



# **Genomic DNA from tissue**

## **User manual**








**NucleoSpin® Tissue XS**

February 2012/Rev.04

# Genomic DNA from tissue

## Protocol-at-a-glance (Rev. 04)

### NucleoSpin® Tissue XS

1 Prepare sample	Up to 2.5 mg tissue
2 Pre-lyse sample	 <p>80 µL T1 8 µL Proteinase K 56 °C, 1–4 h</p>
3 Lyse sample	 <p>80 µL B3 70 °C, 5 min</p>
4 Adjust binding conditions	 <p>80 µL ethanol</p>
5 Bind DNA	 <p>Load lysate  11,000 x g, 1 min</p>
6 Wash silica membrane	 <p>1<sup>st</sup> wash      50 µL B5 11,000 x g, 1 min  2<sup>nd</sup> wash      50 µL B5 11,000 x g, 2 min</p>
7 Elute DNA	 <p>20 µL BE  11,000 x g, 1 min</p>
8 <i>Optional:</i> <i>Remove residual ethanol</i>	 <p><i>Optional:</i> 90 °C, 8 min</p>

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## Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	5
1.3	About this user manual	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Handling of sample material	7
2.4	Elution procedures	8
2.5	Removal of residual traces of ethanol for highest sensitivity in downstream applications	9
3	Storage conditions and preparation of working solutions	11
4	Safety instructions	12
4.1	Risk and safety phrases	12
4.2	GHS classification	13
5	Protocols	15
5.1	Protocol for human or animal tissue	15
5.2	Protocol for cultured cells	18
5.3	Protocol for paraffin-embedded tissue	21
5.4	Protocol for dried blood spots (Guthrie cards)	24
5.5	Protocol for buccal swabs	26
5.6	Protocol for laser-microdissected tissue	28
5.7	Protocol for blood samples	29
6	Appendix	30
6.1	Troubleshooting	30
6.2	Ordering information	31
6.3	Product use restriction/warranty	31

# 1 Components

## 1.1 Kit contents

<b>NucleoSpin® Tissue XS</b>			
<b>REF</b>	<b>10 preps 740901.10</b>	<b>50 preps 740901.50</b>	<b>250 preps 740901.250</b>
Lysis Buffer T1	5 mL	10 mL	50 mL
Lysis Buffer B3	5 mL	10 mL	50 mL
Wash Buffer B5 (Concentrate)*	1 mL	2 mL	6 mL
Elution Buffer BE**	5 mL	5 mL	15 mL
Proteinase K (lyophilized)*	6 mg	20 mg	2 x 50 mg
Proteinase Buffer PB	0.8 mL	1.8 mL	8 mL
NucleoSpin® Tissue XS Columns (green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
User manual	1	1	1

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

\*\*Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

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

### Reagents

- 96–100% ethanol

### Consumables

- 1.5 mL microcentrifuge tubes for sample lysis and DNA elution
- Disposable tips

### Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Thermal heating-block
- Personal protection equipment (lab coat, gloves, goggles)

## 1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Tissue XS** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at [www.mn-net.com](http://www.mn-net.com).

Please contact Technical Service ([tech-bio@mn-net.com](mailto:tech-bio@mn-net.com)) regarding information about changes of the current user manual compared to previous revisions.

## 2 Product description

### 2.1 The basic principle

The **NucleoSpin® Tissue XS** kit is designed for the efficient isolation of genomic DNA from small samples of different kinds of cells and tissues, such as laser-microdissected samples, small amounts of blood, or dried blood spots and forensic samples. Due to a special funnel design the **NucleoSpin® Tissue XS Columns** allow very small elution volumes (5–30 µL) which results in highly concentrated DNA.

Lysis is achieved by incubation of the sample material in a Proteinase K supplemented lysis buffer. Appropriate conditions for binding of the DNA to a silica membrane are created by adding ethanol. The mixture is applied to the **NucleoSpin® Tissue XS Column** and DNA binds to a silica membrane. Two subsequent washing steps efficiently remove contaminations and highly pure DNA is finally eluted with 5–30 µL of a slightly alkaline elution buffer of low ionic strength (5 mM Tris-HCl, pH 8.5).

### 2.2 Kit specifications

- **NucleoSpin® Tissue XS** is recommended for the isolation of genomic DNA from very small samples. Typical sample material comprises fresh or frozen cells, tissues, blood spots on Guthrie/NucleoCard®/FTA cards (see ordering information), buccal swabs, forensic samples, and others, see specifications at a glance (Table 1, page 7).
- **NucleoSpin® Tissue XS** is designed for high recovery of small amounts of DNA due to a special column design.
- The special column design is connected with a significantly reduced dead volume which allows elution in as little as 5–30 µL elution buffer. DNA is ready to use for downstream applications like real-time PCR and others.
- The preparation time is approximately 40–45 min for 6–12 samples (exclusive incubation for lysis).
- The DNA yield strongly depends on the sample type, quality, and amount, see specifications at a glance (Table 1, page 7).
- The length of the purified genomic DNA fragments depends on the quality of the sample material and may vary between 500 bp from laser-microdissected, or forensic samples, and up to 30 kb from fresh tissues or cultured cells.

