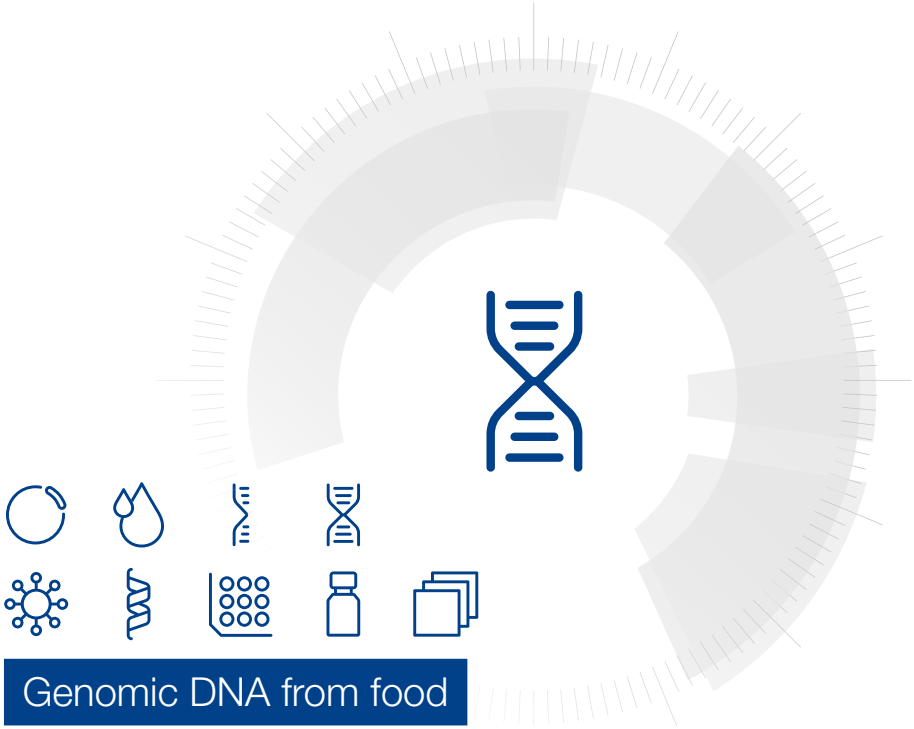
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from food

■ NucleoSpin® Food

May 2023 / Rev. 15

Genomic DNA from food

Protocol at a glance (Rev. 15)

		NucleoSpin® Food
1 Homogenize sample		Homogenize 200 mg material
2 Lyse cells		550 µL CF (65 °C)
		10 µL Proteinase K
		65 °C, 30 min
		> 10,000 x *g*, 10 min
		Take clear supernatant (1 vol) and continue with step 3
3 Adjust DNA binding conditions		1 vol C4
		1 vol ethanol
4 Bind DNA		Load sample stepwise (maximum loading capacity 700 µL)
		11,000 x *g*, 1 min
5 Wash and dry silica membrane		1st wash
		400 µL CQW
		11,000 x *g*, 1 min
		2nd wash
		700 µL C5
		11,000 x g, 1 min
		3rd wash
		200 µL C5
		11,000 x g, 2 min
6 Elute DNA		100 µL CE (70 °C)
		RT, 5 min
		11,000 x *g*, 1 min

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Table of contents

1 Components

1.1 Kit contents

	NucleoSpin® Food		
REF	**10 preps 740945.10**	**50 preps 740945.50**	**250 preps 740945.250**
Lysis Buffer CF	12 mL	100 mL	300 mL
Buffer C4	10 mL	30 mL	150 mL
Wash Buffer CQW	6 mL	30 mL	125 mL
Wash Buffer C5 (Concentrate)*	6 mL	12 mL	50 mL
Elution Buffer CE**	13 mL	13 mL	60 mL
NucleoSpin® Food Columns (plus Collection Tubes)	10	50	250
Proteinase K (lyophilized)*	1.2 mg	6 mg	30 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
Collection Tubes (2 mL)	10	50	250
User manual	1	1	1

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

** Composition of Elution Buffer CE: 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
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating-block for incubation at 65 °C
- Incubator or water bath for preheating Lysis Buffer CF (to 65 °C) and Elution Buffer CE (to 70 °C)
- Equipment for sample disruption and homogenization (see section 2.4)
- 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® Food** 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***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

NucleoSpin® isolation technology from MACHEREY-NAGEL GmbH, and GMO experience from GEN-IAL GmbH were combined to provide an optimal lysis and purification system for nearly all types of food samples. Resulting eluates are ready-to-use for all types of subsequent detection methods, especially for real-time and basic PCR technologies.

GEN-IAL is an established startup company, which offers contract research and molecular testing services in food and feed stuff. Special areas of interest are the development and standardization of detection methods for GMOs, as well as animal and microbial species identification and differentiation.

