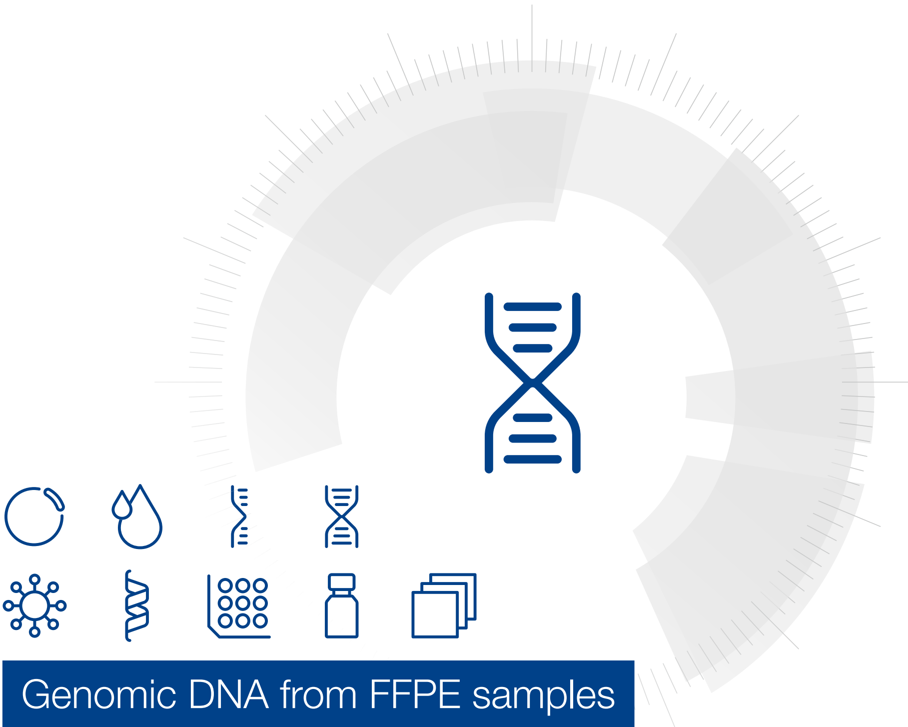
MACHEREY-NAGEL

User manual

Bioanalysis



Genomic DNA from FFPE samples

- NucleoSpin® 96 DNA FFPE

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1 Components

1.1 Kit contents

	NucleoSpin® 96 DNA FFPE	
REF	**1 × 96 preps 740240.1**	**4 × 96 preps 740240.4**
Lysis Buffer FL	30 mL	4 × 30 mL
Binding Buffer BQ1	45 mL	3 × 45 mL
Wash Buffer BW	100 mL	300 mL
Wash Buffer B5	100 mL	2 × 100 mL
Elution Buffer BE	30 mL	125 mL
Proteinase Buffer PB	15 mL	35 mL
Paraffin Dissolver (blue)	60 mL	2 × 125 mL
Decrosslink Buffer D-Link	30 mL	2 × 30 mL
Proteinase K* (lyophilized)	75 mg	4 × 75 mg
NucleoSpin® DNA FFPE Binding Plate	1	4
MN Wash Plate	1	4
Round-well Block (including Cap Strips)	1	4
MN Square-well Block	1	4
Rack of Tube Strips (including Cap Strips)	1	4
Self adhering PE Foil	5	10
User manual	1	1

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

1.2 Material to be supplied by user

Reagents

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

For more detailed information regarding special hardware required for centrifuge or vacuum processing, please see section 2.4.

1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at ***www.mn-net.com***.

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

2 Product description

Formalin-fixed, paraffin-embedded (FFPE) tissue samples are routinely prepared from human surgical tissue samples by fixation with formalin and embedding in paraffin. Thin sections of FFPE samples are commonly subjected to histopathological analysis and remaining paraffin-tissue blocks are usually archived. Existing extensive archives of FFPE tissue samples represent a valuable source for retrospective studies of gene expression patterns and mutation analysis. However, the use of such samples for DNA analysis is limited due to chemical modification by formaldehyde and fragmentation of the DNA during tissue processing (sampling, fixing, embedding) and storage (humidity, time, temperature) of the samples. Standard DNA isolation procedures often result in low DNA yield or poor performance in downstream applications (e.g., PCR).