**Table 1: Kit specifications at a glance**

Parameter	NucleoSpin® Tissue XS
Technology	Silica-membrane technology
Format	Mini spin columns (XS design)
Typical sample size	<b>Tissue samples:</b> 0.025–10 mg tissue (e.g., laser-microdissected, fresh, frozen), <b>Blood samples:</b> 1–30 µL fresh or frozen blood, <b>Cultured cells:</b> 10–10,000 cultured cells, <b>Paraffin-embedded tissue:</b> 0.001–10 mg tissue <b>Guthrie card:</b> spots of 15–30 mm <sup>2</sup> (~ 4.4–6.2 mm), <b>Buccal swab:</b> one
Fragment size	200 bp–approx. 50 kbp
Typical yield	Typical yields for selected samples are listed below: 100 HeLa cells: 0.1–0.5 ng DNA 1000 HeLa cells: 1–5 ng DNA 10000 HeLa cells: 10–50 ng DNA 0.025 mg mouse liver: 20–100 ng DNA 0.25 mg mouse liver: 200–1000 ng DNA 2.5 mg mouse liver: 600–3000 ng DNA
$A_{260}/A_{280}$	1.7–1.9
Elution volume	5–30 µL
Preparation time	~ 20 min/prep (excl. lysis)
Binding capacity	50 µg

## 2.3 Handling of sample material

The **NucleoSpin® Tissue XS** procedure is designed for very small samples and the typical downstream applications are thus very sensitive. It is highly recommended performing sampling and DNA purification with special care, in order to avoid a contamination of the sample and the purified DNA with unwanted DNA-containing material (e.g., fingerprints, hair particles, aerosol, dust).

Moreover, a cross-contamination between samples has to be excluded. The following precautions are recommended:

- Wear personal protection equipment (lab coat, gloves, goggles).
- Use aerosol resistant pipette tips.
- Always change pipette tips between liquid transfers.
- Briefly centrifuge after mixing steps in order to remove droplets from tube lid.

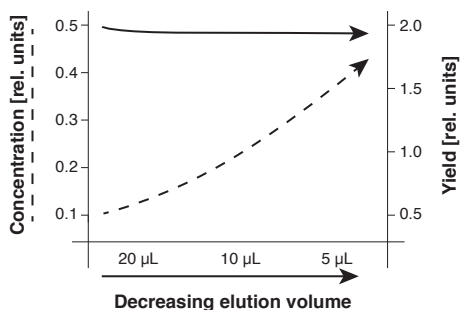
## 2.4 Elution procedures

A high DNA concentration in the elution fraction is of highest importance and desirable for all typical downstream applications. This is of particular interest if the total volume of a reaction mixture is limited as this in turn limits the possible amount of added DNA. Due to a high default elution volume, classical DNA clean-up kits often result in weakly concentrated DNA, if only small samples are processed.

Such DNA often even requires a subsequent concentration before it can be used for typical downstream applications.

In contrast to classical kits, **NucleoSpin® Tissue XS** allows an efficient elution in a very small volume which results in highly concentrated DNA.

An elution volume of 20  $\mu\text{L}$  is recommended by default although volumes as small as 5  $\mu\text{L}$  are feasible. A reduction of the elution volume from 20  $\mu\text{L}$  to 5–15  $\mu\text{L}$  will increase DNA concentration whereas the total DNA yield is only slightly affected. An increase of the elution volume to 30  $\mu\text{L}$  or more will slightly increase total DNA yield but will reduce DNA concentration. Figure 1 gives a graphic description of the correlation between elution volume and DNA concentration and will thus help you to find the optimized elution volume for your individual application.



**Figure 1: Correlation between elution volume and DNA concentration (NucleoSpin® Tissue XS Columns)**



## 2.5 Removal of residual traces of ethanol for highest sensitivity in downstream applications

A reduction of the 20  $\mu\text{L}$  default elution volume will increase the concentration of residual ethanol in the eluate. For 20  $\mu\text{L}$  elution volumes a heat incubation of the elution fraction (incubate eluate with open lid for 8 min at 90 °C) is recommended if the eluate comprises more than 20% of the final PCR volume in order to avoid an inhibition of sensitive downstream reactions. In this context, please mind the remarks below:

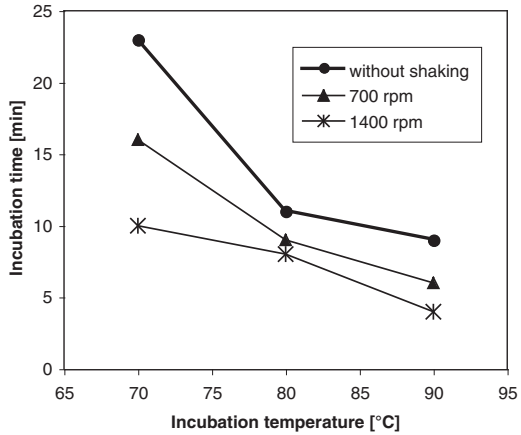
- a) An incubation of the elution fraction at higher temperatures will increase signal output in PCR. This is especially of importance if the template represents more than 20% of the total PCR reaction volume (e.g., more than 4  $\mu\text{L}$  eluate used as template in a PCR reaction with a total volume of 20  $\mu\text{L}$ ).

The template may represent up to 40%\* of the total PCR reaction volume, if the eluate is incubated at elevated temperature as described above.

- b) A volume of 20  $\mu\text{L}$  used for elution will evaporate to 12–14  $\mu\text{L}$  during a heat incubation for 8 min at 90 °C. If a higher final volume is required, please increase the volume of elution buffer, for example from 20  $\mu\text{L}$  to 30  $\mu\text{L}$ .
- c) An incubation of the elution fraction for 8 min at 90 °C will denature DNA. If non denatured DNA is required (for downstream applications other than PCR; for example ligation / cloning), we recommend an incubation for a longer time at a temperature below 80 °C as most of the DNA has a melting point above 80 °C. Suggestion: Incubate for 17 min at 75 °C.
- d) The incubation of the eluate at higher temperatures may be adjusted according to Figure 2. The incubation times and conditions shown will reduce an elution volume of 20  $\mu\text{L}$  to about 12–14  $\mu\text{L}$  and will effectively remove traces of ethanol as described above.
- e) If the initial volume of elution buffer applied to the column is less than 20  $\mu\text{L}$ , heat incubation times should be reduced in order to avoid complete dryness. If the elution volume is, for example 5  $\mu\text{L}$ , a heat incubation of the eluate for 2 min at 80 °C will adequately remove residual ethanol.

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\* The maximum percentage of template volume in a PCR reaction may vary depending on the robustness of the PCR system; 40% template volume were tested using LightCycler™ PCR (Roche) with the DyNAmo™ Capillary SYBR® Green qPCR Kit (Finnzymes).



**Figure 2: Removal of residual ethanol from the elution fraction by heat treatment.**

In order to obtain highest PCR sensitivity, a heat incubation of the eluate is recommended. Heat incubation may be performed at temperatures of 70–90 °C in a heat block with or without shaking. Effective conditions (temperature, time, and shaking rate) for ethanol removal can be read from the diagram; an initial volume of 20 µL will evaporate to 12–14 µL during the incubation shown.

### 3 Storage conditions and preparation of working solutions

#### Attention:

*Buffer B3 contains chaotropic salt and detergents. Wear gloves and goggles!*

*CAUTION: Buffer B3 contains guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.*

- All kit components can be stored at room temperature (18–25 °C) and are stable up to one year.
- Upon storage, especially at low temperatures, a white precipitate may form in Buffers T1 or Buffer B3. Such precipitates can be easily dissolved by incubating the bottle at 50–70 °C before use.

Before starting any **NucleoSpin® Tissue XS** protocol prepare the following:

- **Wash Buffer B5:** Add the indicated volume (see bottle or table below) of ethanol (96–100 %) to **Buffer B5 Concentrate**. 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.
- Before first use of the kit, add the indicated volume (see bottle or table below) of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for 6 months.

NucleoSpin® Tissue XS			
REF	10 preps 740901.10	50 preps 740901.50	250 preps 740901.250
Wash Buffer B5 (Concentrate)	1 mL Add 4 mL ethanol	2 mL Add 8 mL ethanol	6 mL Add 24 mL ethanol
Proteinase K	6 mg Add 260 µL Proteinase Buffer	20 mg Add 1 mL Proteinase Buffer	2 x 50 mg Add 2.5 mL Proteinase Buffer to each vial

## 4 Safety instructions

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

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

### 4.1 Risk and safety phrases

Component	Hazard contents	Hazard symbol	Risk phrases	Safety phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>Gefahrstoff-symbol</i>	<i>R-Sätze</i>	<i>S-Sätze</i>
B3	Guanidine hydrochloride <i>Guanidinhydrochlorid</i>	✘ Xn*	R 22-36	26-39
Proteinase K	Proteinase K, lyophilized <i>Proteinase K, lyophilisiert</i>	✘ Xn	R 36/37/38-42	S 22-24-26-36/37

#### Risk phrases

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



#### Safety phrases

- S 22 Do not breathe dust.  
*Staub nicht einatmen.*
- S 24 Avoid contact with skin.  
*Berührung mit der Haut vermeiden.*
- S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.  
*Bei Berührung mit den Augen gründlich mit Wasser abspülen und Arzt konsultieren.*
- S 36/37 Wear suitable protective clothing and gloves.  
*Bei der Arbeit geeignete Schutzhandschuhe und Schutzkleidung tragen.*
- S 39 Wear eye / face protection.  
*Schutzhandschuhe / Gesichtsschutz tragen.*

\* 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.

