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# Genomic DNA from FFPE samples

## User manual

NucleoMag<sup>®</sup> DNA FFPE

May 2015 / Rev. 01

**MACHEREY-NAGEL**

[www.mn-net.com](http://www.mn-net.com)



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# 1 Components

## 1.1 Kit contents

<b>NucleoMag® DNA FFPE</b>		
<b>REF</b>	<b>1x 96 preps</b>	<b>4 x 96 preps</b>
	<b>744320.1</b>	<b>744320.4</b>
NucleoMag® B-Beads	1.7 mL	4 x 1.7 mL
Lysis Buffer FL	30 mL	4 x 30 mL
Binding Buffer MB2	75 mL	300 mL
Wash Buffer MB4	75 mL	300 mL
Elution Buffer MB6	30 mL	125 mL
Proteinase K (lyophilized)*	75 mg	4 x 75 mg
Proteinase Buffer PB	15 mL	35 mL
Paraffin Dissolver (blue)	60 mL	2 x 125 mL
Decrosslink Buffer D-Link	30 mL	2 x 30 mL
User manual	1	1

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

## 1.2 Reagents, equipment and consumables to be supplied by user

### Reagents

- 80 % ethanol

### Equipment/Consumables

Product	REF	Pack of
<b>Magnetic separation system</b> e.g., NucleoMag <sup>®</sup> SEP (see section2.4)	744900	1
<b>Separation plate for magnetic beads separation,</b> e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
<b>Lysis tubes for incubation of samples and lysis,</b> e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
<b>Elution plate for collecting purified nucleic acids,</b> e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 µL u-bottom wells)	740486	24
e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 300 µL flat-bottom wells)	740673	20
<b>For use of kit on KingFisher<sup>®</sup> 96 instrument:</b> e.g., KingFisher <sup>®</sup> 96 Accessory Kit A (Square-well Blocks, Deep-well Tip Combs, Elution Plates for 4 x 96 NucleoMag <sup>®</sup> DNA FFPE preps using KingFisher <sup>®</sup> 96 platform)	744950	1 set

## 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 **NucleoMag® 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. **NucleoMag® DNA FFPE** employs the odorless Paraffin Dissolver (patent pending) and allows effective lysis in a convenient two-phase system. This kit provides reagents and magnetic beads 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 to solubilize the fixed tissue and release DNA into solution. Subsequently, heat incubation with a specially formulated buffer effectively eliminates crosslinks from the previously released DNA. For binding of the DNA to the paramagnetic beads, Binding Buffer MB2 and the NucleoMag® B-Beads are added to the lysate.

The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers MB4 and 80 % ethanol. Residual ethanol from previous wash steps is removed by airdrying. Finally, highly pure DNA is eluted with low-salt Elution Buffer MB6. Purified DNA can directly be used for downstream applications, e.g., qPCR. The **NucleoMag® DNA FFPE** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

### 2.2 Kit specifications

The **NucleoMag® 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. **NucleoMag® DNA FFPE** is suitable for up to 5 mg tissue. The amount of paraffin is limited to 15 mg, when using the standard protocol with Paraffin Dissolver (approx. 7 sections of 10 µm x 250 mm<sup>2</sup>). However,

larger amounts of paraffin samples may be processed by using either additional Paraffin Dissolver or by deparaffinization using xylene.

- **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 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.

**NucleoMag® DNA FFPE** is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information, section 6.2) or other magnetic separation systems (see section 2.4). Manual preparation time of 96 samples is about 120 minutes.

**NucleoMag® DNA FFPE** allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on the automation platform.

## 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 Magnetic separation systems

For use of **NucleoMag® DNA FFPE**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information, section 6.2). The kit can also be used with other common separators

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481 / .24)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

### Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

### Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins / rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

### Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

## 2.5 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

### Adjusting shaker speed for binding and wash steps:

- Load 600 µL dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.

- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing step.

**Adjusting shaker speed for the elution step:**

- Load 100 µL dyed water to the wells of the collection plate and proceed as described above.

## 2.6 Handling of beads

### Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

### Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

### Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

+: acceptable, ++: good, +++: excellent, \* 8-channel pipetting device



## 2.7 Handling of Proteinase K

For dispensing the Proteinase K solution to each sample it is recommended to predispense the needed amount to a separate reaction tube. Using a liquid handling device it is recommended to dispense the needed Proteinase K solution (25  $\mu\text{L}$  per prep) with 10% extra volume in a suitable tube for the correspondent robot. Unused Proteinase K solution should be stored at -20 °C for further extractions.