2.1 The basic principle

The **NucleoSpin® 96 DNA FFPE** kit is designed for the isolation of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue specimen. The procedure replaces the use of flammable and malodorous xylene or d-limonene commonly used for deparaffinization. Further, the procedure does not require the difficult removal of organic solvent from often barely visible tissue pellets. **NucleoSpin® 96 DNA FFPE** employs the odorless Paraffin Dissolver (patented) and allows effective lysis in a convenient two-phase system. This kit provides reagents for a convenient, reliable, and fast method to isolate DNA from 96 or 384 samples. First, the paraffin of FFPE sections is dissolved in the Paraffin Dissolver. Tissue is then digested by Proteinase K to solubilize the fixed tissue and release DNA into solution.

Appropriate conditions for binding of DNA to the silica membrane in the NucleoSpin® DNA FFPE Binding Plate 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.

2.2 Kit specifications

The **NucleoSpin® 96 DNA FFPE** kit is recommended for the isolation of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Samples are typically thin sections (approx. 3–20 µm thickness) of human or animal origin usually obtained by tissue resection or biopsy.

- **Sample amount:** The maximum sample size is determined by a) the amount of tissue and b) by the amount of paraffin. **NucleoSpin® 96 DNA FFPE** is suitable for up to 10 mg tissue. The amount of paraffin is limited to 15 mg (approx 17 µL), when using the standard protocol with Paraffin Dissolver. However, larger amounts of paraffin samples may be processed by using either additional Paraffin Dissolver or by deparaffinization using xylene. Typically, one or two sections of 10 µm to 20 µm thickness are sufficient for DNA purification. DNA yield depends on the amount or size of the embedded tissue samples. Excess of paraffin may be removed by trimming the paraffin sections or the paraffin embedded sample blocks before slicing.
- **DNA yield** strongly depends on the sample type, quality, quantity as well as conditions and duration of storage. Further, measured DNA yield may vary considerably between different quantification methods. Yield determined by absorption measurement at 260 nm or by a fluorescent dye (e.g., PicoGreen®) may deviate from values obtained by quantification with PCR. Even quantification values obtained via PCR with a short (e.g.,

80 bp) and a long (e.g., 300 bp) amplicon may also differ considerably. The deviation of quantification also depends on DNA size distribution as well as on efficiency of decrosslinking (or extent of remaining crosslinks).

- **DNA size distribution:** DNA isolated from formalin-fixed, paraffin-embedded tissue shows a size distribution from 50 to 5,000 bases. Predominantly DNA of approx. 100 – 300 bases is observed, especially when the sample material is old. However, samples which were subjected to good tissue fixation, embedding, and storage conditions can yield DNA even larger than 5,000 bp.
- **DNA preparation time** strongly depends on the sample and the required lysis time. For best results lysis is performed at room temperature for at least three hours. For some kinds of samples a longer lysis (e.g., overnight) will even result in remarkably higher DNA yield.
- **NucleoSpin® 96 DNA FFPE** can be processed by vacuum or by centrifugation. The kit allows easy automation on common liquid handling instruments.
- The **NucleoSpin® 96 DNA FFPE** kits allow for the purification of multiples of 96 samples. The 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.
- **Use – For research use only**

2.3 Handling, preparation, and storage of starting materials

Many factors influence the yield and quality of DNA obtained from FFPE samples. The procedure of tissue sampling, post sampling delay before fixation, fixation time, embedding, and storage conditions have a high impact on DNA quality and yield. Starting from a paraffin embedded tissue block, samples should be sectioned under clean conditions. Paraffin sections may be stored at +4 °C or lower for at least several weeks without observable effects on DNA yield or usability. Long term storage of paraffin sections may have a negative effect on the DNA due to air oxidation. Wear gloves at all times during the preparation. Change gloves frequently.