## 4.2 GHS classification

Only harmful features need not be labeled with H and P phrases until 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
B3	Guanidine hydrochloride 36–50 % <i>Guanidinhydrochlorid 36–50 %</i>		Warning <i>Achtung</i>	302, 319  280, 301+312, 305+351+338, 330, 337+313
Proteinase K	Proteinase K, lyophilized <i>Proteinase K, lyophilisiert</i>		Danger <i>Gefahr</i>	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 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 261	Avoid breathing dust. <i>Einatmen von Staub vermeiden.</i>
P 280	Wear protective gloves / eye protection. <i>Schutzhandschuhe / Augenschutz tragen.</i>
P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor / physician if you feel unwell. <i>BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.</i>
P 302+352	IF ON SKIN: Wash with soap and water.
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. <i>BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.</i>

### Precaution phrases

- P 304+341 IF INHALED: If breathing is difficult, remove to fresh air and keep at rest in a position comfortable for breathing.  
*BEI EINATMEN: Bei Atembeschwerden an die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
- P 305+351+338 IF IN EYES: Rinse cautiously 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 spülen.*
- P 312 Call a POISON CENTER or doctor / physician if you feel unwell.  
*Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.*
- P 330 Rinse mouth.  
*Mund ausspülen.*
- P 332+313 IF skin irritation occurs: Get medical advice / attention.  
*Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 337+313 If eye irritation persists: Get medical advice / attention.  
*Bei anhaltender Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM oder Arzt anrufen.*
- P 403+233 Store in a well ventilated place. Keep container tightly closed.  
*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 Protocols

### 5.1 Protocol for human or animal tissue

#### Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
- Adjust thermal heating block temperature to 56 °C and equilibrate sample to room temperature (18–25 °C).

#### 1 Prepare sample

Place the sample of **up to 2.5 mg** into a 1.5 mL microcentrifuge tube (not provided).

*For samples from 2.5–10 mg double the volumes of Buffer T1, Buffer B3, and ethanol in steps 2, 3, and 4 to 160 µL each.*

#### 2 Pre-lyse sample

Add **80 µL Buffer T1** and **8 µL Proteinase K** solution and mix by vortexing 2 x 5 s. Be sure that the sample is completely covered with lysis solution.

*If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do never mix Buffer T1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer T1 without substrate.*

Incubate at **56 °C** until complete lysis is obtained (approximately **1–4 h** or **overnight**). Vortex occasionally during incubation or use a shaking incubator. At the end of the incubation, adjust the thermal heating block temperature to 70 °C for the following step.

*If RNA-free DNA is crucial for downstream applications, an RNase digest may be performed: Add 20 µL RNase A (20 mg/mL) solution (not included; see ordering information) and incubate for additional 5 min at room temperature.*



**+ 80 µL T1**

**+ 8 µL  
Proteinase K**

**56 °C,  
1–4 h**

**or**

**56 °C,  
overnight**

### 3 Lyse sample

Add **80 µL Buffer B3**, vortex 2 x 5 s and incubate at **70 °C for 5 min**. Vortex briefly at the end of the incubation.

*Optional: Adjust the thermal heating block temperature to 90 °C for the last step of the protocol.*

Let the lysate cool down to room temperature.

*A white precipitate may form in the lysate upon addition of Buffer B3, especially if very small samples are used. Precipitates will dissolve during the incubating step at 70 °C.*

*If insoluble particles are visible after the heat incubation steps, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a new microcentrifuge tube (not provided).*



**+ 80 µL B3**  
**70 °C,**  
**5 min**

### 4 Adjust DNA binding conditions

Add **80 µL ethanol (96–100 %)** to the lysate and mix by vortexing 2 x 5 s.

Spin down briefly to clear the lid.



**+ 80 µL**  
**ethanol**

### 5 Bind DNA

For each sample, place one **NucleoSpin® Tissue XS Column** into a **Collection Tube (2 mL)**. Apply the sample to the column. Centrifuge for **1 min at 11,000 x g**. Discard the flow-through and place the column into a new Collection Tube (2 mL).

*If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g.*



**Load lysate**



**11,000 x g,**  
**1 min**

### 6 Wash silica membrane

#### **1<sup>st</sup> wash**

Add **50 µL Buffer B5** to NucleoSpin® Tissue XS Column. Centrifuge for **1 min at 11,000 x g**. It is not necessary to discard the flow-through. Reuse the Collection Tube.



