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Genomic DNA from Tissue

User Manual

NucleoSpin® 8 Tissue
NucleoSpin® 96 Tissue
NucleoSpin® 96 Tissue Core Kit

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSpin® 8 Tissue	
	12 x 8 preps	60 x 8 preps
Cat. No.	740740	740740.5
Lysis Buffer T1	25 ml	125 ml
Binding Buffer BQ1	25 ml	125 ml
Wash Buffer B5 (Concentrate)[1]	50 ml	2 x 100 ml
Wash Buffer BW	75 ml	3 x 125 ml
Elution Buffer BE[2]	50 ml	2 x 125 ml
Proteinase K (lyophilized)[1]	75 mg	5 x 75 mg
Proteinase Buffer PB	3.6 ml	18 ml
NucleoSpin® Tissue Binding Strips (green rings)	12	60
MN Square-well Blocks	2	10
MN Wash Plates[3]	1	5
Rack of Tube Strips[4]	1	5
Self-adhering PE Foil	5	25
User Manual	1	1

Material supplied by the user: Suitable lysis tubes or plates, e.g. Rack of Tube Strips with Cap Strips (Cat. No. 740477, 4 sets; see ordering information).

[1] For preparation of working solutions and storage conditions see section 3.

[2] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

[3] For use with vacuum only

[4] Set of 1 rack, 12 strips with 8 tubes each, Cap Strips included

1.1 Kit contents *continued*

	NucleoSpin® 96 Tissue		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**[1]
Cat. No.	**740741.2**	**740741.4**	**740741.24**
Lysis Buffer T1	50 ml	100 ml	6 x 100 ml
Binding Buffer BQ1	50 ml	100 ml	6 x 100 ml
Wash Buffer B5 (Concentrate)[2]	100 ml	2 x 100 ml	12 x 100 ml
Wash Buffer BW	125 ml	2 x 125 ml	12 x 125 ml
Elution Buffer BE[3]	50 ml	100 ml	6 x 100 ml
Proteinase K (lyophilized)[2]	2 x 75 mg	4 x 75 mg	24 x 75 mg
Proteinase Buffer PB	8 ml	15 ml	6 x 15 ml
NucleoSpin® Tissue Binding Plates (green rings)	2	4	24
Round-well Blocks[4]	2	4	24
MN Square-well Blocks	2	4	24
MN Wash Plates[5]	2	4	24
Rack of Tube Strips[6]	2	4	24
Cap Strips	24	48	288
Self-adhering PE Foil	5	10	60
User Manual	1	1	6

[1] The kit for 24 x 96 preparations (Cat.No. 740741.24) consists of 6 x Cat. No. 740741.4.

[2] For preparation of working solutions and storage conditions see section 3.

[3] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

[4] Including 12 Cap Strips for each block

[5] For use with vacuum only

[6] Sets of 1 rack, 12 strips with 8 tubes each, including Cap Strips

1.1 Kit contents *continued*

	NucleoSpin® 96 Tissue Core Kit
	4 x 96 preps
Cat. No.	**740454.4**
Lysis Buffer T1	100 ml
Binding Buffer BQ1	100 ml
Wash Buffer B5 (Concentrate)[1]	2 x 100 ml
Wash Buffer BW	2 x 125 ml
Elution Buffer BE[2]	100 ml
Proteinase K (lyophilized)[1]	4 x 75 mg
Proteinase Buffer PB	15 ml
NucleoSpin® Tissue Binding Plates (green rings)	4
User Manual	1

Additional material required (see section 1.3).

1.2 Reagents to be supplied by the user

- 96-100% ethanol (for preparation of working solutions; see section 3)

[1] For preparation of working solutions and storage conditions see section 3.

[2] Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.3 Accessories supplied by the user - NucleoSpin® 96 Tissue Core Kit

The **NucleoSpin® 96 Tissue Core Kit** provides the buffers, Proteinase K, and NucleoSpin® Tissue Binding Plates. Accessory plates (e.g. lysis plates, elution plates) are not provided with the core kit. The user can individually select additional consumables from a variety of suitable accessory plates according to his requirements for highest flexibility.

For use of **NucleoSpin® 96 Tissue Core Kit** follow the standard protocol (see section 5.2 and 5.4).

Recommended accessories for use of the **NucleoSpin® 96 Tissue Core Kit** are available from MACHEREY-NAGEL (see ordering information):

Protocol step	Suitable consumables, not supplied with the core kits	Remarks
Lysis	4 x Round-well Block with Cap Strips per 4x96 preps or 4 x Rack of Tube Strips with Cap Strips per 4x96 preps	If residual hair and/or bones in the lysate must be removed by centrifugation and transfer of the supernatant an additional Round-well Block per 96 preps is necessary.
Adjustment of binding conditions	48 x Cap Strips per 4x96 preps	When using Round-well Block or Tube Strips for lysis, new Cap Strips are required for sealing of wells after adding Buffer BQ1 and ethanol.
	4 x MN Square-well Block per 4x96 preps	Recommended for automated processing only
Binding of DNA to the membrane	4 x MN Wash Plate per 4x96 preps	MN Wash Plate minimizes the risk of cross contamination (vacuum processing only).
	2 x MN Square-well Block	For waste collection during centrifugation (reusable)
Elution	4 x Rack of Tubes Strips with Cap Strips per 4x96 preps or 4 x Round-well Block with Cap Strips per 4x96 preps	

1.4 Required hardware

Centrifugation

For centrifugation a microtiterplate centrifuge is required which is able to accommodate the **NucleoSpin® Tissue Binding Strips/Plate** stacked on a Round- or Square-well Block which reaches accelerations of 5,600 - 6,000 x *g* (bucket height: 85 mm).

For processing the 8-well Strips the Starter Set C (see ordering information), containing Column Holders C, NucleoSpin® Dummy Strips, MN Square-well Blocks, and Rack of Tube Strips is required.

Vacuum processing

The **NucleoSpin® 8/96 Tissue** kits can be used manually with the NucleoVac 96 Vacuum Manifold (see ordering information). Additionally, a suitable centrifuge for sample preparation steps may be required.

For processing the 8-well Strips the Starter Set A (see ordering information), containing Column Holders A and NucleoSpin® Dummy Strips is required. For automation on laboratory platforms with standard 96-well plate vacuum chambers the use of the Starter Set A is also required.