NucleoSpin® "silica-membrane technology" from MACHEREY-NAGEL allows fast and effective purification of nucleic acids from various matrices. The silica membranes are optimized for high DNA recovery and low binding efficiency for impurities.

2.1 The basic principle

After the food samples have been homogenized, the DNA can be extracted with lysis buffers containing chaotropic salts, denaturing agents, and detergents. The standard isolation ensures lysis using Lysis Buffer CF, which was especially developed by GEN-IAL for food matrices (patent pending). Lysis mixtures should be cleared by centrifugation or filtration in order to remove contaminants and residual cellular debris. The clear supernatant is then mixed with binding buffer and ethanol to create conditions for optimal binding to the **NucleoSpin®** silica membrane, which was selected for this purpose due to its unique DNA-binding properties. After washing with two different buffers for efficient removal of potential PCR inhibitors, DNA can be eluted in low salt buffer or water (see section 2.5 for details), and is ready-to-use for subsequent reactions.

Food samples are very heterogeneous and contain many different compounds like fat, cocoa, or polysaccharides, which can lead to suboptimal extraction or subsequent processing of DNA. **NucleoSpin® Food** guarantees good recovery for small genomic DNA fragments (< 1 kbp) out of processed, complex food matrices (e.g., ketchup or spices), which generally have very low DNA contents, as well as poor quality, degraded DNA. Because of this, we recommend the selection of primers, which amplify only short DNA fragments (80 – 150 bp).

2.2 Kit specifications

- **NucleoSpin® Food** is designed for isolation of genomic DNA from food samples preferably of plant or animal origin. However, bacteria can also be processed (see section 5.1 for details).
- **The NucleoSpin® Food** kit can be used for the identification of GMO-DNA or animal components in food and feed.
- **NucleoSpin® Food** standard procedure allows processing of up to 200 mg material. Depending on the individual sample, typical yields for **NucleoSpin® Food** are in the range of 0.1 – 10 µg DNA.
- Ketchup, sauce, and similar fluid samples (0.2 g equivalents) can be mixed with lysis buffer (500 – 1000 µL each) and incubated with Liquid Proteinase K as described in the protocol (see ordering information for additional Lysis Buffer CF).
- The eluted DNA is ready-to-use for subsequent reactions like real-time PCR, GMO detection, etc.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Food
Format	Mini spin column
Target	DNA
Sample material	5 – 200 mg
Fragment size	300 bp–approx. 50 kbp
Typical yield	0.1 – 10 µg
A_{260}/A_{280}	1.6 – 1.9
Elution volume	100 µL
Preparation time	30 min/6 preps
Binding capacity	30 µg
Use	For research use only

2.3 Storage and homogenization of samples

The lysis procedure is most effective when well homogenized, powdered samples are used. To achieve this, we recommend grinding with a pestle and mortar in the presence of liquid nitrogen or using steel beads. Commercial homogenizers can also be used. After homogenization and treatment of the sample with lysis buffer, mixtures can be cleared easily and effectively by either centrifugation or with **NucleoSpin® Filters** (see ordering information).

2.4 Methods to homogenize samples

- Pestle and mortar in the presence of liquid nitrogen
- Commercial homogenizers, for example bead mills
- VA steel beads (diameter: 7 mm, sample available on request): Put 4 – 5 beads and food material together in a 15 mL plastic tube (Falcon), chill the tube in liquid nitrogen. Vortex for about 30 s (e.g., with a Multi Pulse Vortexer, contact Schütt Labortechnik GmbH, Postfach 3454, D-37024 Göttingen, Germany). Repeat this chilling and vortexing procedure until the entire sample is ground to a powder. Chill the tube once more and remove the beads by rolling them out gently or by using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube! This leads to sticking and loss of sample as it attaches to the beads.

2.5 Elution procedures

It is possible to adapt the elution method and volume of elution buffer for the subsequent application of interest:

- **Complete yields:** By performing two elution steps with 2 × 100 µL, 90 – 100 % of bound nucleic acids can be eluted. Finally, combine eluates and measure yield.
- **Highly concentrated eluates:** With minimal elution volumes (25 – 50 µL) about 60 – 80 % of bound nucleic acids can be eluted, resulting in highly concentrated eluates.

Elution Buffer CE (5 mM Tris/HCl, pH 8.5) can be replaced by TE buffer or water as well. If water is used, the pH should be checked and adjusted to pH 8 – 8.5 since deionized water usually exhibits a pH below 7. Furthermore, absorption of CO_2 leads to a decrease in pH of unbuffered solutions.

3 Storage conditions and preparation of working solutions

Attention:

Buffers C4 and CQW contain guanidine hydrochloride and detergents! Wear gloves and goggles!

CAUTION: Buffers C4 and CQW contain 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.