**+ 50 µL B5**



**11,000 x g,**  
**1 min**



**2<sup>nd</sup> wash**

Add **50 µL Buffer B5** to the NucleoSpin® Tissue XS Column. Centrifuge for **2 min** at **11,000 x g**. Discard Collection Tube with flow-through.



**+ 50 µL B5**



**11,000 x g,  
2 min**

**7 Elute DNA**

Place the NucleoSpin® Tissue XS Column in a new 1.5 mL microcentrifuge tube (not provided) and apply **20 µL Buffer BE** directly onto the center of the silica membrane of the column. Centrifuge for **1 min** at **11,000 x g**.



**+ 20 µL BE**



**11,000 x g,  
1 min**

*Elution volume may be varied from approximately 5–30 µL. For a correlation of elution volume, DNA concentration and DNA amount eluted from the column see section 2.4–2.5.*

**8 Optional: Remove residual ethanol**

*Incubate elution fraction with open lid for **8 min** at **90 °C**.*

*See section 2.5 for further comments and alternative incubation times and temperatures for a removal of residual ethanol.*



**Optional:**

**8 min,  
90 °C**

## 5.2 Protocol for cultured cells

### Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
- Adjust thermal heating block temperature to 56 °C and equilibrate sample to room temperature (18–25 °C).

### 1 Prepare sample

Resuspend up to 10<sup>5</sup> cells in a final volume of **80 µL Buffer T1**.



**+ 80 µL T1**

### 2 Pre-lyse sample

Add **8 µL Proteinase K** solution and mix by vortexing 2 x 5 s.

Incubate at **56 °C for 10 min**. Adjust the thermal heating block temperature to 70 °C at the end of the incubation for the following step.

*If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do never mix Buffer T1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer T1 without substrate.*



**+ 8 µL Proteinase K**

**56 °C,  
10 min**

### 3 Lyse sample

Add **80 µL Buffer B3**, vortex 2 x 5 s and incubate at **70 °C for 5 min**. Vortex briefly at the end of the incubation.

*Optional: Adjust the thermal heating block temperature to 90 °C for the last step of the protocol.*

Let the lysate cool down to room temperature.

*A white precipitate may form in the lysate upon addition of Buffer B3, especially if very small samples are used. Precipitates will dissolve during the incubating step at 70 °C.*

*If insoluble particles are visible after the heat incubation, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a new microcentrifuge tube (not provided).*



**+ 80 µL B3**

**70 °C,  
5 min**

**4 Adjust binding conditions**

Add **80 µL ethanol** (96–100 %) to the lysate and mix by vortexing 2 x 5 s.



**+ 80 µL ethanol**

Spin down briefly to clear the lid.

**5 Bind DNA**

For each sample, place one **NucleoSpin® Tissue XS Column** into a **Collection Tube (2 mL)**. Apply the sample to the column. Centrifuge for **1 min** at **11,000 x g**. Discard the flow-through and place the column into a new Collection Tube (2 mL).



**Load lysate**



**11,000 x g,  
1 min**

*If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g.*

**6 Wash silica membrane**

**1<sup>st</sup> wash**

Add **50 µL Buffer B5** to NucleoSpin® Tissue XS Column. Centrifuge for **1 min** at **11,000 x g**. It is not necessary to discard the flow-through. Reuse the Collection Tube.



**+ 50 µL B5**

**11,000 x g,  
1 min**

**2<sup>nd</sup> wash**

Add **50 µL Buffer B5** directly onto the membrane of the NucleoSpin® Tissue XS Column. Centrifuge for **2 min** at **11,000 x g**. Discard flow-through with Collection Tube.



**+ 50 µL B5**

**11,000 x g,  
2 min**

**7 Elute DNA**

Place the NucleoSpin® Tissue XS Column in a new 1.5 mL microcentrifuge tube (not provided) and apply **20 µL Buffer BE** directly onto the center of the silica membrane of the column. Centrifuge for **1 min** at **11,000 x g**.



**+ 20 µL BE**

*Elution volume may be varied from approximately 5–30 µL. For a correlation of elution volume, DNA concentration and DNA amount eluted from the column see section 2.4–2-5.*



**11,000 x g,  
1 min**

**8 Optional: Remove residual ethanol**

*Incubate elution fraction with open lid for **8 min** at **90 °C**.*

*See section 2.5 for further comments and alternative incubation times and temperatures for a removal of residual ethanol.*

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**Optional:**

**8 min,  
90 °C**

## 5.3 Protocol for paraffin-embedded tissue

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### 1 Prepare sample

As an alternative to this protocol, the NucleoSpin® FFPE DNA kit (see ordering information) is recommended for FFPE samples.

Prepare small sections (up to 3 mg; for larger samples please see the indications below) from blocks of fixed, embedded tissue. If possible, trim excess paraffin from the block before slicing. Handle the sections with tweezers or toothpicks and place the samples into microcentrifuge tubes (not provided).