1.5 Suitable vacuum manifolds

The **NucleoSpin® 8/96 Tissue** kits can be used with the NucleoVac 96 Vacuum Manifold or other common vacuum devices. For further details see list below.

Vacuum manifold	Suitability	Additional equipment
NucleoVac 96	Yes	Starter Set A for NucleoSpin® 8 Tissue
Qiagen/QIAvac 96[1]	Yes	MN Frame (see ordering information), Starter Set A for NucleoSpin® 8 Tissue
Promega/Vac-Man® 96[2]	Yes	NucleoSpin® 96 Tissue Core Kit only!

[1] In general the QIAvac 96 is suitable for the use with the NucleoSpin® Tissue Binding Strips/Plate. Nevertheless, it is recommended to use the MN Frame to adjust the proper height of the MN Wash Plate and Elution Plate in order to ensure best performance.

[2] MN Wash Plate cannot be used.

2 Product description

2.1 The basic principle

The **NucleoSpin® 8/96 Tissue** kit is designed for the efficient isolation of high molecular weight genomic DNA from tissue samples or cells. With the **NucleoSpin® 8/96 Tissue** method, sample lysis is achieved by incubation of the samples in a solution containing SDS and Proteinase K. Appropriate conditions for binding of DNA to the silica membrane in the **NucleoSpin® 8 Tissue Strips/96 Tissue Plates** are created by addition of large amounts of chaotropic salt and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. While DNA is kept on the silica membrane contaminations are removed by washing with two different wash buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.
In the following all information given about the **NucleoSpin® 8/96 Tissue** applies as well to the **NucleoSpin® 8/96 Tissue Core Kit** (except for the kit content).

2.2 Kit specifications

- **NucleoSpin® 8/96 Tissue** is designed for the rapid preparation of highly pure genomic DNA from tissue, e.g. mouse and rat tails, organ tissue or animal or bacterial cells. The purified DNA can be used directly as template for PCR, blotting, or any kind of enzymatic reactions.

- This kit provides reagents and consumables for purification of up to 40 µg (average 20 µg) of pure genomic DNA from up to 20 mg tissue samples with an A_{260}/A_{280} ratio between 1.8 and 1.9 and a typical concentration of 100 - 200 ng/µl.

- From up to two 0.5 cm long mouse tail tip section (age of mice: 4 - 6 weeks), up to 35 µg of pure genomic DNA can be prepared (typical yields: 15 - 25 µg).

- **NucleoSpin® 8/96 Tissue** can be processed by vacuum or in a centrifuge. The kits allow 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 2.4 and contact your local distributor or MN directly.

- The **NucleoSpin® 8/96 Tissue** kits allow for the purification of multiples of 8 **(NucleoSpin® 8 Tissue)** or 96 samples **(NucleoSpin® 96 Tissue)**. Both kits are supplied with accessory plates for highest convenience. The kits are designed for manual or automated use in a centrifuge or for use with a vacuum manifold. The **NucleoSpin® 96 Tissue Core Kit** provides the buffers, Proteinase K and NucleoSpin® Tissue Binding Plate only. Accessory components (e.g. lysis plates, elution plates) are not provided with the core kit but can be individually selected from a variety of suitable accessories (see section 1.3 for further information). This allows highest flexibility for the user.

Table 1: Kit specifications at a glance	
Parameters	**NucleoSpin® 8/96 Tissue (Core)**
Sample material	Up to 20 mg tissue, up to 10^6 cultured cells, bacteria
Typical DNA yield	15 - 25 µg
Elution volume	100 - 200 µl
DNA binding capacity	40 µg
A_{260}/A_{280}	1.8 - 1.9
Preparation time for 12 strips or 2 plates	1 h (after lysis)

Application data

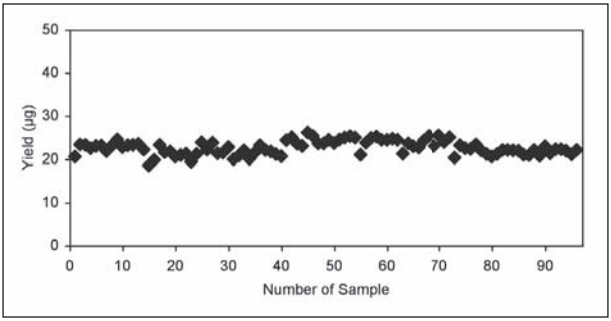


Figure 1: Yield of genomic DNA isolation from mouse tails

DNA was isolated from a mouse tail lysate representing 20 mg mouse tail per sample. DNA was isolated by centrifugation. Following elution the recovered DNA quantity was determined by UV spectroscopy. Average yield was 22.61 ± 1.58 µg. A high reproducibility with a low CV of 7% was achieved.

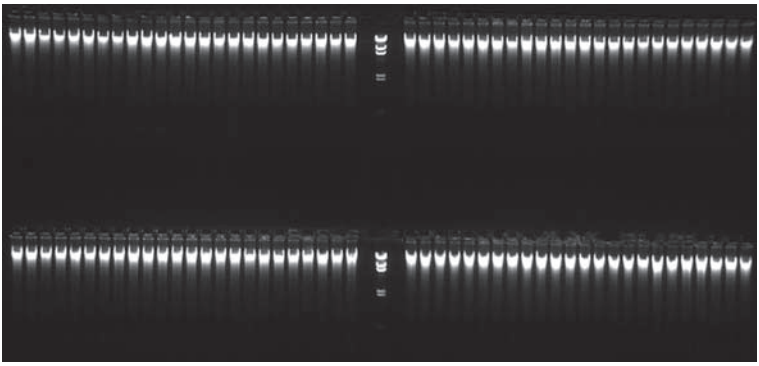


Figure 2: Analysis of genomic DNA isolated from mouse tails by agarose gel electrophoresis

8 µl out of 200 µl eluate from 48 samples were loaded on a 0.7% agarose gel (ethidium bromide stain). High molecular weight DNA was obtained. Absence of low molecular weight smear indicate the high structural integrity of the isolated DNA. DNA size standard: λ *Hin*dIII.

