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Genomic DNA from Food

User manual

NucleoSpin® 8 Food
NucleoSpin® 96 Food

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MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoSpin® 8 Food[1]	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740975**	**740975.5**
Buffer CF	100 ml	500 ml
Buffer C2	50 ml	2 x 100 ml
Buffer C3	12.5 ml	50 ml
Buffer CQW	125 ml	3 x 125 ml
Buffer C5[1] (concentrate)	50 ml	2 x 100 ml
Buffer CE	25 ml	125 ml
Buffer PB	1.8 ml	15 ml
Proteinase K[2]	30 mg	2 x 75 mg
NucleoSpin® Food Binding Strips (blue)	12	60
Rack with MN Tube Strips (Lysis) with Cap Strips	1	5
Rack with MN Tube Strips (Elution) with Cap Strips	1	5
MN Wash Plate[3]	2	10
Gas-permeable Foils	6	18
Round-well Block	1	5
User manual	1	1

[1] The use of NucleoSpin 8 Food requires a Starter Set containing additional hardware (see section 2.3).
[2] For preparation of working solutions and storage conditions see section 3.
[3] For vacuum processing only.

1 Kit contents continued

	NucleoSpin® 96 Food		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps[4]**
Cat.No.	**740976.2**	**740976.4**	**740976.24**
Buffer CF	2 x 100 ml	3 x 100 ml	12 x 100 ml
Buffer C2	80 ml	200 ml	6 x 200 ml
Buffer C3	20 ml	50 ml	6 x 50 ml
Buffer CQW	125 ml	240 ml	6 x 240 ml
Buffer C5 (concentrate)[5]	80 ml	2 x 80 ml	12 x 80 ml
Buffer CE	50 ml	75 ml	6 x 75 ml
Buffer PB	3.6 ml	8 ml	6 x 8 ml
Proteinase K	2 x 30 mg	4 x 30 mg	24 x 30 mg
NucleoSpin® Food Binding Plate (blue)	2	4	24
Rack with MN Tube Strips (Lysis) with Cap Strips	2	4	6 x 4
Rack with MN Tube Strips (Elution) with Cap Strips	2	4	6 x 4
MN Wash Plate[6]	2	4	6 x 4
Round-well Block with Cap Strips	2	4	6 x 4
Gas-permeable Foils	6	12	6 x 12
MN Square-well Block	2	2	6 x 2
User manual	1	1	6 x 1

[4] The kit for 24x96 preparations Cat. No. 740976.24 consists of 6 x Cat. No. 740976.4
[5] For preparation of working solutions and storage conditions see section 3.
[6] For vacuum processing only.

2 Product description

2.1 The basic principle

NucleoSpin® 8/96 Food is designed for the isolation of genomic DNA from food samples preferably of plant or animal origin. In the kit the **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 in all types of subsequent detection methods, especially in real-time and basic PCR technologies.

GEN-IAL is a 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 spin technology" from MACHEREY-NAGEL allows fast and effective purification of nucleic acids from various matrices. The silica membranes are optimized for high DNA recoveries and low unspecific binding of impurities. For further questions regarding DNA or RNA purification feel free to contact us (see cover page for contact details).

Nucleic acid extraction: 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 buffer CF, a proprietary buffer developed by GEN-IAL for food matrices (patent pending). Lysis mixtures have to 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 adjust binding conditions for optimal binding of DNA 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, and is ready-to-use in 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® 8/96 Food** guarantees good recovery rates for small genomic DNA fragments (< 1 kb) from processed, complex food matrices (e.g. ketchup or spices), which generally have very low DNA contents as well as poor quality, degraded DNA. We thus recommend the selection of primers, which amplify only short DNA fragments (80-150 bp).

2.2 Kit specifications

- **NucleoSpin® 8/96 Food** is designed for the isolation of genomic DNA from food samples preferably of plant or animal origin. However, bacteria can also be processed (see section 4 for details).

- **NucleoSpin® 8/96 Food** kit can be used for the identification of GMO-DNA or animal components in food and feed.

- **NucleoSpin® 8/96 Food** 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 (also see table 1).

- The eluted DNA is ready-for-use in subsequent reactions like real-time PCR, GMO detection etc.