## 2.8 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer MB6. Elution can be carried out in a volume of  $\geq 25 \mu\text{L}$ . It is essential to cover the NucleoMag<sup>®</sup> Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, higher elution volumes might be necessary to cover the whole pellet.

Elution is possible at room temperature. Yield can be increased by 15–20% if elution is performed at 55 °C.

### 3 Storage conditions and preparation of working solutions

**Attention:** Buffers MB2 and MB4 contain chaotropic salt! Wear gloves and goggles!

Storage conditions:

- All components of the NucleoMag® DNA FFPE kit should be stored at room temperature (18–25 °C) and are stable for up to one year.
- All buffers are delivered ready-to-use.

Before starting any **NucleoMag® DNA FFPE** protocol, prepare the following:

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

NucleoMag® DNA FFPE		
REF	1 x 96 preps 744320.1	4 x 96 preps 744320.4
Proteinase K (lyophilized)	75 mg Add 2.8 mL Proteinase Buffer	75 mg Add 2.8 mL Proteinase Buffer

- **80 % ethanol:** Use molecular biology grade ethanol, dilute with appropriate nuclease-free water to 80 %.

## 4 Safety instructions




The following components of the **NucleoMag® DNA FFPE** kits contain hazardous contents.

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

### GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

*Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	P-Sätze
MB2	Sodium perchlorate 20–40 % + ethanol 35–55 % <i>Natriumperchlorat 20–40 % + Ethanol 35–55 %</i> CAS 7601-89-0, 64-17-5	 WARNING ACHTUNG	226, H302	210, P233, P301+312, P330, P370+378, P403+235
BW	Sodium perchlorate 5–20 % + ethanol 20–35 % <i>Natriumperchlorat 5–20 % + Ethanol 20–35 %</i> CAS 7601-89-0, 64-17-5	 WARNING ACHTUNG	226	210, 233, 403+235
Proteinase K	Proteinase K, lyophilized <i>Proteinase K, lyophilisiert</i> CAS 39450-01-6	 DANGER GEFAHR	315, 319, 334, 335	261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 403+233

### Hazard phrases

H 226	Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i>
H 302	Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i>
H 315	Causes skin irritation. <i>Verursacht Hautreizungen.</i>
H 319	Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i>
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. <i>Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.</i>
H 335	May cause respiratory irritation. <i>Kann die Atemwege reizen.</i>

## Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
- P 233 Keep container tightly closed.  
*Behälter dicht verschlossen halten.*
- P 261 Avoid breathing dust.  
*Einatmen von Staub vermeiden.*
- P 280 Wear protective gloves / eye protection.  
*Schutzhandschuhe / Augenschutz tragen.*
- P 301+312 IF SWALLOWED: Call a POISON CENTER / doctor / ... / if you feel unwell.  
*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.*
- P 302+352 IF ON SKIN: Wash with plenty of water / ...  
*BEI KONTAKT MIT DER HAUT: Mit viel Wasser / ... waschen.*
- P 304+340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.  
*BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
- P 305+351+313 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing  
*BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
- P 312 Call a POISON CENTER / doctor / ... / if you feel unwell.  
*Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.*
- P 330 Rinse mouth.  
*Mund ausspülen.*
- P 333+313 IF skin irritation or a rash occurs: Get medical advice / attention.  
*Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER / doctor / ...  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.*
- P 337+313 Get medical advice / attention.  
*Bei anhaltender Augenreizung: Ärztliche Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 403+233 Store in a well ventilated place. Keep container tightly closed.  
*Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*
- P 403+235 Store in a well ventilated place. Keep cool.  
*Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ([www.mn-net.com](http://www.mn-net.com)).  
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ([www.mn-net.com](http://www.mn-net.com)).

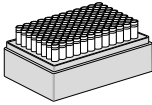
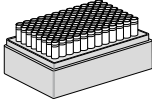

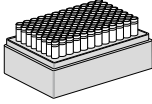


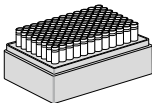
## 5 Protocol for the isolation of genomic DNA from tissue

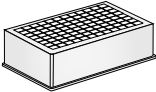
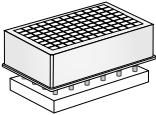
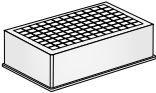
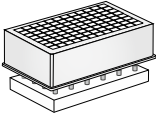

### Protocol-at-a-glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.4, respectively.
- For detailed information on each step, see page 17.

### Before starting the preparation:

- Check if Proteinase K was prepared according to section 3.