2.3 Elution procedures

It is possible to adjust the elution method and the volume of the elution buffer to the subsequent application of interest. In addition to the standard method described in section 5 (recovery rate about 70 - 90%) there are several modifications possible. Use elution buffer preheated at 70°C for one of the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90 - 100% of bound nucleic acids can be eluted.
- **High concentration:** Perform one elution step with only 60% of the volume indicated in the individual protocol. Concentration of DNA will be about 30% higher than with the standard elution procedure. Maximum yield of bound nucleic acids is about 80%.
- **High yield and high concentration:** Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85 - 100% of bound nucleic acids are eluted in the standard elution volume at a high concentration.
- **Convenient elution:** For convenience, elution buffer of ambient temperature may be used. This will result in a slightly lower yield (approximately 20%) compared to elution with heated elution buffer.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi use storage at 4°C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in downstream applications we recommend elution with the supplied elution buffer and storage, especially long term, at -20°C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g. >10 kb) or the detection limit of trace amount of DNA species may be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4°C or room temperature due to shearing of DNA or adsorption to surfaces.

Due to the dead volume of the silica membrane please note that the difference between the dispensed elution buffer volume and the recovered elution buffer volume containing genomic DNA is approx. 20 µl (recovered elution volume = dispensed elution volume -20 µl).

2.4 Automated processing on robotic platforms

NucleoSpin® 8/96 Tissue can be fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 8/96 Tissue** 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. However, a final elution step by centrifugation is recommended in order to achieve higher concentrated eluted DNA.

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 Tissue Binding Strips/96 Binding Plate**.

Drying of the **NucleoSpin® 8 Tissue Binding Strips/96 Binding Plate** 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. Thus, if possible the MN Wash Plate should be integrated into the automated procedure. The MN Frame (see ordering information) can be used to position the MN Wash Plate inside the vacuum chamber. Thorough cleaning of the vacuum chamber 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. Several application notes of the **NucleoSpin® 8/96 Tissue** kit on various automation workstations can also be found at *www.mn-net.com* at Bioanalyis/Literature.

3 Storage conditions and preparation of working solutions

Attention:

Buffers BQ1 and BW contain guanidinium hydrochloride! Wear gloves and goggles when handling them!

- All components of the **NucleoSpin® 8/96 Tissue** kits should be stored at room temperature for a maximum of 1 year. Storage at lower temperatures may cause precipitation of salts. If a salt precipitation is observed, incubate the bottle at 30 - 40°C for some minutes and mix well until all of the precipitate is redissolved. The performance of the kits is not affected by the salt precipitates.

Before starting with any **NucleoSpin® 8/96 Tissue** kit procedure prepare the following:

- **Wash Buffer B5:** Add the indicated volume of 96 - 100% ethanol to the **Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol is added. Store Wash Buffer B5 at room temperature (20 - 25°C) for up to one year.

- Before first use of the kit, add the indicated volume of **Proteinase Buffer PB** to lyophilized **Proteinase K**. Proteinase K solution is stable at -20°C for 6 months.

	NucleoSpin® 8 Tissue	
	12 x 8 preps	**60 x 8 preps**
Cat. No.	**740740**	**740740.5**
Wash Buffer B5 (Concentrate)	50 ml Add 200 ml ethanol	2 x 100 ml Add 400 ml ethanol to each bottle
Proteinase K (lyophilized)	75 mg Add 2.6 ml Proteinase Buffer PB	5 x 75 mg Add 2.6 ml Proteinase Buffer PB to each vial

	NucleoSpin® 96 Tissue		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
Cat. No.	**740741.2**	**740741.4**	**740741.24**
Wash Buffer B5 (Concentrate)	100 ml Add 400 ml ethanol	2 x 100 ml Add 400 ml ethanol to each bottle	12 x 100 ml Add 400 ml ethanol to each bottle
Proteinase K (lyophilized)	2 x 75 mg Add 2.6 ml Proteinase Buffer PB to each vial	4 x 75 mg Add 2.6 ml Proteinase Buffer PB to each vial	24 x 75 mg Add 2.6 ml Proteinase Buffer PB to each vial

	NucleoSpin® 96 Tissue Core Kit
	4 x 96 preps
Cat. No.	**740454.4**
Wash Buffer B5 (Concentrate)	2 x 100 ml Add 400 ml ethanol to each bottle
Proteinase K (lyophilized)	4 x 75 mg Add 2.6 ml Proteinase Buffer to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® 8/96 Tissue** 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
BQ1	Guanidinium hydrochloride	 Xn*	Harmful if swallowed - Irritating to eyes and skin	R 22-36/38	S 22
BW	Guanidinium hydrochloride + isopropanol <25%	 Xn*	Flammable - Harmful if swallowed - Irritating to eyes and skin	R 10-22-36/38	S 7-16-25
Proteinase K	Proteinase K, lyophilized	Xn Xi*	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 sensitization 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 25	Avoid contact with the eyes
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

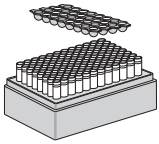
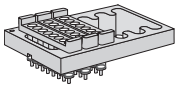
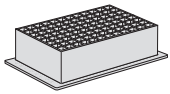
* Hazard labeling not necessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

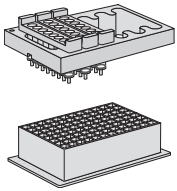
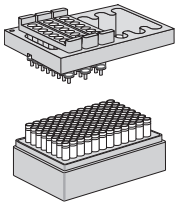
5 General procedure

NucleoSpin® 8 Tissue, centrifuge processing
(For details on each step see section 5.1.)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

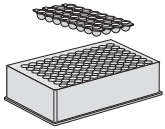
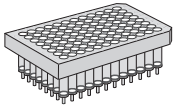
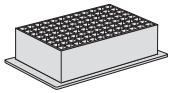
1	**Lyse** samples	**2 x 0.5 cm mouse tail** **or** **up to 20 mg tissue,** **10^6 cultured cells, or bacteria** **180 µl T1** **25 µl Proteinase K** **Mix** **56°C, ≥6 h**	 e.g. Rack of Tube Strips with Cap Strips (not supplied with the kit)
2	**Adjust** DNA binding condition	**200 µl BQ1** **200 µl ethanol (90-100%)** **Mix**	
3	**Load** samples	**Transfer samples to NucleoSpin® Tissue Binding Strips**	
4	**Bind** DNA to silica membrane	**5,600 x *g*** **10 min**	 Column Holder C with NucleoSpin® Tissue Binding Strips and MN Square-well Block