2.4 Required hardware

NucleoSpin® 96 DNA FFPE can be processed under vacuum or by centrifugation. Certain hardware for processing is required.

Centrifugation
For centrifugation, a microtiterplate centrifuge is required. This centrifuge must be able to accomodate the NucleoSpin® DNA FFPE Binding Plate stacked on a Round- or Square-well Block and reach accelerations of 5,600 – 6,000 x *g* (bucket height: 85 mm). Regarding waste collection, suitable consumables (e.g., MN Square-well Blocks) are necessary and they are not included in the kit. For the most convenient handling, without the need of emptying and reusing the MN Square-well Blocks, it is recommended using six MN Square-well Blocks if two 96-well plates are processed at once (see ordering information, section 6.2). Alternatively, it is possible to empty the MN Square-well Blocks after every centrifugation step, reducing the amount of MN Square-well Blocks needed.

Vacuum processing

The **NucleoSpin® 96 DNA FFPE** kit can be used with the NucleoVac 96 Vacuum Manifold (see ordering information, section 6.2). When using **NucleoSpin® 96 DNA FFPE** with less than 96 samples, Self adhering PE Foil (see ordering information, section 6.2) should be used in order to close and protect non-used wells of the NucleoSpin® DNA FFPE Binding Plate and thus guarantee proper vacuum. Establish a reliable vacuum source for the NucleoVac 96 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. It is recommended a vacuum of - 0.2 to - 0.4 bar (reduction of atmospheric pressure). The use of the NucleoVac Vacuum Regulator (see ordering information, section 6.2) is recommended. Alternatively, adjust the vacuum so that during the purification the sample flows through the column with a rate of 1 – 2 drops per second. Depending on the amount of sample being used, the vacuum times may need to be increased for complete filtration. Additionally, a suitable centrifuge for sample preparation steps may be required.

For general consumables and equipment needed, please see section 1.2.

2.5 Automated processing on robotic platforms

NucleoSpin® 96 DNA FFPE can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 96 DNA FFPE** on a certain workstation, please contact MN. Full processing under vacuum enables complete automation without the need for centrifugation steps for drying of the binding 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® DNA FFPE Binding Plate. Drying of the NucleoSpin® DNA FFPE Binding Plates under vacuum is sufficient because the bottom of the plate is protected by the MN Wash Plate during the washing steps. It is recommended to integrate the MN Wash Plate into the automated procedure to avoid cross-contamination by residual wash buffer. The MN Frame (see ordering information, section 6.2) 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 chamber is recommended after each run to prevent DNA-containing aerosols from forming.

Visit MN online 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 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 the protocols (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 during long term or multi-use storage at 4 °C (or ambient temperature) by inhibiting omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in downstream applications, it is recommended eluting with the supplied elution buffer and storing it, especially for 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. This is 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 approximately 20 μL (recovered elution volume = dispensed elution volume -20 μL).

3 Storage conditions and preparation of working solutions

Attention:

Buffers BQ1 and BW contain chaotropic salt! Wear gloves and goggles!

All components of the NucleoSpin® 96 DNA FFPE kit should be stored at room temperature (18 – 25 °C) and are stable for up to one year.

Before starting any NucleoSpin® DNA FFPE NucleoSpin® 96 DNA FFPE protocol, prepare the following:

Wash Buffer B5: Add the indicated volume of ethanol (96 – 100 %) to **Buffer B5 Concentrate** before use. Mark the label of the bottle to indicate that ethanol was added. Store **Wash Buffer B5** at room temperature (18 – 25 °C) for up to one year.

