



# **Genomic DNA from Tissue**

## **User Manual**






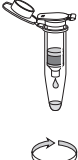

NucleoSpin® Tissue XS

October 2009/Rev.02

# Genomic DNA from Tissue

## Protocol-at-a-glance (Rev. 02)

### NucleoSpin® Tissue XS

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

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# 1 Components

## 1.1 Kit contents

<b>NucleoSpin® Tissue XS</b>			
<b>Cat. No.</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).

## 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/NucleoSave/FTA cards (see ordering on-formation), 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												
Typical sample size	<p><b>Tissue samples:</b> 0.025 - 10 mg tissue (e.g. laser-microdissected, fresh, frozen),</p> <p><b>Blood samples:</b> 1 - 30 µl fresh or frozen blood,</p> <p><b>Cultured cells:</b> 10 - 10,000 cultured cells,</p> <p><b>Paraffin-embedded tissue:</b> 0.001 - 10 mg tissue</p> <p><b>Guthrie card:</b> spots of 15 - 30 mm<sup>2</sup> (~ 4.4 - 6.2 mm),</p> <p><b>Buccal swab:</b> one</p>												
Typical yield	<p>Typical yields for selected samples are listed below:</p> <table> <tr> <td>100 HeLa cells:</td> <td>0.1 - 0.5 ng DNA</td> </tr> <tr> <td>1000 HeLa cells:</td> <td>1 - 5 ng DNA</td> </tr> <tr> <td>10000 HeLa cells:</td> <td>10 - 50 ng DNA</td> </tr> <tr> <td>0.025 mg mouse liver:</td> <td>20 - 100 ng DNA</td> </tr> <tr> <td>0.25 mg mouse liver:</td> <td>200 - 1000 ng DNA</td> </tr> <tr> <td>2.5 mg mouse liver:</td> <td>600 - 3000 ng DNA</td> </tr> </table>	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
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												
Elution volume	5 - 30 µl												
Preparation time	Approximatively 40 - 45 min for 6 - 12 samples (exclusive incubation for lysis)												
Format	XS spin columns												

## 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.

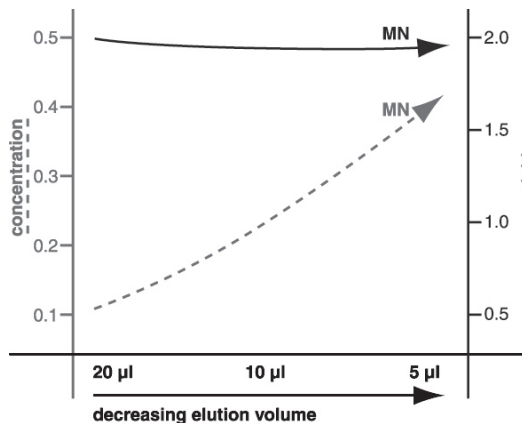
## 2.4 Elution procedures

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 µl default elution volume will increase the concentration of residual ethanol in the eluate. For 20 µl 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:

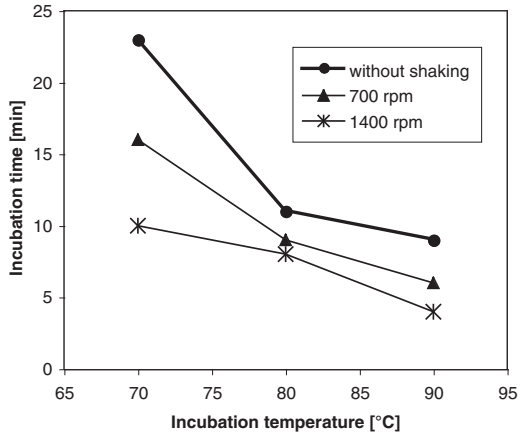
- a) An incubation of the elution fraction at higher temperatures will increase signal output in PCR. This is especially of importance if the template represents more than 20% of the total PCR reaction volume (e.g. more than 4 µl eluate used as template in a PCR reaction with a total volume of 20 µl).