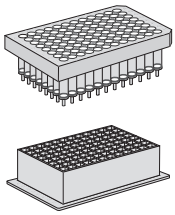
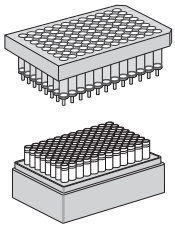
5	**Wash** silica membrane	**500 µl BW** **5,600 x *g*** **2 min** **700 µl B5** **5,600 x *g*** **4 min**	 Column Holder C with NucleoSpin® Tissue Binding Strips and MN Square-well Block
6	**Dry** silica membrane	**70°C** **10 min**	
7	**Elute** DNA	**100 µl BE (70°C)** **5,600 x *g*** **2 min** *Optional: Repeat elution step once.*	 Column Holder C with NucleoSpin® Tissue Binding Strips on Rack of Tube Strips

NucleoSpin® 96 Tissue, centrifuge processing
(For details on each step see section 5.2.)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

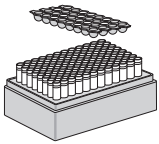
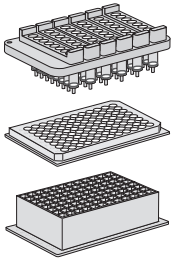
1	**Lyse** samples	**2 x 0.5 cm mouse tail or up to 20 mg tissue, 10^6 cultured cells, or bacteria** **180 µl T1** **25 µl Proteinase K** **Mix** **56°C, ≥6 h**	 Round-well Block with Cap Strips
2	**Adjust** DNA binding condition	**200 µl BQ1** **200 µl ethanol (90-100%)** **Mix**	
3	**Load** samples	**Transfer samples to NucleoSpin® Tissue Binding Plate**	
4	**Bind** DNA to silica membrane	**5,600 x *g* 10 min**	 NucleoSpin® Tissue Binding Plate and MN Square-well Block

5	**Wash** silica membrane	**500 µl BW** **5,600 x *g*** **2 min** **700 µl B5** **5,600 x *g*** **4 min**	 NucleoSpin® Tissue Binding Plate and MN Square-well Block
6	**Dry** silica membrane	**70°C** **10 min**	
7	**Elute** DNA	**100 µl BE (70°C)** **5,600 x *g*** **2 min** *Optional: Repeat elution step once.*	 NucleoSpin® Tissue Binding Plate on Rack of Tube Strips

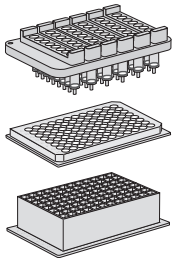
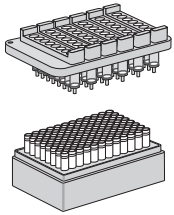
NucleoSpin® 8 Tissue, vacuum processing
(For details on each step see section 5.3.)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

1	**Lyse** samples	**2 x 0.5 cm mouse tail or up to 20 mg tissue, 10^6 cultured cells, or bacteria** **180 µl T1** **25 µl Proteinase K** **Mix** **56°C, ≥6 h**	 e.g. Rack of Tube Strips with Cap Strips (not supplied with the kit)
2	**Adjust** DNA binding condition	**200 µl BQ1** **200 µl ethanol (70%)** **Mix**	
3	**Load** samples	**Transfer samples to NucleoSpin® Tissue Binding Strips**	 Column Holder A with NucleoSpin® Tissue Binding Strips and MN Wash Plate on MN Square-well Block *(optional)*
4	**Bind** DNA to silica membrane	**- 0.2 bar* 5 min**	

* Reduction of atmospheric pressure

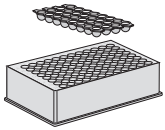
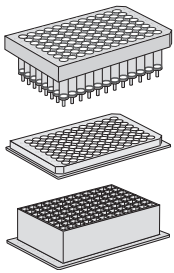
5	**Wash** silica membrane	**600 µl BW** **- 0.2 bar*** **5 min** **900 µl B5** **- 0.2 bar*** **5 min** **900 µl B5** **- 0.2 bar*** **5 min**	 Column Holder A with NucleoSpin® Tissue Binding Strips and MN Wash Plate on MN Square-well Block *(optional)*
6	**Dry** silica membrane	**Remove MN Wash Plate** **- 0.6 bar*** **10 min**	
7	**Elute** DNA	**100 µl BE (70°C)** **- 0.4 bar*** **2 min** *<u>Optional</u>: Repeat elution step once.*	 Column Holder A with NucleoSpin® Tissue Binding Strips on Rack of Tube Strips

* Reduction of atmospheric pressure

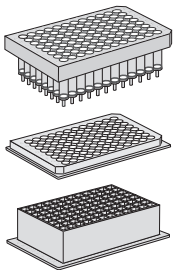
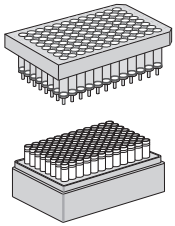
NucleoSpin® 96 Tissue, vacuum processing
(For details on each step see section 5.4.)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

1	**Lyse** samples	**2 x 0.5 cm mouse tail** **or** **up to 20 mg tissue,** **10^6 cultured cells, or bacteria** **180 µl T1** **25 µl Proteinase K** **Mix** **56°C, ≥6 h**	 Round-well Block with Cap Strips
2	**Adjust** DNA binding condition	**200 µl BQ1** **200 µl ethanol (90-100%)** **Mix**	
3	**Load** samples	**Transfer samples to NucleoSpin® Tissue Binding Plate**	 NucleoSpin® Tissue Binding Plate and MN Wash Plate on MN Square-well Block *(optional)*
4	**Bind** DNA to silica membrane	**- 0.2 bar*** **5 min**	

* Reduction of atmospheric pressure

5	**Wash** silica membrane	**600 µl BW** **- 0.2 bar*** **5 min** **900 µl B5** **- 0.2 bar*** **5 min** **900 µl B5** **- 0.2 bar*** **5 min**	 NucleoSpin® Tissue Binding Plate and MN Wash Plate on MN Square-well Block *(optional)*
6	**Dry** silica membrane	**Remove MN Wash Plate** **- 0.6 bar*** **10 min**	
7	**Elute** DNA	**100 µl BE (70°C)** **- 0.4 bar*** **2 min** *Optional: Repeat elution step once.*	 NucleoSpin® Tissue Binding Plate on Rack of Tube Strips