Add **300 µL n-octane or xylene** to each tube. Vortex vigorously and incubate at room temperature for about 30 min. Vortex occasionally.

Centrifuge at **11,000 x g** for **3 min**. Pipette off supernatant.

Add **1 mL ethanol** (96–100 %) to each tube. Close and mix by inverting several times. Centrifuge at **11,000 x g** for **3 min**. Pipette off supernatant.

Repeat the ethanol washing step. Pipette off as much of the ethanol as possible.

Incubate the open tube at **37 °C** until the ethanol has evaporated (~ **15 min**).

*Note: For samples from 3–10 mg the volumes of Buffer T1, B3, and ethanol in steps 2, 3, and 4 should be doubled (160 µL each). However, also for larger samples the indicated volume (300 µL) of n-octane or xylene can be used.*

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## 2 Pre-lyse sample

Add **80 µL Buffer T1** and **8 µL Proteinase K** solution and mix by vortexing 2 x 5 s. Be sure that the sample is completely covered with lysis solution.

*If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do never mix Buffer T1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer T1 without substrate.*

Incubate at **56 °C** until complete lysis is obtained (approximately **1–4 h or overnight**). Vortex occasionally during incubation or use a shaking incubator. At the end of the incubation, adjust the thermal heating block temperature to 70 °C for the following step.

+ 80 µL T1

+ 8 µL  
Proteinase K



56 °C,  
1–4 h

or

56 °C,  
overnight

---

## 3 Lyse sample

Add **80 µL Buffer B3**, vortex 2 x 5 s and incubate at **70 °C for 5 min**. Vortex briefly at the end of the incubation.

*Optional: Adjust the thermal heating block temperature to 90 °C for the last step of the protocol.*

Let the lysate cool down to room temperature.

*A white precipitate may form in the lysate upon addition of Buffer B3, especially if very small samples are used. Precipitates will dissolve during the incubating step at 70 °C.*

*If insoluble particles are visible after the heat incubation, centrifuge for 5 min at high speed (e.g. 11,000 xg) and transfer the supernatant to a new microcentrifuge tube (not provided).*



+ 80 µL B3

70 °C,  
5 min

---

## 4 Adjust binding conditions

Add **80 µL ethanol** (96–100 %) to the lysate and mix by vortexing 2 x 5 s.

Spin down briefly to clear the lid.



+ 80 µL  
ethanol

**5 Bind DNA**

For each sample, place one **NucleoSpin® Tissue XS Column** into a **Collection Tube (2 mL)**. Apply the sample to the column. Centrifuge for **1 min** at **11,000 x g**. Discard the flow-through and place the column into a new Collection Tube (2 mL).

*If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g.*



**Load lysate**



**11,000 x g,  
1 min**

**6 Wash silica membrane**

**1<sup>st</sup> wash**

Add **50 µL Buffer B5** to NucleoSpin® Tissue XS Column. Centrifuge for **1 min** at **11,000 x g**. It is not necessary to discard the flow-through. Reuse the Collection Tube.



**+ 50 µL B5**

**11,000 x g,  
1 min**

**2<sup>nd</sup> wash**

Add **50 µL Buffer B5** directly onto the membrane of the NucleoSpin® Tissue XS Column. Centrifuge for **2 min** at **11,000 x g**. Discard Collection Tube with flow-through.



**+ 50 µL B5**

**11,000 x g,  
2 min**

**7 Elute DNA**

Place the NucleoSpin® Tissue XS Column in a new 1.5 mL microcentrifuge tube (not provided) and apply **20 µL Buffer BE** directly onto the center of the silica membrane of the column. Centrifuge for **1 min** at **11,000 x g**.



**+ 20 µL BE**

*Elution volume may be varied from approximately 5–30 µL. For a correlation of elution volume, DNA concentration and DNA amount eluted from the column see section 2.4–2.5.*



**11,000 x g,  
1 min**

**8 Optional: Remove residual ethanol**

*Incubate elution fraction with open lid for **8 min** at **90 °C**.*

*See section 2.5 for further comments and alternative incubation times and temperatures for a removal of residual ethanol.*



**Optional:**

**8 min,  
90 °C**

## 5.4 Protocol for dried blood spots (Guthrie cards)

### Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
  - Adjust thermal heating block temperature to 56 °C and equilibrate sample to room temperature (18–25 °C).
- 

### 1 Prepare sample

Cut out one dried blood spot. Use only blood soaked paper. Cut spots into small pieces and place them in a 1.5 mL microcentrifuge tube (not provided).

The area of the dried blood spot should be less than 30 mm<sup>2</sup> (corresponds to approximately 20 µL blood).

---

### 2 Pre-lyse sample

Add **160 µL Buffer T1** and mix by vortexing for 2 x 5 s.