- All kit components can be stored at 15 – 25 °C and are stable until: see package label.
- If there is any precipitate present in the buffers, warm the buffer to 25 – 37 °C to dissolve the precipitate before use.

Before starting any **NucleoSpin® Food** protocol prepare the following:

- **Wash Buffer C5:** Add the indicated volume of ethanol (96 – 100 %) to **Buffer C5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer C5 at 15 – 25 °C for at least one year.
- Before first use of the kit, add the indicated volume of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable for 6 months at -20 °C.

	NucleoSpin® Food		
REF	**10 preps** **740945.10**	**50 preps** **740945.50**	**250 preps** **740945.250**
Wash Buffer C5 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol
Proteinase K	1.2 mg Add 120 µL Proteinase Buffer	6 mg Add 600 µL Proteinase Buffer	30 mg Add 3.0 mL Proteinase Buffer

4 Safety instructions

When working with the **NucleoSpin® Food** 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 ***www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer C4 and CQW 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® Food** 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 and Proteinase K 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 General remarks

5.1 Important information and advice

- Due to the low DNA content in processed food, this protocol should be started with up to 200 mg of material.
- Lysis buffer was tested (see list on the next page) for extraction of DNA from various types of samples including food of plant and animal origin, and bacteria. To detect bacterial DNA in food samples, we recommend an overnight preculture of sample and appropriate culture medium. Centrifuge an aliquot of the culture and start the preparation with the bacterial pellet.
- RNase A (not included in the kit) addition may be recommended for RNA-rich samples. Add 10 µL (20 mg/mL stock solution) per 550 µL lysis buffer in step 2 of the protocol or perform an RNase A digestion in the eluate before further use.
- A vacuum manifold can optionally be used for acceleration of washing steps. Loading and elution steps should be done by centrifugation as described in the protocol.
- Ketchup, sauce, and similar fluid samples (200 mg equivalents) can be mixed with lysis buffer (500 – 1000 µL each) and incubated with Proteinase K as described in the protocol (see ordering information for separately available Lysis Buffer CF).
- For powdered hygroscopic samples, more lysis buffer than indicated in the protocol can be used until the lysis solution is at least semi fluid and can be pipetted (see ordering information for separately available Lysis Buffer CF). Extraction can be improved by preincubation of sample with lysis buffer for 1 – 2 h.
- According to local law regulations different amounts of sample have to be analyzed for GMO detection, for example up to 1 – 2 g of sample can be used with upscaled lysis buffer volumes. We recommend to use a single 300 µL aliquot (section 6, step 3) of the clear supernatant for further processing with **NucleoSpin® Food Columns**. Otherwise, prepare 2 aliquots as described in the protocol and load them step by step onto the **NucleoSpin® Food Column**.

Positively tested samples (PCR)*	
Food (plant origin)	Raw products: maize, soja, rape etc. (powder or oil) Chocolate products, cocoa, nougat products Breakfast cereals, muesli, nut / chocolate spread Jam and fruit concentrates Cookies, cakes and biscuits Pollen Lecithine Spices Bread
Food (animal origin)	Raw and processed products (meat, sausage, pie)
Cosmetics	Plant and animal ingredients (e.g., in crème or powder)
Bacteria	Starter cultures, etc.

6 Protocol for genomic DNA purification from food

Before starting the preparation:

- Check if Wash Buffer C5 was prepared according to section 3.
- Preheat Lysis Buffer CF to 65 °C and Elution Buffer CE to 70 °C.
- Ethanol (96 – 100 %)

1 Homogenize sample

Homogenize about **200 mg material** with a commercial homogenizer.

Homogenize samples

2 Lyse cells

Transfer the resulting powder to a Collection Tube (2 mL) and add **550 µL Buffer CF (preheated to 65 °C)**. Mix carefully (15 s), add **10 µL Proteinase K** and mix again (2 – 3 s).

+ 550 µL CF (65 °C)

+ 10 µL Proteinase K

If the lysis buffer volume is not large enough to dissolve the sample completely add more buffer (and Proteinase K proportionally) until sample has been totally resuspended.

Incubate at **65 °C** for **30 min**. Afterwards, centrifuge the mixture for **10 min (> 10,000 x *g*)** to pellet contaminants and cell debris.

65 °C, 30 min

> 10,000 x *g*, 10 min

<u>Optional:</u> If RNA-free DNA is crucial for downstream applications an RNase digest may be performed: After incubation at 65 °C for 30 min, add 10 µL RNase A (20 mg/mL stock solution, not provided, see ordering information) per 550 µL lysis buffer, mix well, and incubate at RT (18 – 25 °C) for 30 min. Proceed with the protocol with the centrifugation step.