* Reduction of atmospheric pressure

5.1 NucleoSpin® 8 Tissue, centrifuge processing

The use of NucleoSpin® Tissue Binding Strips in a Column Holder C allows the isolation of up to n x 8 samples (n = 1 to 6). Insert as many of the NucleoSpin® Tissue Binding Strips as required into the same positions of each one of the two reusable column holders and place column holders onto the MN Square-well Blocks. Label the column holders or 8-well strips for later identification. Always use 2 Column Holders C containing identical numbers of NucleoSpin® Tissue Binding Strips for centrifugation. This avoids the need to balance the centrifuge, and allows multiples of 16 samples to be processed in parallel. We recommend inserting the NucleoSpin® Tissue Binding Strips around the center of the column holder.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

For each preparation, cut up to two 0.5 cm-pieces (20 mg) of mouse tail into appropriate lysis tubes or plates. If preparing DNA from rat tails, one 0.5 cm-piece is sufficient. Lysis tubes are not supplied with the NucleoSpin® 8 Tissue kit, we recommend usage of Rack of Tube Strips with Cap Strips (see ordering information). Tissue samples should not exceed 20 mg, cultured cells and bacteria should not exceed 10^6 cells.

1 Lyse samples

Prepare a Proteinase K working solution: For each sample, mix **25 µl Proteinase K** with **180 µl Buffer T1** and vortex. Transfer 200 µl of the resulting solution to each lysis tube containing the samples. Close the individual tubes and mix by vigorous shaking for 10 - 15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tube. The samples must be submerged in the solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in Buffer T1 without substrate.

Incubate the tubes/plate containing the samples at **56°C** for **at least 6 h** (for mammalian cells reduce incubation to 10 min, bacterial cells may require prelysis with e.g. lysozyme) or overnight until the samples are completely lysed. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes/plate are securely closed. When using Rack of Tube Strips place a weight on top in order to prevent the Cap Strips from popping off occasionally.

After lysis, set incubator to 70°C for the membrane drying step.

Centrifuge the tubes/plate (15 s; 1,500 x *g*) to collect any condensate from the lid of the tube/plate.

Residual hair and/or bones in the lysate can be removed by centrifugation (2 min; 5,600 - 6,000 x g) and transfer of the supernatant to new microtubes or to a new Rack of Tube Strips (not supplied with the kit).

2 Adjust DNA binding condition

Add **200 µl Buffer BQ1** and **200 µl 96 - 100% ethanol** to each sample. Again take care not to moisten the rims of the individual wells while dispensing the buffer. Close the tubes/plate. Mix by vigorous shaking for 10 - 15 s. Spin briefly (10 s; 1,500 x *g*) to collect any sample from the lid.

Ethanol and Buffer BQ1 can be premixed before addition to the samples, if the mixture is to be used up during the next 3 months. Never centrifuge at higher g-forces or for longer periods as DNA will precipitate.

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

3 Load samples

Transfer the lysates resulting from step 2 carefully into the wells of the NucleoSpin® Tissue Binding Strips. When using the Rack of Tube Strips for lysis, remove the first Cap Strip and transfer lysates before removing the next Cap Strip. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination during centrifugation. After transfer seal the openings of the inserted NucleoSpin® Tissue Binding Strips with Self-adhering PE Foil.

4 Bind DNA to silica membrane

Place the MN Square-well Block with Column Holder C onto the centrifuge carriers and insert them into the rotor buckets. Centrifuge at **5,600 - 6,000 x *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.

5 Wash silica membrane

1st wash

Remove the Self-adhering PE Foil and add **500 µl Buffer BW** to each well of the NucleoSpin® Tissue Binding Strips. Seal the plate with a new Self-adhering PE Foil and centrifuge again at **5,600 - 6,000 x *g*** for **2 min**.

2nd wash

Remove the Self-adhering PE Foil and add **700 µl Buffer B5** to each well of the NucleoSpin® Tissue Binding Strips. Seal the plate with a new Self-adhering PE Foil and centrifuge again at **5,600 - 6,000 x *g*** for **4 min**.

During this step, as much ethanolic Buffer B5 as possible is removed by centrifugation.

6 Dry silica membrane

Remove the Self-adhering PE Foil and place the Column Holder C holding the NucleoSpin® Tissue Binding Strips on an opened Rack of Tube Strips. Place it in an incubator for **10 min** at **70°C** to evaporate residual ethanol.

Removal of ethanol by evaporation at 70°C is more effective than prolonged centrifugation.

Note: The ethanol in Buffer B5 may inhibit enzymatic reactions and should be removed completely before eluting DNA.

7 Elute DNA

Dispense **100 µl preheated Buffer BE (70°C)** to each well of the NucleoSpin® Tissue Binding Strips. Dispense the buffer directly onto the membrane. Incubate at room temperature for **1 min** and centrifuge at **5,600 - 6,000 x *g*** for **2 min**. Repeat elution step once. Remove the Column Holder C with inserted NucleoSpin® Tissue Binding Strips from the Rack of Tube Strips. For alternative elution procedures see section 2.3.

If elution in small volume tubes is desired place a 96 PCR plate (not supplied) on top of a Round-well Block or a Rack of Tube Strips and elute into the PCR plate.

5.2 NucleoSpin® 96 Tissue, centrifuge processing

Place NucleoSpin® Tissue Binding Plate on an MN Square-well Block. The use of a second plate placed on an MN Square-well Block avoids the need to balance the centrifuge.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

For each preparation, cut up to two 0.5 cm-pieces (20 mg) of mouse tail into appropriate lysis tubes or plates. If preparing DNA from rat tails, one 0.5 cm-piece is sufficient. Tissue samples should not exceed 20 mg, cultured cells and bacteria should not exceed 10^6 cells.

1 Lyse samples

Prepare a Proteinase K working solution: For each sample, mix **25 µl Proteinase K** with **180 µl Buffer T1** and vortex. Transfer 200 µl of the resulting solution to each well of the Round-well Block containing the samples. Close the wells with Cap Strips. Mix by vigorous shaking for 10-15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the wells.

