>• Add the indicated volume of ethanol (96–100 %) to Wash Buffer DW Concentrate and mix thoroughly (see section 5 for more information).</li> <li>• Keep bottles tightly closed in order to prevent evaporation or contamination.</li> </ul>
Suboptimal performance of DNA in downstream experiments	<p><i>Carry-over of ethanol or salt</i></p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• Check if Buffer DW has been equilibrated to room temperature (18–25 °C) before use. Washing at lower temperatures decreases the efficiency of salt removal.</li> </ul>

### 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](http://www.mn-net.com) for more detailed product information.

### 6.3 Product use restriction/warranty

**NucleoSpin® gDNA 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.

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DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

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