Proteinase K: Before first use of the kit, add indicated volume of Proteinase Buffer PB to each vial of the **lyophilized Proteinase K**. Dissolved Proteinase K solution should be stored at- 20 °C and is stable for 6 months

	NucleoSpin® 96 DNA FFPE	
REF	**1 × 96 preps 740240.1**	**4 × 96 preps 740240.4**
Wash Buffer B5 (Concentrate)	100 mL Add 400 mL ethanol	2 × 100 mL Add 400 mL ethanol to each bottle
Proteinase K (lyophilized)	1 vial (75 mg) Add 2.8 mL Proteinase Buffer	4 vials (75 mg/vial) Add 2.8 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoSpin® 96 DNA FFPE** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***http://www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer BQ1 and BW can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® 96 DNA FFPE** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

5.1 Centrifuge processing

Protocol at a glance

For hardware requirements refer to section 2.4.
For detailed information on each step see page 14.

Before starting the preparation:

- Check if Proteinase K and buffer B5 were prepared according to section 3.

1	**Deparaffinize** sample	**400 µL Paraffin Dissolver** **60 °C, 3 min** **Mix hot sample** **Cool down to RT**
2	**Lyse** sample	**Add to each sample:** **200 µL FL** **25 µL Proteinase K** **Mix** **11,000 x *g*, 1 min** **56 °C, for 1 – 3 h or overnight**
3	**Decrosslink**	**11,000 x *g*, 30 s** **100 µL D-Link** **Mix** **11,000 x *g*, 30 s, 90 °C for 30 min** **11,000 x *g*, 1 min** **Load aqueous phase (lower) in Square-well Block (approx. 300 µL)**
4	**Adjust** DNA binding conditions	**300 µL BQ1** **300 µL Ethanol (96 – 100 %)** **Mix**
5	**Transfer** lysates to NucleoSpin® DNA FFPE Binding plate	
6	**Bind** DNA to silica membrane of the NucleoSpin® FFPE DNA Binding Plate	**5,600 x *g*, 10 min**

7	**Wash** silica membrane	**500 µL BW** **5,600 x *g*, 2 min** **700 µL B5** **5,600 x *g*, 4 min**
8	**Dry** silica membrane	**70 °C, 10 min**
9	**Elute** DNA	**100 µL BE (70 °C)** **5,600 x *g*, 2 min** *Optional: Repeat elution step once.*

Detailed protocol

- For hardware requirements, refer to section 2.4.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 60 °C.
- Preheat Elution Buffer BE to 70 °C.

1 Deparaffinize sample

Place each sample into a suitable 1.5 mL microreaction tube.

Add **400 μL Paraffin Dissolver** to the sample. Incubate **3 min** at **60 °C** (to melt the paraffin). **Vortex or shake** the sample immediately (at 60 °C) at a vigorous speed to dissolve the paraffin. Cool down sample to room temperature.

Make sure that paraffin completely melts during the heat incubation step and mix well after melting to completely dissolve the paraffin.

Insufficient mixing of the heated sample may cause recurrence of solid paraffin particles. Make sure the sample does not comprise more than 15 mg paraffin or adjust the volume of Paraffin Dissolver.

2 Lyse sample

Add **200 μL Lysis Buffer FL** and **25 μL Proteinase K** to the sample. Mix well by repeated pipetting up and down or pulse vortexing or shaking, centrifuge at **11,000 x *g*** for **1 min** and incubate at **56 °C** for **1 – 3 h** or overnight with shaking.

3 Decrosslink

Set heating block to 90 °C.

Centrifuge at **11000 x *g*** for **30 s**.

Decrosslinking step is strongly recommended for short time lysis (< 3 h) and may be omitted after overnight lysis.

Add **100 μL Buffer D-Link**, **mix** by repeated pipetting up and down, vortexing for 5 s or shaking.

Centrifuge for **1 min** at **11000 x *g*** to obtain phase formation.

Incubate at **90 °C** for **30 min**, **mix** by vortexing for 5 s. **Cool** down samples to room temperature. Centrifuge samples for **1 min** at **11000 x *g***.

Transfer 300 μL of the lower aqueous phase from each sample to a Square-well Block for further processing.

