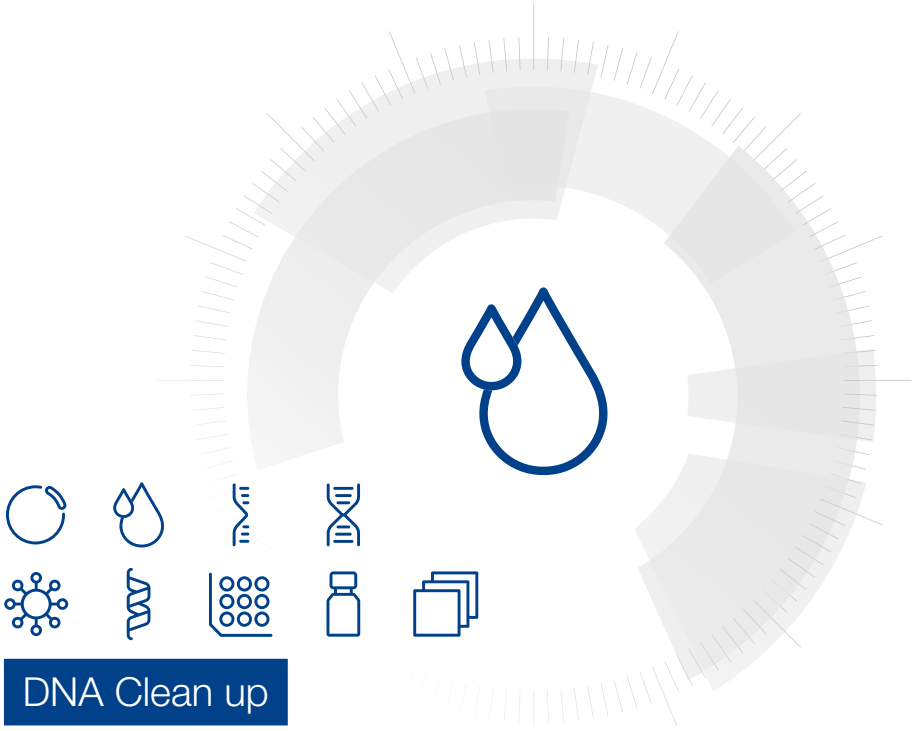
Bioanalysis

MACHEREY-NAGEL

User manual



DNA Clean up

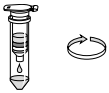
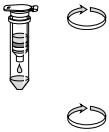
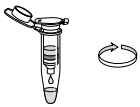
- NucleoSpin® Inhibitor Removal

June 2023 / Rev. 04

DNA Clean up

Protocol at a glance (Rev. 04)

Method 5.1 for samples with moderate contaminations of humic substances or other types of inhibitors

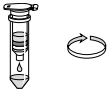
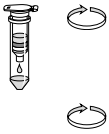
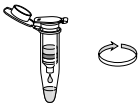
		NucleoSpin® Inhibitor Removal
1	Prepare sample 	100 µL sample in a 1.5 mL or 2.0 mL tube
2	Adjust DNA binding conditions 	300 µL IR1X Mix and incubate 1 min at RT 210 µL ethanol
3	Bind DNA 	10,000 × g 30 sec
4	Wash silica membrane 	500 µL IRW 10,000 × g 30 sec 300 µL IRW 10,000 × g 2 min
5	Prepare elution 	100 µL BE RT, 1 min
6	Elute highly pure DNA 	11,000 × g 1 min

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-333 · support@mn-net.com · *www.mn-net.com*

DNA Clean up

Protocol at a glance (Rev. 04)

Method 5.2 for samples with considerable contaminations of humic substances (intense brownish color)

		NucleoSpin® Inhibitor Removal
1	Prepare sample	 100 µL sample in a 1.5 mL tube
2	Adjust DNA binding conditions	 500 µL IR1 mix
3	Bind DNA	 10,000 × g 30 sec
4	Wash silica membrane	 500 µL IRW 10,000 × g 30 sec 300 µL IRW 10,000 × g 2 min
5	Prepare elution	 100 µL BE RT, 1 min
6	Elute highly pure DNA	 11,000 × g 1 min