Incubate the sample for **10 min** at **94 °C**. Subsequently, adjust the thermal heating block temperature to 56 °C for the following step. Let the sample cool down to room temperature. Add **16 µL Proteinase K** solution. Mix by vortexing and spin the sample down briefly.

Incubate at **56 °C** for **1 h**. Vortex occasionally during incubation or use a shaking incubator. Adjust the thermal heating block to 70 °C for the following step.

*Be sure that the sample is completely covered with lyses buffer during incubation.*

*If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do never mix Buffer T1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer T1 without substrate.*

---



## 2a Separate lysis solution from paper pieces

### Alternative A:

Place a **NucleoSpin® Filter** (not provided; see ordering information) into a Collection Tube (2 mL). Transfer the complete lysate including paper pieces with a 1 mL pipette tip onto the NucleoSpin® Filter. Centrifuge for **1 min** at **11,000 x g**. Discard the NucleoSpin® Filter. Continue with flow-through.

### Alternative B:

Transfer as much as possible of the lysate solution to a 1.5 mL microcentrifuge tube (not provided). Discard paper pieces and continue with recovered solution.

---

## 3 Lyse sample

Add **160 µL Buffer B3**, vortex 2 x 5 s and incubate at **70 °C** for **5 min**. Vortex briefly after the incubation.

Let the lysate cool down to room temperature.

---

## 4 Adjust binding conditions

Add **160 µL ethanol (96–100 %)** to the sample and mix by vortexing 2 x 5 s.

Spin down briefly to clear the lid.

---

Proceed with step 5 (Bind DNA) of the standard protocol (see section 5.1).

## 5.5 Protocol for buccal swabs

### Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
  - Adjust thermal heating block temperature to 56 °C and equilibrate sample to room temperature (18–25 °C).
- 

### 1 Prepare sample

Collect the samples with cotton, dacron® (Daigger) or C.E.P. swabs (Gibco BRL).

Scrape firmly against the inside for each cheek several times and let the swabs air dry.

*The respective individual should not have consumed food or drink within 30 min before collection of the samples.*

---

### 2 Pre-lyse sample

Place the dry swab material in 1.5 mL microcentrifuge tubes (not provided).

Add a mixture of **200–400 µL Buffer T1** and **20–40 µL Proteinase K** solution.

*Additional amount of Buffer T1 and Proteinase K is required for this application (see ordering information, section 6.2).*

*The suitable amount of Buffer T1 depends on the actual size of the buccal swab type. Be sure that the buccal swab is completely covered with lysis buffer during incubation.*

Mix by vortexing **2–5 s** and incubate **10 min** at **56 °C**.

---

### 2a Separate lysis solution from buccal swabs

#### Alternative A:

Place a **NucleoSpin® Filter** (not provided; see ordering information) into a Collection Tube (2 mL). Transfer the swab tip (cut off swab shaft) and the remaining solution onto the NucleoSpin® Filter. Centrifuge for **1 min** at **11,000 x g**. Discard the NucleoSpin® Filter. Continue with flow-through.

#### Alternative B:

Transfer as much as possible of the lysate solution to a 1.5 mL microcentrifuge tube (not provided). Discard swab and continue with recovered solution.

---

### 3 Lyse sample

Add **one volume of Buffer B3** (200–400 µL), vortex 2 x 5 s and incubate at **70 °C** for **5 min**. Vortex briefly after the incubation.

Let the lysate cool down to ambient temperature.

---

### 4 Adjust binding conditions

Add **one volume of ethanol** (96–100%; 200–400 µL) to each sample and mix by vortexing 2 x 5 s.

Spin down briefly to clear the lid.

---

Proceed with step 5 (Bind DNA) of the standard protocol (see section 5.1).

## 5.6 Protocol for laser-microdissected tissue

### Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
  - Adjust thermal heating block temperature to 56 °C and equilibrate sample to room temperature (18–25 °C).
  - The extraction of genomic DNA from laser-microdissected samples is a challenge: the sample amount is very small and the DNA quality is adversely affected by fixation and staining procedures. The use of cryosections or different fixation and staining procedures should always be considered as alternatives.
- 

### 1 Prepare sample

Place laser-microdissected sample into a 1.5 mL microcentrifuge tube (not provided).

---

### 2 Pre-lyse sample

Add **80 µL Buffer T1** and **8 µL Proteinase K** solution.

Mix by vortexing **2–5 s** and incubate for approximately **1–4 h or overnight at 56 °C**.

*The optimal incubation time may vary depending on sample type and amount.*

---

### 3 Lyse sample

Add **80 µL Buffer B3**, vortex 2 x 5 s and incubate at **70 °C** for **5 min**. Vortex briefly after the incubation.

Let the lysate cool down to room temperature.

---

### 4 Adjust binding conditions

Add **80 µL ethanol (96–100%)** to each sample and mix by vortexing 2 x 5 s.

Spin down briefly to clear the lid.

---

Proceed with step 5 (Bind DNA) of the standard protocol (see section 5.1).

