



Genomic DNA from food

User manual

NucleoSpin® Food

February 2011 / Rev. 09

Genomic DNA from food

Protocol-at-a-glance (Rev.09)

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 <i>g</i> 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 750 µL) 11,000 x <i>g</i> 1 min
5 Wash and dry silica membrane	 	400 µL CQW 1 st wash 11,000 x <i>g</i> 1 min 700 µL C5 2 nd wash 11,000 x <i>g</i> 1 min 200 µL C5 3 rd wash 11,000 x <i>g</i> 2 min
6 Elute DNA	 	100 µL CE (70 °C) RT 5 min 11,000 x <i>g</i> 1 min

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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 C2	6 mL	24 mL	120 mL
Buffer C3	1.5 mL	6 mL	30 mL
Wash Buffer CQW	6 mL	30 mL	125 mL
Wash Buffer C5 (Concentrate)*	6 mL	20 mL	3 x 20 mL
Elution Buffer CE**	5 mL	15 mL	2 x 25 mL
NucleoSpin® Food Columns (plus Collection Tubes)	10	50	250
Proteinase K (lyophilized)*	1.2 mg	6 mg	30 mg
Proteinase Buffer PB	0.8 mL	0.8 mL	3.6 mL
Collection Tubes (2 mL)	10	50	250
Label for Binding Buffer C4	1	1	1
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.

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.
- 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
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

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 x 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 CO₂ leads to a decrease in pH of unbuffered solutions.

3 Storage conditions and preparation of working solutions

Attention:

Buffers C2, C3, C4, and CQW contain guanidine hydrochloride and detergents! Wear gloves and goggles!

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

- **Binding Buffer C4:** Transfer the total content of **Buffer C2** to **Buffer C3** and mix well. The resulting **Binding Buffer C4** is stable for up to one year at room temperature. For a better dissolving of both components a 5 min incubation at 45 °C is recommended. If the kit will only be used occasionally it is also possible to mix small quantities of Buffer C3 and C2 in a 1:4 ratio. For example, mix 100 µL Buffer C3 and 400 µL Buffer C2. Mix by pipetting up and down.
- **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 room temperature (18–25 °C) for up to 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	20 mL Add 80 mL ethanol	3 x 20 mL Add 80 mL ethanol to each vial
Proteinase K	1.2 mg Add 120 µL Proteinase Buffer	6 mg Add 600 µL Proteinase Buffer	30 mg Add 2.7 mL Proteinase Buffer

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® Food** kits contain hazardous contents.

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

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>Gefahrstoffsymbol</i>		<i>R-Sätze</i>	<i>S-Sätze</i>
C2	Guanidine hydrochloride <i>Guanidinhydrochlorid</i>	✘ Xn*	Harmful if swallowed – Irritating to eyes and skin <i>Gesundheitsschädlich beim Verschlucken – Reizt die Augen und die Haut</i>	R 22–36/38	
CQW	Guanidine hydrochloride + ethanol < 40 % <i>Guanidinhydrochlorid + Ethanol < 40 %</i>	✘ Xn*	Flammable – Harmful if swallowed – Irritating to eyes and skin. <i>Entzündlich – Gesundheitsschädlich beim Verschlucken – Reizt die Augen und die Haut</i>	R 10–22–36/38	S 7-16
Proteinase K	Proteinase K, lyophilized <i>Proteinase K, lyophilisiert</i>	✘ Xn*	Irritating to eyes, respiratory system and skin may cause sensitization by inhalation <i>Reizt die Augen, Atmungsorgane und die Haut – Sensibilisierung durch Einatmen möglich</i>	R 36/37/38–42	S 22–24–26–36/37

Risk phrases / R-Sätze

R 10	Flammable <i>Entzündlich</i>
R 22	Harmful if swallowed <i>Gesundheitsschädlich beim Verschlucken</i>
R 36/37/38	Irritating to eyes, respiratory system and skin <i>Reizt die Augen, Atmungsorgane und die Haut</i>
R 36/38	Irritating to eyes and skin <i>Reizt die Augen und die Haut</i>
R 42	May cause sensitization by inhalation <i>Sensibilisierung durch Einatmen möglich</i>

* 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.

Safety phrases / S-Sätze

- S 7 Keep container tightly closed
Behälter dicht geschlossen halten
- S 13 Keep away from food, drink and animal feedstuffs
Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten
- S 22 Do not breathe dust
Staub nicht einatmen
- S 24 Avoid contact with the skin
Berührung mit der Haut vermeiden
- S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren
- S 36/37 Wear suitable protective clothing and gloves
Bei der Arbeit geeignete Schutzhandschuhe und Schutzkleidung tragen

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 for extraction of DNA from various types of samples including food of plant and animal origin, and bacteria (see list on the next page). 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**.

5.2 Table of positively tested samples (PCR)

Food (plant origin)	Raw products: maize, soy, 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)
Pharmaceuticals	Plant (starch) compounds in pharmaceuticals (e.g., tablets) Vitamins (e.g., pills)
Cosmetics	Plant and animal ingredients in for example cream or powder
Bacteria	E.g., starter cultures

6 Protocol for genomic DNA purification from food

Before starting the preparation:

- Check if Wash Buffer B5 was prepared according to section 3.
- Preheat Lysis Buffer CF to 65 °C and Elution Buffer CE to 70 °C.

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.

Optional: 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.



**65 °C
30 min**

**> 10,000 x g
10 min**

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

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

+ 400 µL
CQW

11,000 x g
1 min

2nd wash

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



+ 700 µL C5

11,000 x g
1 min



3rd wash

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.

+ 200 µL C5

11,000 x g
2 min

Residual ethanol from Wash Buffer C5 may inhibit enzymatic reactions.

6 Elute DNA

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 (18–25 °C)**. Centrifuge for **1 min** at **11,000 x g** to elute the DNA.



Load sample



11,000 x g
1 min

For alternative elution procedures see section 2.6.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
DNA yield is low	<p><i>Homogenization of food material was not sufficient</i></p> <ul style="list-style-type: none"> For most species we recommend grinding with steel beads (see section 2.4) or with commercial bead mills, mixers, or homogenizers.
	<p><i>Extraction of DNA from food material during lysis was not sufficient</i></p> <ul style="list-style-type: none"> To obtain higher yields of DNA, the incubation time in lysis buffer can be prolonged (up to overnight).
	<p><i>Sample contains too much RNA</i></p> <ul style="list-style-type: none"> 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.
DNA is degraded	<p><i>Suboptimal elution</i></p> <ul style="list-style-type: none"> 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).
	<p><i>Sample was contaminated with DNase</i></p> <ul style="list-style-type: none"> Check working area and pipettes.
DNA quality is low	<p><i>Centrifugation speed was too high</i></p> <ul style="list-style-type: none"> Centrifuge at the speed indicated in the protocol. Higher velocities and prolonged vortexing can lead to shearing of the DNA.
	<p><i>Sample contains DNA-degrading contaminants (e.g., phenolic compounds, metabolites)</i></p> <ul style="list-style-type: none"> 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 (100 mL C2 + 25 mL C3)	740935	125 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

NucleoSpin® Food 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.

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

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!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

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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