3 Adjust DNA binding conditions

Transfer clear supernatant from step 2 into a microcentrifuge tube (not provided) capable of holding at least 3 sample volumes. Add **1 vol Buffer C4** and **1 vol ethanol** (e.g., take 300 µL sample and add 300 µL Buffer C4 and 300 µL ethanol). Vortex the mixture for 30 s.

1 vol C4

1 vol ethanol

4 Bind DNA

For each preparation take one NucleoSpin® Food Column placed in a Collection Tube. Pipette 700 µL mixture onto the column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through. Repeat the procedure to load the remaining sample.

Load sample

11,000 x *g*, 1 min

5 Wash and dry silica membrane

1st wash

+ 400 µL CQW

11,000 x *g*, 1 min

Pipette **400 µL Buffer CQW** onto the NucleoSpin® Food Column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through.

2nd wash

+ 700 µL C5

11,000 x *g*, 1 min

Pipette **700 µL Buffer C5** onto the NucleoSpin® Food Column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through.

3rd wash

+ 200 µL C5

11,000 x *g*, 2 min

Pipette another **200 µL Buffer C5** onto the NucleoSpin® Food Column. Centrifuge for **2 min** at **11,000 x *g*** in order to remove Buffer C5 completely.

Residual ethanol from Wash Buffer C5 may inhibit enzymatic reactions.

6 Elute DNA

Load sample

RT, 5 min

11,000 x *g*, 1 min

Place the NucleoSpin® Food Column in a new 1.5 mL microcentrifuge tube (not provided). Pipette **100 µL Elution Buffer CE (preheated to 70 °C)** onto the membrane. Incubate for **5 min** at **room temperature (15 – 25 °C).** Centrifuge for **1 min** at **11,000 x *g*** to elute the DNA.

For alternative elution procedures see section 2.5.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
DNA yield is low	*Homogenization of food material was not sufficient* • For most species we recommend grinding with steel beads (see section 2.4) or with commercial bead mills, mixers, or homogenizers. *Extraction of DNA from food material during lysis was not sufficient* • To obtain higher yields of DNA, the incubation time in lysis buffer can be prolonged (up to overnight). *Sample contains too much RNA* • Add 10 – 20 µL RNase A solution to the lysis buffer after heat incubation. If this is not successful, add the enzyme to the cleared lysate and incubate for 30 min at 37 °C. *Suboptimal elution* • The DNA can be either eluted in higher volumes (up to 300 µL) or by repeating the elution step up to three times. Remember that the elution buffer must be preheated to 70 °C prior to elution. • Also check the pH of the used elution buffer, which should be in the range of pH 8.0 – 8.5. To ensure correct pH, use supplied Elution Buffer CE (5 mM Tris / HCl, pH 8.5).
DNA is degraded	*Sample was contaminated with DNase* • Check working area and pipettes. *Centrifugation speed was too high* • Centrifuge at the speed indicated in the protocol. Higher velocities and prolonged vortexing can lead to shearing of the DNA.
DNA quality is low	*Sample contains DNA-degrading contaminants (e.g., phenolic compounds, metabolites)* • Repeat washing step with Buffer CQW.

7.2 Ordering information

Product	REF	Pack of
NucleoSpin® Food	740945.10/.50/.250	10/50/250
NucleoSpin® Filters	740606	50
Buffer CF	740946	1 L
Buffer C4	740366.250	250 mL
Proteinase K	740506	100 mg
RNase A	740505.50 740505	50 mg 100 mg
Collection Tubes (2 mL)	740600	1000

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

7.3 Product use restriction / warranty

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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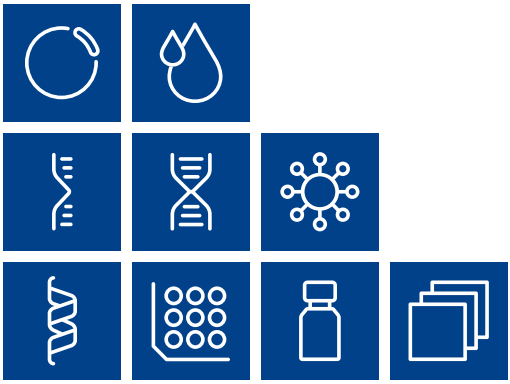

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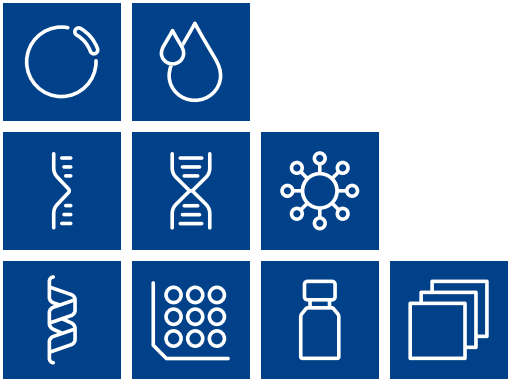

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