- **NucleoSpin® 8/96 Food** allow parallel purification of multiples of 8/96 samples

- **NucleoSpin® 8/96 Food** can be processed by centrifugation or under vacuum. Processing under vacuum allows easy automation on common liquid handling instruments. For more information about the automation process and the availability of ready-to-run scripts for certain platforms please refer to section 2.5 and contact your local distributor or MN directly.

- Any unused wells of the **NucleoSpin® 8/96 Food Binding Module** should be covered with Self-adhering PE Foil (see ordering information) in order to guarantee a proper vacuum and to protect the unused wells from being contaminated.

Table 1: Kit specifications at a glance

	NucleoSpin® 8/96 Food
Sample size	up to 200 mg
typical DNA yield	0.1 - 10 µg
Elution volume	100 µl - 200 µl
Binding capacity	30 µg
Size of DNA	> 300 bp
Time/ 6 strips or 1 plate	< 2 h (without lysis)
Column type	8-well strip or 96-well plate

2.3 Required hardware

Centrifugation

NucleoSpin® 8 Food and NucleoSpin® 96 Food:

For **centrifugation** a microtiterplate centrifuge which is able to accommodate the NucleoSpin® Food Binding Strips/Plate stacked on a round or square-well block and reaches accelerations of 5,600 – 6,000 x g is required (bucket height: 85 mm), e.g. Hermle Z 513/Z 513 K, Jouan KR4i, Heraeus Kendro Multifuge 3/3-R, Beckman Coulter Allegra 25R, Hettich Rotanta 460 series, Sigma 4-15/4K15/6-15/6K15.

NucleoSpin® 8 Food:

For processing the NucleoSpin® 8well strips the Starter Set C (see ordering information), containing Column Holders C, Dummy Strips, MN Square-well Blocks, Tube Strips is required, too. For detailed information refer to the Starter Set C manual.

Vacuum processing

NucleoSpin® 8 Food and NucleoSpin® 96 Food:

For vacuum application a NucleoVac 96 vacuum manifold (Cat.No. 740681) or other suitable manifolds are required. If the Qiavac 96 vacuum manifold is used the MN Frame (see ordering information) is additionally required.

NucleoSpin® 8 Food:

Using the NucleoSpin® 8 Food kit Starter Set A containing Column Holders A and Dummy Strips and NucleoVac 96 vacuum manifold or similar suitable vacuum manifolds are required (see ordering information).

2.4 Elution procedures

It is possible to adapt the elution method and volume of the elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 80 - 90 %) described in the protocols there are 3 modifications possible:

- **High yields**: 90-100% of bound nucleic acids can be eluted by performing two elution steps with volumes as indicated in the protocol e.g. 2 x 100 µl. Finally, combine eluates and measure yield.

- **Highly concentrated eluates**: Using a minimal elution volume (about 75 µl) about 70-80% of bound nucleic acids can be eluted, resulting in highly concentrated eluates.

- **Preheated elution buffer (70°C):** Preheat elution buffer to increase yield. After loading half of the preheated elution buffer (75 µl) onto the membrane, incubate the **NucleoSpin® Food Binding Strip/Plate** for 3 min at 60-70°C. Afterwards add another volume elution buffer (e.g. 50-75 µl) and incubate further 2 min at room temperature before elution.

- Recovery of gDNA from the membrane depends on the elution volume. Elution volumes of 75 – 200 µl are possible, with an optimum of 100 – 125 µl dispensed volume. The dead volume of the membrane is approx. 45 µl and the recovered elution buffer can thus easily estimated.

2.5 Automation

NucleoSpin® 8/96 Food can be fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8/96 Food** on a certain workstation please contact MN. Full processing under vacuum enables complete automation without the need for centrifugation steps for drying of the membrane or for elution.

The risk of cross-contamination is reduced by optimized vacuum settings during the elution step and by the improved shape of the outlets of the **NucleoSpin® 8/96 Food Binding Module** and the use of the MN Wash Plate that is included in the kit.