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 µl used for elution will evaporate to 12-14 µl during a heat incubation for 8 min at 90°C. If a higher final volume is required, please increase the volume of elution buffer e.g. from 20 µl to 30 µl.
- 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; e.g. 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 µl to about 12-14 µl 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 µl, heat incubation times should be reduced in order to avoid complete dryness. If the elution volume is e.g. 5 µl, 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:

*Buffers B3 and B5 contain guanidine hydrochloride! Wear gloves and goggles!*

- All kit components can be stored at room temperature (20-25°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 C5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer C5 at room temperature (20-25°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			
	<b>10 preps</b>	<b>50 preps</b>	<b>250 preps</b>
<b>Cat. No.</b>	<b>740902.10</b>	<b>740902.50</b>	<b>740902.250</b>
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 – risk and safety phrases

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.*

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
B3	Guanidine hydrochloride	✘ Xn*		Harmful if swallowed - Irritating to eyes and skin	
Proteinase K	Proteinase K, lyophilized	✘ Xn Xi*		Irritating to eyes, respiratory system and skin, may cause sensitization by inhalation	S 22-24-26-36/37

### Risk phrases

R 22	Harmful if swallowed
R 36/37/38	Irritating to eyes, respiratory system and skin
R 36/38	Irritating to eyes and skin
R 42	May cause sensitization by inhalation

### Safety phrases

S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 36/37	Wear suitable protective clothing and gloves

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

## 5 Protocols

### 5.1 Standard 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°C-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, 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

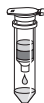


1 min  
11,000 x g

### 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.



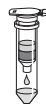
+ 50 µl B5



1 min  
11,000 x g

**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**



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

**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.*



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

**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°C-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.

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**



**1 min  
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.



**1 min  
11,000 x g**

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

#### 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**.

*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.*



**+ 20 µl BE**



**1 min  
11,000 x g**

**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**

## 5.3 Protocol for paraffin-embedded tissue

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### 1 Prepare sample

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, 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 x g) and transfer the supernatant to a new microcentrifuge tube (not provided).*



+ 80 µl B3

70 °C  
5 min

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## 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.

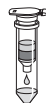


+ 80 µl  
ethanol

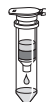
**5 Bind DNA**

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

*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.*

**+ 20 µl BE****1 min**  
**11,000 x g****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 Support 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°C-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 Support protocol for purification of genomic DNA from 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°C-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.

*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 Support protocol for purification of genomic DNA from laser-microdissected tissues

### 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°C-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 **10 min** at **56°C** for approximately **1-4 h** or **overnight**.

*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 Support 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°C-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="370 344 689 367"><i>Low DNA content of the sample</i></p> <ul data-bbox="370 379 981 427" style="list-style-type: none"> <li data-bbox="370 379 981 427">• The content of DNA depends very much on sample type, amount, and quality.</li> </ul>
Column clogging	<p data-bbox="370 472 813 494"><i>Sample contains residual cell debris or cells</i></p> <ul data-bbox="370 507 981 603" style="list-style-type: none"> <li data-bbox="370 507 981 603">• 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="370 647 633 670"><i>Residual ethanol in eluate</i></p> <ul data-bbox="370 683 981 730" style="list-style-type: none"> <li data-bbox="370 683 981 730">• 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="370 858 723 880"><i>Silica abrasion from the membrane</i></p> <ul data-bbox="370 893 981 1241" style="list-style-type: none"> <li data-bbox="370 893 981 1241">• 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® fluorescent dye).</li> </ul>
Unexpected $A_{260}/A_{280}$ ratio	<p data-bbox="370 1279 969 1302"><i>Measurement not in the range of photometer detection limit</i></p> <ul data-bbox="370 1315 981 1455" style="list-style-type: none"> <li data-bbox="370 1315 981 1455">• 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	Cat. No.	Pack of
NucleoSpin® Tissue XS	740901.10/.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
NucleoSave	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 were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY**. They are suitable **FOR IN-VITRO USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

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

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