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1 Components

1.1 Kit contents

NucleoSpin® Inhibitor Removal		
REF	**740408.10**	**740408.50**
NucleoSpin® Inhibitor Removal Column	10	50
Binding Buffer IR1*	10 mL	30 mL
Additive IRX*	1 mL	4 mL
Wash Buffer IRW* (Concentrate)	6 mL	12 mL
Elution Buffer BE**	13 mL	13 mL
Collection Tubes (2 mL)	10	50
Collection Tubes (1.5 mL)	10	50
User manual	1	1

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

Reagents

- 96 – 100 % ethanol (for preparation of Wash Buffer IRW and adjustment of binding conditions for protocols 5.1)

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer (e.g. Vortex-Genie 2 from Scientific Industries)
- Personal protection equipment (lab coat, gloves, goggles)

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

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

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the NucleoSpin® Inhibitor Removal 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.

2 Product description

2.1 The basic principle

The NucleoSpin® Inhibitor Removal kit is designed for fast and efficient clean up of pre-purified DNA samples contaminated with PCR inhibitors. Samples might contain PCR inhibitors such as humic substances, heme, polyphenols, tannins, or dyes. Such inhibitors might originate from insufficient purification procedures or challenging sample materials such as soil, blood, fruit, brownish water, processed food (e.g. tea, coffee) or other.

Due to the diverse nature and amount of inhibitors that might be present in DNA preparations from problematic samples, the NucleoSpin® Inhibitor Removal kit provides two alternative procedures: method 5.1 is recommended for samples with slight contamination of humic acids or other PCR inhibitors. Method 5.2 is recommended for samples considerably contaminated with humic substances (strong brownish color).

2.2 Kit specifications

Kit specifications at a glance

Parameter	NucleoSpin® Inhibitor Removal
Technology	Silica membrane technology
Format	Mini spin column
Sample material	DNA solutions contaminated with PCR inhibitors
Sample amount	100 µL
DNA recovery	Typically > 75 %
Elution volume	50 – 100 µL
Preparation time	15 min (6 preps)
Binding capacity	60 µg*
Use	For research use only

2.3 Handling, preparation, and storage of starting materials

DNA containing eluates should be kept on ice for short term storage and frozen at -20 °C or below for long term storage.

*theoretical value

2.4 Elution procedures

In addition to the standard elution method, several modifications are possible to increase yield, concentration, and convenience.

- Convenient elution (standard elution): Elution can be performed by a single addition of 100 µL Elution Buffer onto the column.
- High yield: Elution can be performed in two serial elutions of 100 µL each, resulting in a total volume of 200 µL.
- High concentration: Elution can be performed by application of 100 µL Elution Buffer, which is then re-used in a second elution step, resulting in 100 µL eluate with a high DNA concentration. Alternatively, the elution volume can be reduced down to 50 µL. Please note that this typically will reduce the total amount of DNA recovered.

3 Storage conditions and preparation of working solutions

Attention: Buffer IR1 contains chaotropic salt. Wear gloves and goggles!

CAUTION: Buffer IR1 contains guanidine hydrochloride 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.

Before starting the NucleoSpin® Inhibitor Removal procedure, prepare the following:

Wash Buffer IRW: Add the indicated volume (see on the bottle or table below) of ethanol (96 – 100 %) to IRW concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer IRW at room temperature (15 – 25 °C) for up to one year.

	NucleoSpin® Inhibitor Removal	
REF	**10 preps** **740408.10**	**50 preps** **740408.50**
Wash Buffer IRW	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol

Preparation of buffer IR1X by supplementation of Buffer IR1 with additive IRX for use in procedure 5.1

Per preparation, combine 240 µL of buffer IR1 with 60 µL additive IRX. For convenience, a master mix sufficient for several preparations can be prepared.

Number of preps	Vol of IR1	Vol of IRX	Total Vol of IR1X
1	240 µL	60 µL	300 µL
2	480 µL	120 µL	600 µL
3	720 µL	180 µL	900 µL
4	960 µL	240 µL	1.2 mL
5	1.2 mL	300 µL	1.5 mL
10	2.4 mL	600 µL	3 mL
12	2.88 mL	720 µL	3.6 mL
20	4.8 mL	1.2 mL	6 mL

When preparing buffer IR1X for multiple preparations, it is recommended to prepare mix for one additional preparation to compensate for pipetting errors and attain sufficient mix for the planned number of preparations.