Drying of the **NucleoSpin® 8/96 Food Binding Module** under vacuum is sufficient because the bottom of the plate/strip is protected from spraying wash buffer during the washing steps by the MN Wash Plate. So, if possible the MN Wash Plate should be integrated into the automated procedure. The MN Frame (Cat. No. 740 680) can be used to position the disposable MN Wash Plate inside the vacuum chamber. This also reduces the risk of cross-contamination, as common metal adaptors tend to get contaminated by gDNA. Thorough cleaning of the vacuum manifold is recommended after each run to prevent forming of gDNA-containing aerosols.

Visit MN on the internet at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol.

2.6 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 a **NucleoSpin® Filter** (Cat. No. 740606).

Methods to homogenize samples

- Commercial homogenizers, for example Crush Express for 96-well homogenization (contact Saaten-Union Resistenzlabor GmbH, D-33818 Leopoldshöhe), Tissue Striker (www.KisanBiotech.com) or Geno/Grinder 2000 (www.spexcsp.com or for Germany www.c3-analysentechnik.de) are suitable.

- Homogenizing samples by VA steel beads (diameter: 7 mm): Put 4-5 beads and food material together into a 15 ml plastic tube (Falcon), chill the tube in liquid nitrogen and vortex for about 30 seconds (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 material is ground to a powder. Chill the tube once more and remove the beads by rolling them out gently or with 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 material attached to the beads.

3 Storage conditions and preparation of working solutions

Attention:

Buffers C2, C3, C4 and CQW contain guanidinium hydrochloride and/or detergents! Wear gloves and goggles!

Before starting any **NucleoSpin® 8/96 Food** protocol prepare the following:

- **Buffer C4:** Transfer the total contents of buffer C3 to buffer C2 and mix well. The resulting buffer C4 is stable for 4 months at room temperature (to be stored in the dark). 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 one volume of buffer C3 with four volumes of buffer C2, e.g. 100 µl buffer C3 and 400 µl buffer C2. Mix by pipetting up and down

- **Buffer C5:** Add the given volume of ethanol (indicated on the bottle) to buffer C5 before use.

- **Proteinase K:** Before first use of the kit, add the indicated volume of Proteinase Buffer to dissolve lyophilized proteinase K. Proteinase K solution is stable at +4°C for up to 6 months. Dividing the solution into small aliquots and storage at –20°C is recommended if the solution will not be used up during this period.

	NucleoSpin® 8 Food	
	12 x 8 preps	**60 x 8 preps**
Cat.No.	**740975**	**740975.5**
Buffer C5 concentrate	50 ml add 200 ml ethanol	100 ml add 400 ml ethanol
Proteinase K (lyophilized)	30 mg dissolve in 1.35 ml buffer PB	2 x 75 mg dissolve in 3.35 ml buffer PB each

NucleoSpin® 96 Food		
	2 x 96 preps	**4 x 96 preps**
Cat.No.	**740976.2**	**740976.4**
Buffer C5 concentrate	80 ml add 320 ml ethanol	80 ml add 320 ml ethanol
Proteinase K (lyophilized)	2 x 30 mg dissolve in 1.35 ml buffer PB each	4 x 30 mg dissolve in 1.35 ml buffer PB each

The kit for 24 x 96 preparations (Cat.No. 740976.24) consists of 6 x Cat.No. 740976.4.

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® 8/96 Food kits contain hazardous contents.

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

Buffer/ Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
C2	guanidine hydrochloride	 Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	
CQW	guanidine hydrochloride	 Xn	Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16
Proteinase K	proteinase K, lyophilized	 Xn	Irritating to eyes, respiratory system and skin, may cause sensitization by inhalation	R 36/37/38-42	S 22-24-26-36/37

Risk Phrases

R 10 Flammable

R 22 Harmful if swallowed

R 36/37/38 Irritating to eyes, respiratory system and skin

R 36/38 Irritating to eyes and skin

R 42 May cause sensitisation by inhalation

Safety Phrases

S 7 Keep container tightly closed

S 16 Keep away from sources of ignition – No Smoking!

S 22 Do not breathe dust

S 24 Avoid contact with the skin

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S 36/37 Wear suitable protective clothing and gloves

* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 General Procedure NucleoSpin® 8/96 Food

5.1 Important information and advice

Due to the low DNA content in processed food, this protocol should be started with up to 0.2 g 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 a RNase A digestion in the eluate before further use.