4 Adjust DNA binding conditions

Add **300 µL Buffer BQ1** and **300 µL 96 – 100 % ethanol** to each sample. Take care not to moisten the rims of the individual wells while dispensing the buffer. Mix by pipetting up and down 5 times.

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.

5 Transfer lysates

Place a NucleoSpin® DNA FFPE Binding Plate on a MN Square-well Block.

If using more than one plate, label the plates for later identification. The use of a second plate placed on a MN Square-well Block avoids the need to balance the centrifuge.

Transfer the lysates resulting from step 4 carefully into the wells of the NucleoSpin® FFPE DNA Binding Plate. Continue with the next 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.

6 Bind DNA to silica membrane

Place the MN Square-well Blocks with NucleoSpin® FFPE DNA Binding Plates onto the centrifuge carriers and insert them into the rotor buckets. Centrifuge at **5,600 – 6,000 x *g*** for **2 min**.

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 10 min, if the lysates have not passed completely.

7 Wash silica membrane

1st wash

Remove the Self adhering PE Foil and add **500 µL Buffer BW** to each well of the NucleoSpin® FFPE DNA 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® FFPE DNA 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.

8 Dry silica membrane

Remove the Self adhering PE Foil and place the NucleoSpin® FFPE DNA 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.

9 Elute DNA

Dispense **100 µL** preheated **Buffer BE** (70 °C) to each well of the NucleoSpin® FFPE DNA 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**. *Optional: Repeat elution step once.*

Remove the NucleoSpin® FFPE DNA 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® FFPE DNA Binding Plate. For alternative elution procedures see section 2.6.

Alternatively, use the Round-well Block to collect the eluted DNA.

5.2 Vacuum processing

Protocol at a glance

For hardware requirements refer to section 2.4.
For detailed information on each step see page 20.

Before starting the preparation:

- Check if Proteinase K and buffer B5 were prepared according to section 3.

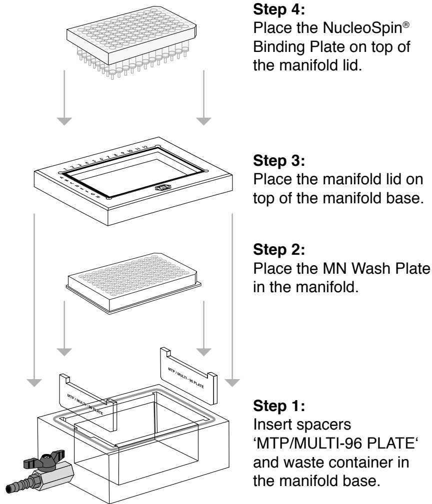
1	**Deparaffinize** sample	**400 µL Paraffin Dissolver** **60 °C, 3 min** **Mix** **Cool down to RT**
2	**Lyse** sample	**Add to each sample** **200 µL FL** **25 µL Proteinase K** **Mix** **11,000 x *g*, 1 min** **56 °C, for 1 – 3 h or overnight**
3	**Decrosslink**	**11,000 x *g*, 30 s** **100 µL D-Link** **mix** **11,000 x *g*, 30 s** **90 °C, 30 min**
	Load sample to processing plate	**11,000 x *g*, 1 min** **Load aqueous phase (lower) in Square-well Block (approx. 300 µL)**
4	**Adjust** DNA binding conditions	**300 µL BQ1** **300 µL Ethanol (96 – 100 %)** **Mix** **Prepare the NucleoVac 96 Vacuum Mantifold**
5	**Transfer** lysates to NucleoSpin® DNA FFPE Binding plate	

6	**Bind** DNA to silica membrane of the NucleoSpin® FFPE DNA Binding Plate	**-0.2 bar*,** **5 min**
7	**Wash silica membrane**	**600 µL BW** **900 µL B5** **900 µL B5** **-0.2 bar*,** **5 min each step** **Remove MN Wash Plate**
8	**Dry** silica membrane	**-0.6 bar*,** **10 min**
9	**Elute** DNA	**100 µL BE (70 °C)** **-0.4 bar*,** **2 min** *Optional: Repeat elution step once*

* Reduction of atmospheric pressure

Setup of vacuum manifold:

Binding / Washing steps

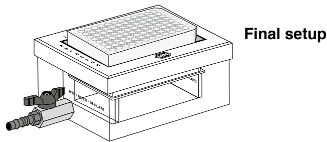


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

Step 3:
Place the manifold lid on top of the manifold base.