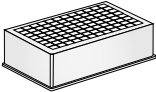
<b>1</b>  <b>Deparaffinize sample</b>	<b>400 <math>\mu</math>L Paraffin Dissolver</b> <b>60 °C, 3 min</b> <b>Mix hot sample by vortexing</b> <b>Cool down to RT</b>	
<b>2</b>  <b>Lyse sample</b>	<b>Add to each sample:</b> <b>200 <math>\mu</math>L FL</b> <b>25 <math>\mu</math>L Proteinase K</b> <b>Mix by vortexing for 5 s</b> <b>11,000 x g, 1 min</b> <b>56 °C, 1–3 h</b>	 
<b>3</b>  <b>Decrosslink sample</b>	<b>11,000 x g, 30 s</b> <b>100 <math>\mu</math>L D-Link</b> <b>Mix by vortexing for 5 s</b> <b>11,000 x g, 1 min</b> <b>90 °C, 30 min</b>	 
<b>4</b>  <b>Transfer sample</b>	<b>11,000 x g, 1 min</b> <b>Load aqueous phase (lower)</b> <b>in Square-well Block</b>	 

5	<p><b>14 µL NucleoMag® B-Beads</b> <b>600 µL MB2</b></p>	
	<p><b>Bind DNA to NucleoMag® B-Beads</b></p>	<p><b>Mix by shaking at 1,000 rpm for 5 min at RT</b> <i>(Optional: Mix by pipetting up and down)</i></p> <p>↔</p>
	<p><b>Remove supernatant after 2 min separation</b></p>	
6	<p>Remove Square-well Block from NucleoMag® SEP</p> <p><b>600 µL MB4</b></p>	
	<p><b>Wash with MB4</b></p>	<p><b>Shake 1–3 min at RT</b> <i>(Optional: Mix by pipetting up and down)</i></p> <p>↔</p>
	<p><b>Remove supernatant after 2 min separation</b></p>	
7	<p>Remove Square-well Block from NucleoMag® SEP</p> <p><b>600 µL 80% ethanol</b></p>	
	<p><b>Wash with 80% ethanol (1<sup>st</sup>)</b></p>	<p><b>Shake 1–3 min at RT</b> <i>(Optional: Mix by pipetting up and down)</i></p> <p>↔</p>
	<p><b>Remove supernatant after 2 min separation</b></p>	

**8** **Wash with 80 % ethanol (2<sup>nd</sup>)**

**Remove Square-well Block from NucleoMag® SEP**

**600 µL 80 % ethanol**



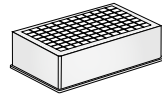
**Shake 1–3 min at RT**  
*(Optional: Mix by pipetting up and down)*



**Remove supernatant after 2 min separation**

**9** **Air-dry magnetic beads**

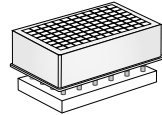
**Air-dry for 10 min at RT**



**10**

**Remove Square-well Block from NucleoMag® SEP**

**25–100 µL MB6**  
*(Optional: Elute at 56 °C)*

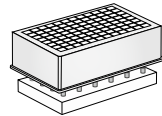


**Elute DNA**

**Shake 5 min at RT**  
*(Optional: Mix by pipetting up and down)*



**Separate 2 min and transfer DNA into elution plate / tubes**



## Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.4). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

### Before starting the preparation:

- Check if Proteinase K was prepared according to section 3.
- 

#### 1 Deparaffinize sample

Place the 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 lower aqueous phase. 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 sample

Centrifuge for **1 min** at **11000 x g**.

Set heating block to 90 °C. Add **100 µL Buffer D-Link**, mix by repeated pipetting up and down, vortexing for 5 s, or shaking.

Centrifuge for **1 min** at **11,000 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 **11,000 x g**.

*Decrosslinking step is strongly recommended for short time lysis (1–3 h) and may be omitted after overnight lysis.*

---

#### 4 Load sample to Square-well Block

Transfer **400 µL** of the lower aqueous phase from each sample to a Square-well Block for further processing.

---



## 5 Bind DNA to NucleoMag® B-Beads

Add **14 µL of NucleoMag® B-Beads** and **600 µL Buffer MB2** to the lysed sample.

**Mix** by pipetting up and down 6 times and **shake** for **5 min at room temperature**.

*Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.*

*Note: Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.*

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP a magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

*Note: Do not disturb the attracted beads while aspirating the supernatant.*

---

## 6 Wash with MB4

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MB4** to each well and mix the beads by shaking until the beads are resuspended completely (**1–3 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

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## 7 Wash with 80 % ethanol (1<sup>st</sup>)

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and mix the beads by shaking until the beads are resuspended completely (**1–3 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

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**8 Wash with 80 % ethanol (2<sup>nd</sup>)**

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**1–3 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

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**9 Air-dry**

Air dry the magnetic bead pellet for **10 min** at **room temperature**.