Ketchup, sauce and similar fluid samples (0.2 g 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 additional 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 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, e.g. up to 1-2 g of sample can be used with up scaled lysis buffer volumes. We recommend to use a single 300 µl aliquot (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**.

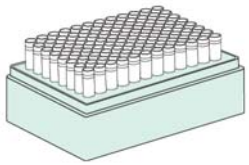
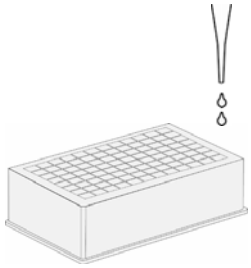
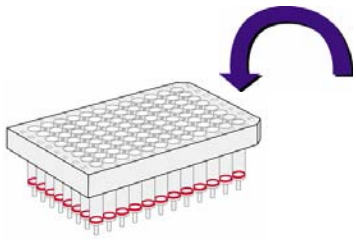
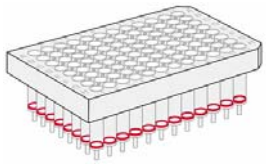
For processing large samples of e.g. 1 g lysis buffer and proteinase K have to be upscaled: per 0.1 g of sample add 275 µl buffer CF and 5 µl proteinase K. After incubation and clearance of the lysate proceed with step 3 in the protocol and further use of 300 µl of the lysate. In order to increase the sensitivity a repeated loading (repeated performance of step 3-5) is possible.

5.2 Table of positive tested samples (PCR)*

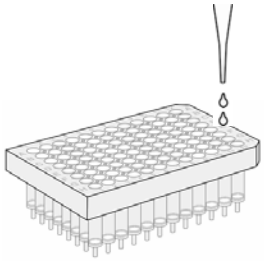
Food (plant origin)	raw products: maize, soja, rape etc. (powder or oil) chocolate products, cocoa, nougat products breakfast cereals, muesli, nut/chocolate spread jam and fruit concentrates cookies, cakes and biscuits pollen lecithine spices bread
Food (animal origin)	raw and processed products (meat, sausage, pie)
Cosmetics	plant and animal ingredients in e.g. crème or powder
Bacteria	e.g. starter cultures

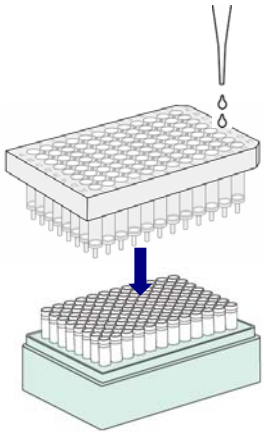
* including data obtained from NucleoSpin® Food kit

5.3 General procedure (overview)

1	Homogenize samples	**0.2 g sample,** **550 µl preheated (65°C) buffer CF** **10 µl proteinase K** **mix** **65°C, 30 min**	
2	Clear lysate	**5,600 × g, 20 min**	
3	Adjust DNA binding conditions	**300 µl clear lysate, add 300 µl buffer C4 and 200 µl ethanol, mix**	
4	Load samples	**transfer samples to NucleoSpin® Food Binding Module (blue)**	
5	Bind DNA to silica membrane	**5,600 × g, 10 min** **or** **ca. –0.2 bar (5 min)**	

6	Wash silica membrane	**500 µl CQW** **5,600 × g, 2 min** **or** **ca. –0.2 bar (5 min)** **900 µl C5** **5,600 × *g*, 5 min** **or** **ca. –0.2 bar (5 min)** **vacuum processing only:** **repeat C5 wash step once**
	Remove MN Wash Plate (vacuum processing only)	
	Dry silica membrane	**5,600 x g, 15 min** **or** **37°C, 20 min** **or** **ca. –0.6 bar, 10 min**
7	Elution	**100 µl CE, 70°C** **5,600 × g, 2 min** **or** **ca. –0.6 bar, 2 min** **optional** **repeat elution step once**





5.4 NucleoSpin® 8 Food Protocol – purification of DNA under centrifugation

Before starting with preparation, prepare buffers C4 and C5 and proteinase K solution (see section 3 for details). Equilibrate buffer CF to 65°C and buffer CE to 65-70°C.