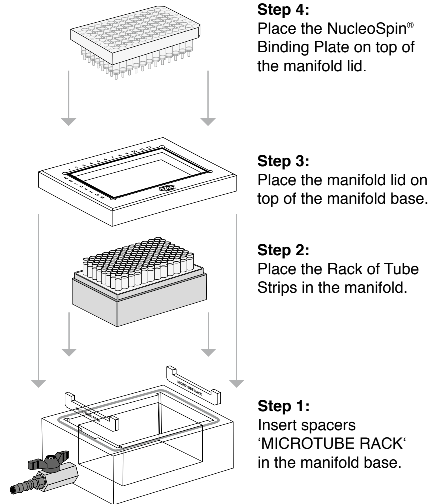
Step 2:
Place the MN Wash Plate in the manifold.

Step 1:
Insert spacers 'MTP/MULTI-96 PLATE' and waste container in the manifold base.



Final setup

Elution step

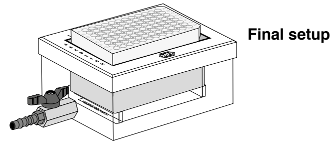


Step 4:
Place the NucleoSpin® Binding Plate on top of the manifold lid.

Step 3:
Place the manifold lid on top of the manifold base.

Step 2:
Place the Rack of Tube Strips in the manifold.

Step 1:
Insert spacers 'MICROTUBE RACK' in the manifold base.



Final setup

Detailed protocol

- For hardware requirements, refer to section 2.4.

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 60 °C.
- Preheat Elution Buffer BE to 70 °C.

---

1 Deparaffinize sample

Place the sample into a suitable 1.5 mL microreaction tube or into a Round-well Block..

Add **400 µL Paraffin Dissolver** to the sample. Incubate **3 min** at **60 °C** (to melt the paraffin). **Vortex or shake** the sample immediately (at 60 °C) at a vigorous speed to dissolve the paraffin. Cool down sample to room temperature.

Make sure that paraffin completely melts during the heat incubation step and mix well after melting to completely dissolve the paraffin.

Insufficient mixing of the heated sample may cause recurrence of solid paraffin particles. Make sure the sample does not comprise more than 15 mg paraffin or adjust the volume of Paraffin Dissolver.

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2 Lyse sample

Add **200 µL Lysis Buffer FL** and **25 µL Proteinase K** to the sample. Mix well by repeated pipetting up and down or pulse vortexing or shaking, centrifuge at **11,000 x *g*** for **1 min** and incubate at **56 °C** for **1 – 3 h** or overnight with shaking.

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3 Decrosslink

Set heating block to 90 °C.

Centrifuge at **11000 x *g*** for **30 s**.

Decrosslinking step is strongly recommended for short time lysis (< 3 h) and may be omitted after overnight lysis.

Add **100 µL Buffer D-Link**, **mix** by repeated pipetting up and down, vortexing for 5 s or shaking.

Centrifuge for **1 min** at **11000 x *g*** to obtain phase formation.

Incubate at **90 °C** for **30 min**, **mix** by vortexing for 5 s. **Cool** down samples to room temperature. Centrifuge samples for **1 min** at **11000 x *g***.

Transfer 300 µL of the lower aqueous phase from each sample to a Square-well Block for further processing.

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4 Adjust DNA binding conditions

Add **300 µL Buffer BQ1** and **300 µL 96 – 100 % ethanol** to each sample. Take care not to moisten the rims of the individual wells while dispensing the buffer. Mix by pipetting up and down 5 times.