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**10 Elution**

Add desired volume of **Buffer MB6 (25–100 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at **room temperature**. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **10 min** at **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Transfer supernatant containing the purified genomic DNA to either microtubes or Tube Strips (see ordering information, section 6.2).

*Note: Yield can be increased by 15–20 % by using pre-warmed elution buffer (55 °C) or by incubating the bead/elution buffer suspension at 55 °C for 10 min.*

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## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	<i>Elution buffer volume insufficient</i>
	<ul style="list-style-type: none"> <li>• Beads pellet must be covered completely with elution buffer</li> </ul>
	<i>Insufficient performance of elution buffer during elution step</i>
	<ul style="list-style-type: none"> <li>• Remove residual buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step.</li> </ul>
	<i>Beads dried out</i>
	<ul style="list-style-type: none"> <li>• Do not let the beads dry as this might result in lower elution efficiencies.</li> </ul>
	<i>Aspiration of attracted bead pellet</i>
	<ul style="list-style-type: none"> <li>• Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate.</li> </ul>
	<i>Aspiration and loss of beads</i>
	<ul style="list-style-type: none"> <li>• Prolong magnetic separation time or decrease aspiration speed.</li> </ul>
Low purity	<i>Insufficient washing procedure</i>
	<ul style="list-style-type: none"> <li>• Use only the appropriate combinations of separator and plate, for example, Square-well Block in combination with NucleoMag® SEP.</li> <li>• Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.</li> </ul>
Suboptimal performance of DNA in downstream applications	<i>Carry-over of ethanol wash solutions</i>
	<ul style="list-style-type: none"> <li>• Be sure to remove all of the 80% ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications.</li> </ul>
	<i>Ethanol evaporation from wash buffers</i>
	<ul style="list-style-type: none"> <li>• Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.</li> </ul>

Carry-over of beads

*Time for magnetic separation too short*

- Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.

*Aspiration speed too high (elution step)*

- High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.
- 

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 Proteinase K solution according to instructions (see section 3).
- 

RNA contamination

*RNA in sample*

- If DNA free of RNA is desired, cool down to room temperature after lysis incubation and add 20  $\mu$ L of an RNase A solution (20 mg/mL; see ordering information, section 6.2). Incubate for 15 min with moderate shaking.
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Insufficient Paraffin removal

*Paraffin amount too high*

- Using too many paraffin sections or excess of paraffin may cause immediate solidification 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
NucleoMag® DNA FFPE	744320.1	1 x 96 preps
	744320.4	4 x 96 preps
NucleoSpin® 96 DNA FFPE	740240.1	1 x 96 preps
	740240.4	4 x 96 preps
NucleoSpin® FFPE DNA XS	740980.10	10 preps
	740980.50	50 preps
	740980.250	250 preps
Paraffin Dissolver (blue)	740343.60	60 mL
Proteinase K (lyophilized)	740506	100 mg
RNase A (lyophilized)	740505.50	50 mg
	740505	100 mg
NucleoMag® SEP	744900	1
Square-well Blocks	740481	4
	740481.24	24
Self-adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 CapStrips)	740477	4 sets
	740477.24	24 sets
Elution Plate U-bottom	740486.24	24
Elution Plate Flat-bottom	740673	20
KingFisher® 96 Accessory Kit A (set consists of Square-well Blocks, Deep-well Tip combs, Elution Plates; for 4 x 96 NucleoMag® DNA FFPE preps using KingFisher® 96 platform)	744950	1 set

Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information.

### 6.3 Product use restriction/warranty

**NucleoMag® DNA FFPE** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

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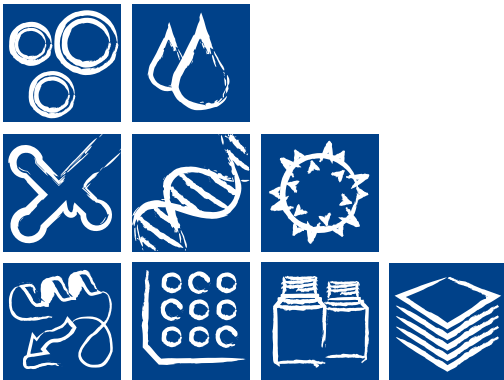
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