For each preparation collect up to 200 mg of sample into an appropriate lysis vessel, e.g. Rack with MN Tube Strips (Lysis, supplied with the kit).

1 Homogenize and lyse samples

Homogenize up to 200 mg sample using a commercial homogenizer. Transfer homogenized samples into MN Tube Strips (Lysis) and add 550 µl buffer CF preheated to 65°C. Add 10 µl of proteinase K solution. Close the MN Tube Strips using Cap Strips and mix by vigorous shaking for 15-30 sec. Spin briefly for 30 sec at 1,500 x g to collect any sample at the bottom of the MN Tube Strips. Incubate at 65°C for 30 min.

2 Clear lysate

Centrifuge the samples for 20 min at full speed (5,600-6,000 x g).

Insert the desired number of NucleoSpin® Food Binding Strips in the Column Holder C and place it on an MN Square-well Block (supplied with Starter Set C) for collection of flow through. If using more than one block label the column holders for later identification.

Always use two column holders with identical numbers of 8-well strips for centrifugation. Doing so there is no need for balancing. We recommend positioning of the 8-well strips around the center of the column holder.

3 Adjust binding conditions

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol, Close the individual wells with Cap Strips. Mix by vigorous vortexing for 15-30 sec (or by pipetting up and down). Spin briefly for 30 sec at 1,500 x g to collect any sample from cap strips.

Buffer C4 and ethanol can be premixed.

4 Loading

Transfer samples from the previous step into the wells of the NucleoSpin® Food Binding Strips. Do not moisten the rims of the individual wells while dispensing the samples. After transfer seal the openings of the strips with Gas-permeable Foil.

This foil can be cut into appropriate pieces due to the number of NucleoSpin® Food Binding Strips which are to be covered.

Note: When not using air permeable foil pierce foil to achieve air permeability.

5 Bind DNA to silica membrane

Place the Column Holder C holding the NucleoSpin® Food Binding Strips onto a MN Square-well Block (supplied with the Starter Set C) and place it into the rotor buckets. Centrifuge at 5,600 – 6,000 × g for 10 min.

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely. The volume of each well of the NucleoSpin® Food Binding Strip is ~ 1 ml. Higher volumes, resulting from steps 1-3, have to be loaded successively until the complete lysis mixture has been applied.

6 Wash silica membrane

1st wash

Remove the Gas-permeable Foil and add 500 µl CQW to each well of the NucleoSpin® Food Binding Strips. Seal the strips with a new Gas-permeable Foil and centrifuge again at 5,600 – 6,000 × g for 2 min. Discard waste collected in the MN Square-well Block after this wash step.

2nd wash

Remove the Gas-permeable Foil and add 900 µl C5 to each well of the NucleoSpin® Food Binding Strips. Centrifuge for 5-15 min at full speed (5,600 - 6,000 x g) in order to remove buffer C5.

For critical ethanol-sensitive applications it is recommended to prolong the centrifugation time up to 15 min or incubate at higher temperature. Remove the adhesive foil and place the column holder with the NucleoSpin® Food Binding Strips into an incubator for 20 min at 37°C to evaporate residual ethanol.

Removal of ethanol by evaporation at 37°C is more effective than additional, prolonged centrifugation (15 min, 6,000 x g).

7 Elute highly pure DNA

Place column holder with NucleoSpin® Food Binding Strips on an opened rack with MN Tube Strips (elution). Dispense 100 µl pre-warmed buffer CE (70°C) to each well of the NucleoSpin® Food Binding Strips. Dispense the buffer directly onto the membrane. Incubate at room temperature for 2-3 min. Centrifuge at 5,600 – 6,000 × g for 2 min. Remove the Column Holder C from the Tube Strips.

Yields will be 10 – 20% higher when eluting in 200 µl buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100 µl. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

Clean the MN Square-well Blocks with detergent and hot water and incubate for 1 - 5 min in 0.4 M HCl. Rinse with water again and autoclave before next use.