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 and place a NucleoSpin® FFPE DNA Binding Plate on top of the manifold

5 Transfer lysates

Place a NucleoSpin® DNA FFPE Binding Plate on a MN Square-well Block.

If using more than one plate, label the plates for later identification. The use of a second plate placed on a MN Square-well Block avoids the need to balance the centrifuge.

Transfer the lysates resulting from step 4 carefully into the wells of the NucleoSpin® FFPE DNA Binding Plate. Continue with the next 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.

6 Bind DNA to silica membrane

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

7 Wash silica membrane

1st wash

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

2nd wash

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

* Reduction of atı

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® FFPE DNA Binding Plate. Release the vacuum.

Remove MN Wash Plate

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

8 Dry silica membrane

Insert the NucleoSpin® FFPE DNA 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.

9 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® FFPE DNA Binding Plate on top. Dispense **100 µL** preheated **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 2.6.

Finally, close the Tube Strips with Cap Strips for storage. Centrifuge Rack of Tube Strips briefly to collect all sample at the bottom of the Tube Strips.

Alternatively, use the Round-well Block for elution. Insert spacers “Round well Block” into the manifold.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Incomplete lysis* • Sample has not completely been submerged during heat incubation. Cut samples into small pieces. Mix well. Be sure that the samples are fully submerged in Buffer FL / Proteinase K mixture. Incubate until the samples are completely lysed. *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® FFPE DNA Binding 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 RNA-free DNA 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, section 6.2). Incubate for 15 min with moderate shaking.

Problem	Possible cause and suggestions
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® DNA FFPE Binding 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.
Insuffcient Paraffin removal	*Paraffin amount too high* • Using too many paraffin sections or excess of paraffin may cause immediate solidfication of the paraffin after heat incubation when cooling lysate down to room temperature. Repeat heat incubation with increased (e.g., doubled) volume of paraffin dissolver.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 DNA FFPE	740240.1 740240.4	1 × 96 preps 4 × 96 preps
NucleoMag® DNA FFPE	744320.1 744320.4	1 × 96 preps 4 × 96 preps
NucleoSpin® FFPE DNA XS	740980.10/.50/.250	10/50/250 preps
Paraffin Dissolver (blue)	740343.60	60 mL
Decrosslink Buffer D-Link	740979.30	30 mL
Buffer BQ1	740923.1	1 L
Buffer B5 Concentrate (for 500 mL Buffer B5)	740921.100	100 mL
Buffer BW	740922.500	500 mL
Proteinase K (lyophilized)	740506	100 mg
RNase A (lyophilized)	740505.50 740505	50 mg 100 mg
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Self adhering PE Foil	740676	50

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

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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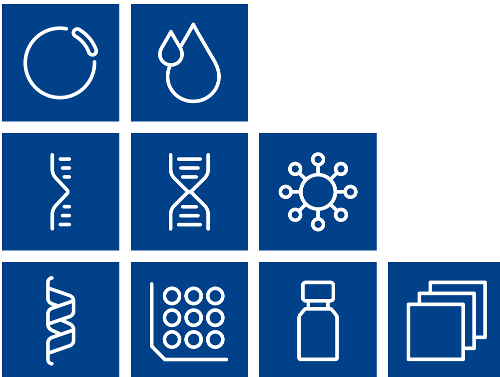
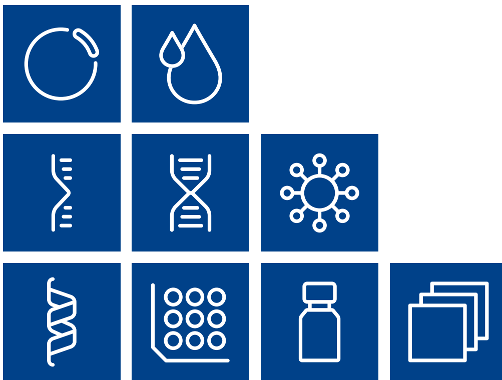


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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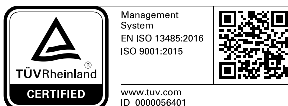

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