The samples must be submerged in the solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in Buffer T1 without substrate.

Incubate the Round-well Block containing the samples at **56°C** for **at least 6 h** (for mammalian cells reduce incubation to 10 min, bacterial cells may require pre-lysis with e.g. lysozyme)or overnight until the samples are completely lysed. For optimal lysis, mix occasionally during incubation. Place a weight on top of the Round-well Block in order to prevent the Cap Strips from popping off occasionally.

After lysis, set the incubator to 70°C for the membrane drying step.

Centrifuge the Round-well Block (15 s; 1,500 x *g*) to collect any condensate from the Cap Strips. Remove Cap Strips.

Residual hair and/or bones in the lysate can be removed by centrifugation (2 min; 5,600-6,000 x g) and transfer of the supernatant to a new Round-well Block (not supplied with the kit).

2 Adjust DNA binding condition

Add **200 µl Buffer BQ1** and **200 µl 96 - 100% ethanol** to each sample. Again take care not to moisten the rims of the individual wells while dispensing the buffer. Close the individual wells with new Cap Strips (supplied). Mix by vigorous shaking for 10 - 15 s. Spin briefly (10 s; 1,500 x *g*) to collect any sample from the Cap Strips.

Ethanol and Buffer BQ1 can be premixed before addition to the samples, if the mixture is to be used during the next 3 months. Never centrifuge at higher g-forces or for longer periods as DNA will precipitate.

Place a NucleoSpin® Tissue Binding Plate on an MN Square-well Block. If using more than one plate, label the plates for later identification.

3 Load samples

Remove the first Cap Strip and transfer the lysates resulting from step 2 carefully from the Round-well Block into the wells of the NucleoSpin® Tissue Binding Plate. Continue with the next eight samples. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination during centrifugation. After transfer seal the openings of the plate with Self-adhering PE Foil.

For transfer of the lysate from the Round-well Block to the NucleoSpin® Tissue Binding Plate, we recommend use of an electronic eight-channel pipetting device with extra long tips capable of holding more than 650 µl.

4 Bind DNA to silica membrane

Place the MN Square-well Blocks with NucleoSpin® Tissue Binding Plates onto the centrifuge carriers and insert them into the rotor buckets. Centrifuge at **5,600 - 6,000 x *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.

5 Wash silica membrane

1st wash

Remove the Self-adhering PE Foil and add **500 µl Buffer BW** to each well of the NucleoSpin® Tissue Binding Plate. Seal the plate with a new Self-adhering PE Foil and centrifuge again at **5,600 - 6,000 x *g*** for **2 min**.

2nd wash

Remove the Self-adhering PE Foil and add **700 µl Buffer B5** to each well of the NucleoSpin® Tissue Binding Plate. Seal the plate with a new Self-adhering PE Foil and centrifuge again at **5,600 - 6,000 x *g*** for **4 min**.

During this step, as much ethanolic Buffer B5 as possible is removed by centrifugation.

6 Dry silica membrane

Remove the Self-adhering PE Foil and place the NucleoSpin® Tissue Binding Plate on an opened Rack of Tube Strips. Place it in an incubator for **10 min** at **70°C** to evaporate residual ethanol.

Removal of ethanol by evaporation at 70°C is more effective than prolonged centrifugation.

Note: The ethanol in Buffer B5 may inhibit enzymatic reactions and should be removed completely before eluting DNA.

7 Elute DNA

Dispense **100 µl preheated Buffer BE (70°C)** to each well of the NucleoSpin® Tissue Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for **1 min**. Centrifuge at **5,600 - 6,000 x *g*** for **2 min**. Repeat elution step once. Remove the NucleoSpin® Tissue Binding Plate from the Rack of Tube Strips by lifting the plate at one side carefully as the Tube Strips may stick to the outlets of the NucleoSpin® Tissue Binding Plate. For alternative elution procedures see section 2.3.

If elution in small volume tubes is desired, place a 96 PCR plate (not supplied) on top of a Round-well Block or a Rack of Tube Strips and elute into the PCR plate.

5.3 NucleoSpin® 8 Tissue, vacuum processing

For processing of NucleoSpin® 8 Tissue under vacuum the NucleoVac 96 Vacuum Manifold and the Starter Kit A are required (see ordering information). Starter Kit A contains the Column Holders A and NucleoSpin® Dummy Strips to seal unused rows.

The use of NucleoSpin® Tissue Binding Strips in a Column Holder A allows the isolation of up to n x 8 samples (n = 1 to 6). Insert as many NucleoSpin® Tissue Binding Strips as required into the reusable column holder and place it onto an MN Square-well Block. We recommend inserting the NucleoSpin® Tissue Binding Strips around the center of the column holder. Seal unused wells of NucleoSpin® Tissue Binding Strips with Self-adhering PE-Foil and close unused wells with Dummy Strips. Place the Column Holder on the NucleoVac 96 manifold.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

For each preparation, cut up to two 0.5 cm-pieces (20 mg) of mouse tail into appropriate lysis tubes or plates. If preparing DNA from rat tails, one 0.5 cm-piece is sufficient. Lysis tubes are not supplied with the NucleoSpin® 8 Tissue kit, we recommend use of Rack of Tube Strips with Cap Strips (see ordering information). Tissue samples should not exceed 20 mg, cultured cells and bacteria should not exceed 10^6 cells.

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1 Lyse samples

Prepare a Proteinase K working solution: For each sample, mix **25 µl Proteinase K** with **180 µl Buffer T1** and vortex. Transfer 200 µl of the resulting solution to each lysis tube containing the samples. Close the individual tubes and mix by vigorous shaking for 10 - 15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tubes.

The samples must be submerged in the solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in Buffer T1 without substrate.

Incubate the tubes/plate containing the samples at **56°C** for **at least 6 h** (for mammalian cells reduce incubation to 10 min, bacterial cells may require pre-lysis with e.g. lysozyme) or overnight until the samples are completely lysed. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes/plate are securely closed. When using Rack of Tube Strips place a weight on top in order to prevent the Cap Strips from popping off occasionally.

Centrifuge the tubes/plate (15 s; 1,500 x *g*) to collect any condensate from the lid of the tube/plate.