Attention: Additive IRX is viscous! Pipet slowly to avoid pipetting errors due to the viscosity of the additive.

Mix the solution by either pipetting up and down several times, incubation for several minutes on a rolling or inverting incubator or by moderate vortexing.

Note: The mixture contains detergent. Do not vortex strongly in order to avoid excessive foaming. Alternatively, you can prepare the master mix several hours in advance and wait until the foam dissolved. The mixture IR1X is stable for at least six months at 15 – 25 °C.

4 Safety instructions

When working with the **NucleoSpin® Inhibitor Removal** 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: Guanidine hydrochloride in Buffer IR1 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® Inhibitor Removal** 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 Protocols

5.1 Protocol for DNA clean up from samples with moderate contaminations of humic substances or other types of inhibitors

This protocol is especially recommended for samples containing inhibitors from e.g. blood (heme) or plant (polyphenols) or other and can also be used for samples with small amounts of inhibitors form water/soil (humic substances, slight brownish to yellow color).

Before starting the preparation:

- Check if buffer IR1X was prepared from IR1 and IRX according to section 3.
- Check if wash buffer IRW was prepared according to section 3.

Procedure

1	Starting material: Supply 100 µL DNA solution (e.g. DNA solution containing moderate contamination of humic substances (slight brownish color)) or other types of inhibitors in a 1.5 mL or 2 mL tube (not provided). *Note: If your starting material consist of less than 100 µL, fill it up to 100 µL with Buffer BE.*	 **100 µL sample in a 1.5 mL or 2.0 mL tube**
2	Add **300 µL IR1X** and mix incubate for 1 min. *Note: Premix IR1 and IRX according to section 3 to obtain IR1X before starting the preparation.* Add **210 µL ethanol** and mix. *Note: Do not premix IR1X and ethanol – a sequential addition of IR1X and ethanol is recommended.* Apply mixture onto **NucleoSpin® Inhibitor Removal Column** resting in a Collection Tube (2 mL, provided).	 **300 µL IR1X** **Mix and incubate 1 min at RT** **210 µL ethanol**
3	Centrifuge for 30 s at 10,000 × g. Discard the flowthrough and reuse the collection tube.	 **10,000 × g 30 sec**

4 Add **500 µL Wash Buffer IRW** onto the column.

Centrifuge for 30 s at 10,000 × g.

Discard the flowthrough and reuse the collection tube.

Add **300 µL Wash Buffer IRW** onto the column.

Centrifuge for 2 min at 10,000 × g.

Note: If flow through contaminates the column outlet upon removal of the assembly from the centrifuge or upon removal of the column from the collection tube, repeat the centrifugation step.

Discard the flow through with collection tube and put column into a fresh 1.5 mL collection tube (provided).

500 µL IRW

10,000 × g
30 sec

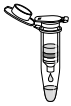
300 µL IRW

10,000 × g
2 min

5 Add **100 µL Elution Buffer BE** onto the column.

Note: See section 2.4 for alternative elution procedures.

Incubate for 1 min at room temperature.



100 µL BE

RT,
1 min

6 Centrifuge for 1 min at 11,000 × g.

Discard the column and use the purified eluate for further analysis.

11,000 × g
1 min

5.2 Protocol for DNA clean up from samples with considerable contamination of humic substances (intense brownish color)

This protocol is especially recommended for samples containing larger amounts of inhibitors originating from e.g. water / soil (humic substances) with moderate to strong brownish color.

Before starting the preparation:

- Check if wash buffer IRW was prepared according to section 3.