5.5 NucleoSpin® 96 Food Protocol – purification of DNA under centrifugation

Before starting with preparation, prepare buffers C4 and C5 and proteinase K solution (see section 3 for details). Equilibrate buffer CF to 65°C and buffer CE to 70°C.

1 Homogenize and lyse sample material

Homogenize up to 200 mg sample using a commercial homogenizer. Transfer homogenized samples into MN Tube Strips (Lysis) and add 550 µl buffer CF preheated to 65°C. Add 10 µl proteinase K solution. Close the wells with Cap Strips. Mix by vigorous shaking for 15-30 sec. Spin briefly for 30 sec at 1,500 x g to collect any sample from the Cap Strips. Incubate at 65°C for 30 min.

2 Clear lysate

Centrifuge the samples for 20 min at a full speed (5,600 - 6,000 x g). Remove Cap Strips.

3 Adjust binding conditions

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol. Close the individual wells with Cap Strips. Mix by vigorous vortexing for 15-30 sec. Spin briefly for 30 sec at 1,500 x g to collect any sample from cap strips.

Buffer C4 and ethanol can be premixed.

4 Loading

Place the NucleoSpin® Food Binding Plate on a MN Square-well Block. Transfer samples from the Round-well Block into the wells of the NucleoSpin® Food Binding Plate. Do not moisten the rims of the individual wells while dispensing samples. After transfer seal the openings of the NucleoSpin® Food Binding Plate with Gas-permeable Foil.

5 Bind DNA to silica membrane

Place the NucleoSpin® Food Binding Plate on a MN Square-well Block and place both in the rotor buckets. Centrifuge at 5,600 – 6,000 × g for 5 min.

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.

The volume of each well of the NucleoSpin® Food Binding Plate is ~ 1 ml. Higher volumes, resulting from steps 1-3, have to be loaded successively until the complete lysis mixture has been applied.

6 Wash silica membrane

1st wash

Remove the Gas-permeable Foil and add 500 µl CQW to each well of the NucleoSpin® Food Binding Plate. Seal the plate with a new Gas-permeable Foil and centrifuge again at 5,600 – 6,000 × g for 2 min. Discard waste collected in the MN Square-well Block after this wash step.

2nd wash

Remove the Gas-permeable Foil and add 900 µl C5 to each well of the NucleoSpin® Food Binding Plate. Centrifuge for 5-15 min at full speed (5,600 - 6,000 x g) in order to remove buffer C5.

For critical ethanol-sensitive applications it is recommended to prolong the centrifugation time up to 15 min or incubate at higher temperature. Remove the adhesive foil and place the NucleoSpin® Food Binding Plates into an incubator for 20 min at 37°C to evaporate residual ethanol.

Removal of ethanol by evaporation at 37°C is more effective than additional, prolonged centrifugation (15 min, 6,000 x g).

7 Elute highly pure DNA

Place the NucleoSpin® Food Binding Plate on an opened Rack with MN Tube Strips (elution). Dispense 100 µl pre-warmed buffer CE (70°C) to each well of the NucleoSpin® Food Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for 2-3 min. Centrifuge at 5,600 – 6,000 × g for 2 min. Remove the plate from the Tube Strips.

Yields will be 10 – 20% higher when eluting in 200 µl buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100 µl. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

Clean the MN Square-well Blocks with detergent and hot water and incubate for 1 - 5 min in 0.4 M HCl. Rinse with water again and autoclave before next use.

5.6 Processing under vacuum

Although the NucleoSpin® 8/96 Food kit was designed for processing under centrifugation, processing under vacuum is also possible.

NucleoSpin® 8 Food:

For manual processing under vacuum the Starter Set A and the NucleoVac 96 vacuum manifold are required (see ordering information). Starter Set A contains the Column Holders A and the Dummy Strips to close unused rows.

NucleoSpin® 96 Food:

For processing under vacuum the NucleoVac 96 vacuum manifold is required (see ordering information).

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1 Homogenize and lyse sample material

Homogenize up to 200 mg sample using a commercial homogenizer. Transfer homogenized samples into MN Tube Strips (Lysis) and add 550 µl buffer CF preheated to 65°C. Add 10 µl proteinase K solution. Close the wells with Cap Strips. Mix by vigorous shaking for 15-30 sec. Spin briefly for 30 sec at 1,500 x g to collect any sample from the Cap Strips. Incubate at 65°C for 30 min.