Residual hair and/or bones in the lysate can be removed by centrifugation (2 min; 5,600 - 6,000 x g) and transfer of the supernatant to new microtubes or to a new Rack of Tube Strips (not supplied with the kit).

2 Adjust DNA binding condition

Add **200 µl Buffer BQ1** and **200 µl 96 - 100% ethanol** to each sample. Again take care not to moisten the rims of the individual wells while dispensing the buffer. Close the tubes/plate. Mix by vigorous shaking for 10 - 15 s. Spin briefly (10 s; 1,500 x *g*) to collect any sample from the lid.

Ethanol and Buffer BQ1 can be premixed before addition to the samples, if the mixture is to be used during the next 3 months. Never centrifuge at higher g-forces or for longer periods as DNA will precipitate.

Prepare the NucleoVac 96 Vacuum Manifold:

Place waste tray into vacuum manifold base. Insert spacers labeled “MTP/Multi-96 plate” notched side up and place the MN Wash Plate on them. Close the manifold with the manifold lid.

Insert desired number of NucleoSpin® Tissue Binding Strips in the Column Holder A. Use NucleoSpin® Dummy Strips to seal unused positions in the column holder.

Place Column Holder A with inserted NucleoSpin® Tissue Binding Strips on top of the manifold.

3 Load samples

Transfer the lysates resulting from step 2 carefully into the wells of the NucleoSpin® Tissue Binding Strips. When using the Rack of Tube Strips remove the first Cap Strip and transfer lysates before removing the next Cap Strip. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross contamination.

4 Bind DNA to silica membrane

Apply vacuum until all lysates have passed the wells of the NucleoSpin® Tissue Binding Strips **(- 0.2 bar*; 5 min)**. Release the vacuum.

* Reduction of atmospheric pressure

5 Wash silica membrane*

1st wash

Add **600 µl Buffer BW** to each well of the NucleoSpin® Tissue Binding Strips. Apply vacuum **(-0.2 bar**; 5 min)** until all buffer has passed through the wells of the NucleoSpin® Tissue Binding Strips. Release the vacuum.

2nd wash

Add **900 µl Buffer B5** to each well of the NucleoSpin® Tissue Binding Strips. Apply vacuum **(-0.2 bar**; 5 min)** until all buffer has passed through the wells of the NucleoSpin® Tissue Binding Strips. Release the vacuum.

3rd wash

Add **900 µl Buffer B5** to each well of the NucleoSpin® Tissue Binding Strips. Apply vacuum **(-0.2 bar**; 5 min**) until all buffer has passed through the wells of the NucleoSpin® Tissue Binding Strips. Release the vacuum.

6 Remove MN Wash Plate

After the final washing step close the valve, release the vacuum and remove the Column Holder A with inserted NucleoSpin® Tissue Binding Strips from the vacuum manifold. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry silica membrane

Insert Column Holder A with the NucleoSpin® Tissue Binding Strips again into the lid and close the manifold. Apply maximum vacuum **(at least -0.6 bar**)** for **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

* Buffer volumes are increased compared to processing under centrifugation to improve washing efficiency under vacuum.

** Reduction of atmospheric pressure

7 Elute DNA

Insert spacers “Microtube rack” into the NucleoVac 96 Vacuum Manifold‘s short sides. Place a Rack of Tube Strips onto the spacer. Close the vacuum manifold and place the Column Holder A with the NucleoSpin® Tissue Binding Strips on top. Dispense **100 µl preheated (70°C) Buffer BE** directly to the bottom of each well. **Incubate** for **3 min** at room temperature. Apply vacuum for elution **(-0.4 bar*; 2 min)**. Release vacuum and repeat elution step once. For alternative elution procedures see section 2.3.

Finally, close Tube Strips with Cap Strips for storage.

Centrifuge the Rack of Tube Strips shortly to collect all sample at the bottom of the Tube Strips.

* Reduction of atmospheric pressure

5.4 NucleoSpin® 96 Tissue, vacuum processing

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

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

For each preparation, cut up to two 0.5 cm-pieces (20 mg) of mouse tail into appropriate lysis tubes or plates. If preparing DNA from rat tails, one 0.5 cm-piece is sufficient. Tissue samples should not exceed 20 mg, cultured cells and bacteria should not exceed 10^6 cells.

1 Lyse samples

Prepare a Proteinase K working solution: For each sample, mix **25 µl Proteinase K** with **180 µl Buffer T1** and vortex. Transfer 200 µl of the resulting solution to each well of the Round-well Block containing the samples. Close the wells with Cap Strips and mix by vigorous shaking for 10 - 15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the wells.

The samples must be submerged in the solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in Buffer T1 without substrate.

Incubate the Round-well Block containing the samples at **56°C** for **at least 6 h** (for mammalian cells reduce incubation to 10 min, bacterial cells may require pre-lysis with e.g. lysozyme) or overnight until the samples are completely lysed. For optimal lysis, mix occasionally during incubation. Place a weight on top of the Round-well Block in order to prevent the Cap Strips from popping off occasionally.

Centrifuge the Round-well Block (15 s; 1,500 x *g*) to collect any condensate from the Cap Strips. Remove Cap Strips.

Residual hair and/or bones in the lysate can be removed by centrifugation (2 min; 5,600 - 6,000 x g) and transfer of the supernatant to a new Round-well Block (not supplied with the kit).

2 Adjust DNA binding condition

Add **200 µl Buffer BQ1** and **200 µl 96 - 100%** ethanol to each sample. Again take care not to moisten the rims of the individual wells while dispensing the buffer. Close the individual wells with new Cap Strips (supplied). Mix by vigorous shaking for 10 - 15 s. Spin briefly (10 s; 1,500 x *g*) to collect any sample from the Cap Strips.

Ethanol and Buffer BQ1 can be premixed before addition to the samples, if the mixture is to be used up during the next 3 months. Never centrifuge at higher g-forces or for longer periods as DNA will precipitate.

Prepare the NucleoVac 96 Vacuum Manifold:

Place waste tray into vacuum manifold base. Insert spacers labeled “MTP/Multi-96 plate” notched side up and place the MN Wash Plate on them. Close the manifold with the manifold lid.

Place a NucleoSpin® Tissue Binding Plate on top of the manifold.