Procedure

1	Starting material: Supply 100 µL DNA solution (e.g. DNA solution containing humic substances (brownish color)) in a 1.5 mL or 2 mL tube (not provided). *Note: If starting material is < 100 µL, fill up to 100 µL with Buffer BE.*		**100 µL sample in a 1.5 mL tube**
2	Add **500 µL IR1** and mix. Apply mixture onto **NucleoSpin® Inhibitor Removal Column** resting in a Collection Tube (2 mL, provided).		**500 µL IR1** **mix**
3	Centrifuge for 30 s at 10,000 × g. Discard the flow through and reuse the collection tube.		**10,000 × g 30 sec**
4	Add **500 µL Wash Buffer IRW** onto the column. Centrifuge for 30 s at 10,000 × g. Discard the flowthrough and reuse the collection tube. Add **300 µL Wash Buffer IRW** onto the column. Centrifuge for 2 min at 10,000 × g. *Note: If flow through contaminates the column outlet upon removal of the assembly from the centrifuge or upon removal of the column from the collection tube, repeat the centrifugation step.* Discard the flow through with collection tube and put column into a fresh 1.5 mL collection tube (provided).		**500 µL IRW** **10,000 × g 30 sec** **300 µL IRW** **10,000 × g 2 min**

5	Add **100 µL Elution Buffer BE** onto the column. *Note: See section 2.4 for alternative elution procedures.* Incubate for 1 min at room temperature.		**100 µL BE** **RT,** **1 min**
6	Centrifuge for 1 min at 11,000 × g. Discard the column and use the purified eluate for further analysis.		**11,000 × g** **1 min**

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or low DNA recovery	*DNA washed off the silica membrane:* • make sure to prepare the Wash Buffer by adding the appropriate amount of ethanol. *Low initial DNA concentration:* • Provide higher initial DNA concentration or uses more sensitive analysis methods *Falsely quantification of DNA in the provided sample:* • Presence of PCR inhibitory substances (e.g. humic substances, polyphenols) in a sample will substantially influence DNA quantification by spectrophotometry and fluorescent methods causing a considerable over-or under-estimation of DNA, especially in colored samples or samples with inacceptable $A_{260/280}$ or $A_{260/230}$ ratios. Do not trust DNA quatification results of impure DNA solutions.
PCR inhibition	*Insufficient PCR inhibitor removal:* • The kit is designed to remove diverse PCR inhibitors like e.g. humic substances (brownish color) from DNA solutions. Because the chemical nature of PCR inhibitory substances is diverse, some inhibitors might not be effectively removed. If procedure 5.1 does not give satisfactory results, try procedure 5.2.
No or insufficient decoloration of the sample	*Brownish eluate:* • If an insufficient decoloration of the sample is observed using procedure 5.1, try procedure 5.2 for DNA clean up.
Low $A_{260/280}$ or $A_{260/230}$ ratio	*Low purity:* • Quality ratio determination strongly depends on a sufficient amount of DNA measured. Make sure to use a sufficient amount of DNA that has been validated to enable a meaningful ratio determination with the photometric systems used.
No improvement of $A_{260/280}$ or $A_{260/230}$ ratio	As above.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Inhibitor Removal	740408.10/.50	10/50
NucleoSpin® eDNA water	740402.10/50	10/50
Collection Tubes (2 mL)	740600	1000
Buffer BE (125 mL)	740306.100	1

6.3 Product use restrictions / 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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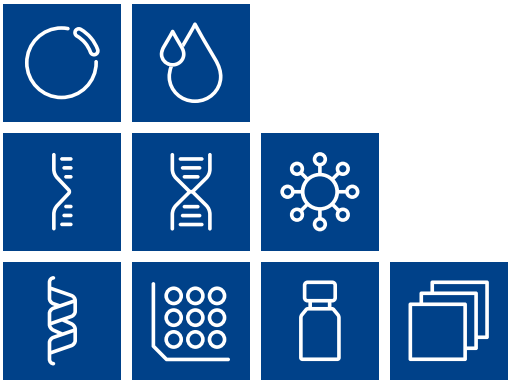
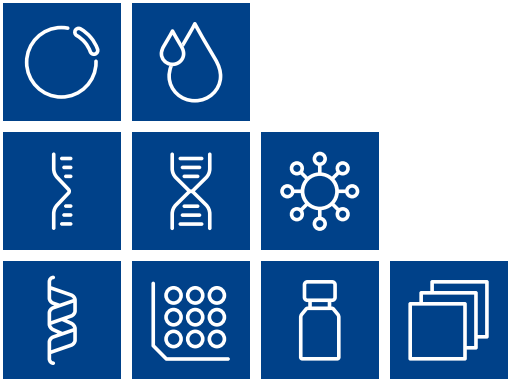
Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-333
e-mail: support@mn-net.com



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



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MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com

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