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2 Clear lysate

Centrifuge the samples for 20 min at a full speed (5,600 - 6,000 x g). Remove cap strips.

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3 Adjust binding conditions

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol. Close the individual wells with cap strips. Mix by vigorous shaking for 15-30 sec (or pipette up and down). Spin briefly for 30 sec at 1,500 x g to collect any sample from cap strips.

Buffer C4 and ethanol can be premixed.

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4 Loading

Place waste tray into vacuum manifold base. Insert spacers labeled “MTP/Multi-96 plate” notched side up and rest the MN Wash Plate on them. Close manifold and place NucleoSpin® Food Binding Module on top of the manifold. Transfer samples to the wells of the NucleoSpin® Food Binding Module.

5 Bind DNA to silica membrane

Apply vacuum of -200 to -400 mbar (reduction of atmospheric pressure) to allow sample to pass through the membrane. Flow-through rate should be about 1-2 drops per second. Adjust vacuum strength accordingly.

6 Wash silica membrane

1st wash

Add 500 µl buffer CQW to each well of the NucleoSpin® Food Binding Module and apply vacuum of –400 mbar (reduction of atmospheric pressure).

2nd wash

Add 900 µl C5 to each well of the NucleoSpin® Food Binding Module**.** Apply vacuum of –400 mbar (reduction of atmospheric pressure) in order to remove buffer C5.

3nd wash

Add 900 µl C5 to each well of the NucleoSpin® Food Binding Module**.** Apply vacuum of –400 mbar (reduction of atmospheric pressure) in order to remove buffer C5.

Remove MN Wash Plate and waste tray.

Dry the membrane by applying maximum vacuum for 15 minutes.

7 Elution

Rest the Rack with MN Tube Strips (elution) on appropriate spacers ("Microtube rack") into manifold base. Close manifold and insert NucleoSpin® Food Binding Module into manifold top. Dispense 100 µl buffer CE (preheated to 70°C) to each well of the NucleoSpin® Food Binding Module. Pipette buffer directly onto the membrane. Incubate at room temperature for 2 - 3 min. Apply vacuum of -400 mbar (reduction of atmospheric pressure) until all the samples have passed.

For optimal yield it is recommended to repeat this step once (incubation not necessary).

Yields will be 10 – 20% higher when eluting in 200 µl buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100 µl. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH ≤ 8.0.

6 Appendix

6.1 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 2.5) 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 before 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.
DNA is degraded	*Sample was contaminated with DNase* • Check working area and pipettes. *Sample dependent problem* • Highly processed samples may be responsible for impossibilty to extract high molecular weight DNA.
DNA quality is low	*Sample contains DNA-degrading contaminants (e.g. phenolic compounds, metabolites)* • Repeat washing step with buffer CQW.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Food kit	740975	12 x 8 preps
NucleoSpin® 8 Food kit	740975.5	60 x 8 preps
NucleoSpin® 96 Food kit	740976.2	2 x 96 preps
NucleoSpin® 96 Food kit	740976.4	4 x 96 preps
NucleoSpin® 96 Food kit	740976.24	24 x 96 preps
Buffer CF	740946	1 l
RNase A 100 mg	740505	100 mg
RNase A 50 mg	740505.50	50 mg
Proteinase K	740506	100 mg
MN Square-well Block	740678	20
Square-well Block	740670	20
Round-well Block	740671	20
MN Tube Strips	740637	5 racks
Cap Strips	740638	30
MN Wash Plates	740674	20
Self-adhering PE Foil	740676	50
MN Frame	740680	1
Starter Set A	740682	1
Starter Set C	740684	1
Vacuum Regulator	740641	1
NucleoVac 96 vacuum manifold	740681	1

6.3 Product use restriction / warranty

NucleoSpin® 8/96 Food kits components were developed, designed, distributed and sold **for RESEARCH PURPOSES ONLY**. They are suitable ***for IN - VITRO USES only***. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather in the responsibility of the user to verify the use of the **NucleoSpin® 8/96 Food** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL guarantees to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

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Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all

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