3 Load samples

Remove the first Cap Strip and transfer the lysates resulting from step 2 carefully from the Round-well Block into the wells of the NucleoSpin® Tissue Binding Plate. Continue with the next eight samples. Do not moisten the rims of the individual wells while dispensing the samples – moistened rims may cause cross-contamination.

For transfer of the lysate from the Round-well Block to the NucleoSpin® Tissue Binding Plate, we recommend use of an electronic eight-channel pipetting device with extra long tips capable of holding more than 650 µl.

4 Bind DNA to silica membrane

Apply vacuum until all lysates have passed through the wells of the NucleoSpin® Tissue Binding Plate **(- 0.2 bar*; 5 min)**. Release the vacuum.

* Reduction of atmospheric pressure

5 Wash silica membrane*

1st wash

Add **600 µl Buffer BW** to each well of the NucleoSpin® Tissue Binding Plate. Apply vacuum **(-0.2 bar**; 5 min)** until all buffer has passed through the wells of the NucleoSpin® Tissue Binding Plate. Release the vacuum.

2nd wash

Add **900 µl Buffer B5** to each well of the NucleoSpin® TissueBinding Plate. Apply vacuum **(-0.2 bar**; 5 min)** until all buffer has passed through the wells of the NucleoSpin® Tissue Binding Plate. Release the vacuum.

3rd wash

Add **900 µl Buffer B5** to each well of the NucleoSpin® Tissue Binding Plate. Apply vacuum **(-0.2 bar**; 5 min)** until all buffer has passed through the wells of the NucleoSpin® Tissue Binding Plate. Release the vacuum.

6 Remove MN Wash Plate

After the final washing step close the valve, release the vacuum and remove the NucleoSpin® Tissue Binding Plate. Put it on a clean paper towel to remove residual EtOH-containing wash buffer. Remove manifold lid, MN Wash Plate, and waste container from the vacuum manifold.

Dry silica membrane

Insert the NucleoSpin® Tissue Binding Plate into the lid and close the manifold. Apply maximum vacuum **(at least -0.6 bar**)** for **10 min** to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

Note: The ethanol in Buffer B5 inhibits enzymatic reactions and has to be removed completely before eluting DNA.

Finally, release the vacuum.

* Buffer volumes are increased compared to processing under centrifugation to improve washing efficiency under vacuum.

** Reduction of atmospheric pressure

7 Elute DNA

Insert spacers “Microtube rack” into the NucleoVac Vacuum Manifold’s short sides. Place a Rack of Tube Strips onto the spacer. Close the vacuum manifold and place the NucleoSpin® Tissue Binding Plate on top. Dispense **100 µl pre-heated Buffer BE** onto the membrane. Incubate for **3 min** at **room temperature**. Apply vacuum for elution **(- 0.4 bar*; 2 min)**. Release the vacuum and repeat the elution step once. For alternative elution procedures see section 2.3.

Finally, close the Tube Strips with Cap Strips for storage.

Centrifuge Rack of Tube Strips shortly to collect all sample at the bottom of the Tube Strips.

* Reduction of atmospheric pressure

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
	Incomplete lysis • Sample not completely submerged during heat incubation. Cut samples into small pieces. Mix well. Be sure that the samples are fully submerged in Buffer T1/Proteinase K mixture. Incubate until the samples are completely lysed. • Buffer T1 and Proteinase K premixed more than 15 min before addition to the substrate. Proteinase K tends to self digestion under optimal reaction conditions in Buffer T1 without substrate.
No or poor DNA yield	*Reagents not applied properly* • Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add Buffer BQ1 and ethanol to the lysates before loading them to the wells of the NucleoSpin® Tissue Binding Strips/Plate. *Suboptimal elution of DNA from the column* • Preheat Buffer BE to 70°C before elution. Apply Buffer BE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7. Use slightly alkaline elution buffer like Buffer BE (pH 8.5).
RNA contamination	*RNA in sample* • If DNA free of RNA is desired, cool down to room temperature after lysis incubation and add 20 µl of an RNase A solution (20 mg/ml; see ordering information). Incubate for 15 min with moderate shaking.

Poor performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol* • After washing with Buffer B5 centrifuge ≥ 4 min at 5,600 - 6,000 x *g* in order to remove ethanolic Buffer B5 completely and evaporate residual ethanol by incubating the NucleoSpin® Tissue Binding Strips/Plate at 70°C for 10 min. • Increase vacuum drying time to 15 min. *Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer BE.
Clogged wells	*Too much starting material* • Repeat the procedure, using two mouse tail sections of maximally 4 - 6 mm length. If processing rat tails, one 0.5 cm-long tail tip section is sufficient. *Hair or bones left in the lysate after step 2* • Centrifuge the Round-well Block for 3 min at 5,600 - 6,000 x *g*. Transfer lysates to a new Round-well Block without disturbing the debris pellet. *Incomplete passage of lysate in step 4* • If no more than 300 - 500 µl of lysate is remaining in the columns, continue with step 5. Through the addition of Buffer BW the sample is diluted and thus the sample will pass the column more easily.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 8 Tissue	740740 740740.5	12 x 8 preps 60 x 8 preps
NucleoSpin® 96 Tissue	740741.2 740741.4 740741.24	2 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® 96 Tissue Core Kit	740454.4	4 x 96 preps
Buffer T1	740940.25	25 ml
Buffer BQ1	740923.1	1 l
Buffer B5 Concentrate	740921.100	100 ml
Buffer BW	740922.500	500 ml
Proteinase K	740506	100 mg
RNase A	740505	100 mg
Rack of Tube Strips with Cap Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
Round-well Block with Cap Strips (set consists of 1 Round-well Block 12 Cap Strips)	740475 740475.24	4 sets 24 sets
MN Wash Plate	740479 740479.24	4 24
Cap Strips	740478	48

Product	Cat. No.	Pack of
Starter Set A (for use of 8-well strips on the NucleoVac 96 and automation platforms)	740682	1 set
Starter Set C (for use of 8-well strips under centrifugation)	740684	1 set
MN Frame (for optimized handling of 96-well plates with vacuum manifold on BioRobot® 9600, 9604, and 3000 (Qiagen), MultiPROBE II, Janus (PerkinElmer), Biomek 2000, 3000 and NX, FX (Beckman Coulter)	740680	1
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Self-adhering PE Foil	740676	50

6.3 References

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

6.4 Product use restriction / warranty

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

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