---

## 5.7 Protocol for blood samples

### Before starting the preparation:

- Check if Wash Buffer B5 and Proteinase K were prepared according to section 3.
  - Adjust thermal heating block temperature to 56 °C and equilibrate sample to room temperature (18–25 °C).
- 

#### 1 Prepare sample

Not necessary

---

#### 2 Lyse blood samples

Pipette **8 µL Proteinase K** solution and **up to 20 µL blood** into a 1.5 mL microcentrifuge tube (not provided).

Add **60 µL Buffer T1**.

*Note: For larger blood samples (20–30 µL), add 16 µL Proteinase K and add 120 µL Buffer T1. The volumes of Buffer B3 and ethanol have to be increased to 160 µL during the following procedure.*

---

#### 3 Lyse sample

Add **80 µL Buffer B3** to the sample and vortex the mixture vigorously (10–20 s).

Incubate samples at **70 °C for 10–15 min**.

---

#### 4 Adjust binding conditions

Add **80 µL ethanol** (96–100%) to the lysate and mix by vortexing 2 x 5 s.

Spin down briefly to clear the lid.

---

#### 5 Bind DNA

For each sample, place one NucleoSpin® Tissue XS Column into a Collection Tube (2 mL). Apply the sample to the column. Centrifuge for **1 min at 11,000 x g**. Discard the flow-through and place the column into a new Collection Tube (2 mL).

---

Proceed with step 6 (Wash silica membrane) of the standard protocol (see section 5.1).

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## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA yield	<p data-bbox="370 341 689 365"><i>Low DNA content of the sample</i></p> <ul data-bbox="370 373 981 429" style="list-style-type: none"> <li data-bbox="370 373 981 429">• The content of DNA depends very much on sample type, amount, and quality.</li> </ul>
Column clogging	<p data-bbox="370 464 813 488"><i>Sample contains residual cell debris or cells</i></p> <ul data-bbox="370 496 981 580" style="list-style-type: none"> <li data-bbox="370 496 981 580">• The lysate may have contained residual particulate matter. Make sure to proceed after the lysis step only with clear lysate before adding ethanol to create binding conditions.</li> </ul>
No increase of PCR signal despite of an increased volume of eluate used as template in PCR	<p data-bbox="370 616 633 639"><i>Residual ethanol in eluate</i></p> <ul data-bbox="370 647 981 703" style="list-style-type: none"> <li data-bbox="370 647 981 703">• Please see the detailed description of removal of residual traces of ethanol in section 2.6.</li> </ul>
Discrepancy between $A_{260}$ quantification values and PCR quantification values	<p data-bbox="370 823 723 847"><i>Silica abrasion from the membrane</i></p> <ul data-bbox="370 855 981 1211" style="list-style-type: none"> <li data-bbox="370 855 981 1211">• Due to the typically low DNA content in very small samples and the resulting low total amount of isolated DNA, a DNA quantification via <math>A_{260}</math> absorption measurement is often hampered due to the low sensitivity of the absorption measurement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect <math>A_{260}</math> quantification of small DNA amounts centrifuge the eluate for 30 s at <math>&gt;11,000 \times g</math> and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen<sup>®</sup> fluorescent dye).</li> </ul>
Unexpected $A_{260}/A_{280}$ ratio	<p data-bbox="370 1246 969 1270"><i>Measurement not in the range of photometer detection limit</i></p> <ul data-bbox="370 1278 981 1422" style="list-style-type: none"> <li data-bbox="370 1278 981 1422">• In order to obtain a significant <math>A_{260}/A_{280}</math> ratio it is necessary that the initially measured <math>A_{260}</math> and <math>A_{280}</math> values are significantly above the detection limit of the photometer used. An <math>A_{280}</math> value close to the background noise of the photometer will cause unexpected <math>A_{260}/A_{280}</math> ratios.</li> </ul>

## 6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Tissue XS	740901.10/.50/.250	10/50/250
NucleoSpin® Tissue	740952.10/.50/.250	10/50/250
NucleoSpin® FFPE DNA	740980.10/.50/.250	10/50/250
Buffer T1	740940.25	25 mL
Buffer B3	740920	100 mL
Buffer B5 Concentrate (for 100 mL Buffer B5)	740921	20 mL
Buffer BE	740306.100	100 mL
Proteinase K	740506	100 mg
RNase A	740505.50 7410505	50 mg 100 mg
NucleoCard®	740403.10/.100	10/100
NucleoSpin® Filters	740606	50
Collection Tubes (2 mL)	740600	1000

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

## 6.3 Product use restriction/warranty

**NucleoSpin® Tissue XS** 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.

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Last updated: 07/2010, Rev. 03

Please contact:  
MACHEREY-NAGEL GmbH & Co. KG  
Tel.: +49 24 21 969-270  
tech-bio@mn-net.com

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