Genomic DNA from tissue

User manual

NucleoSpin® Tissue XS

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MACHEREY-NAGEL
www.mn-net.com

Genomic DNA from tissue

Protocol at a glance (Rev. 08)

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

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1 Components

1.1 Kit contents

	NucleoSpin® Tissue XS		
	10 preps	**50 preps**	**250 preps**
REF	**740901.10**	**740901.50**	**740901.250**
Lysis Buffer T1	5 mL	10 mL	50 mL
Lysis Buffer B3	10 mL	10 mL	60 mL
Wash Buffer B5 (Concentrate)*	6 mL	6 mL	6 mL
Elution Buffer BE**	13 mL	13 mL	13 mL
Proteinase K (lyophilized)*	6 mg	20 mg	2 x 50 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
NucleoSpin® Tissue XS Columns (green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
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* 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***.

Please contact Technical Service (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 20 min/prep (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	**Tissue samples:** 0.025–10 mg tissue (e.g., laser-microdissected, fresh, frozen), Blood samples: 1–30 µL fresh or frozen blood, **Cultured cells:** 10–10^4 cultured cells, **Paraffin-embedded tissue:** 0.001–10 mg tissue **Guthrie card:** spots of 15–30 mm^2 (~ 4.4–6.2 mm), **Buccal swab:** 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

- **Ethylene oxide treated product:**
  NucleoSpin® Tissue XS is often used in highly sensitive applications and therefore the manufacturing process was optimized to reduce the risk of DNA contamination. MACHEREY-NAGEL has a stringently controlled production process to avoid DNA contamination of consumables. Further, MACHEREY-NAGEL uses ethylene oxide (EO) treatment to remove amplifiable DNA, which might still be introduced during the manufacturing process. MACHEREY-NAGEL products carrying the ethylen oxide treated plastic materials seal, contain plastic materials that are EO treated. This means, DNA of any kind, which might still be introduced into plastic consumables during the production process, is inactivated by means of the treatment with ethylene oxide, in order to prevent the generation of accidental human profile by PCR amplification. Ethylene oxide treatment has been shown to be the method of choice to prevent DNA profiles due to DNA contamination (Shaw et al. 2008; Figure 1).

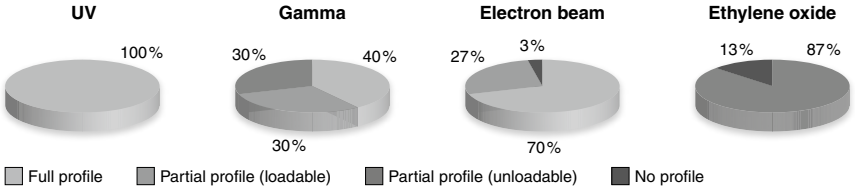

Figure 1 According to Shaw *et al.*, 2008, Comparison of the effects of sterilization techniques on subsequent DNA profiling. Int J Legal Med 122: 29-33.

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 µL is recommended by default although volumes as small as 5 µL are feasible. A reduction of the elution volume from 20 µL to 5–15 µL will increase DNA concentration whereas the total DNA yield is only slightly affected. An increase of the elution volume to 30 µL 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.

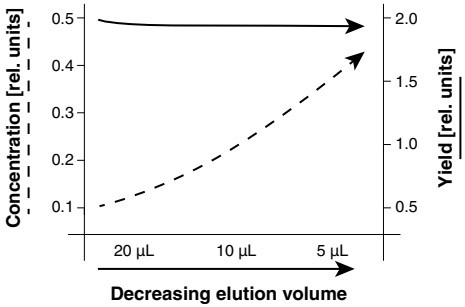

Figure 2 Correlation between elution volume and DNA concentration (NucleoSpin® Tissue XS Columns)

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 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 LightCyclerTM PCR (Roche) with the DyNAmoTM Capillary SYBR® Green qPCR Kit (Finnzymes).

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, for example 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; 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 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, for example 5 µL, a heat incubation of the eluate for 2 min at 80°C will adequately remove residual ethanol.

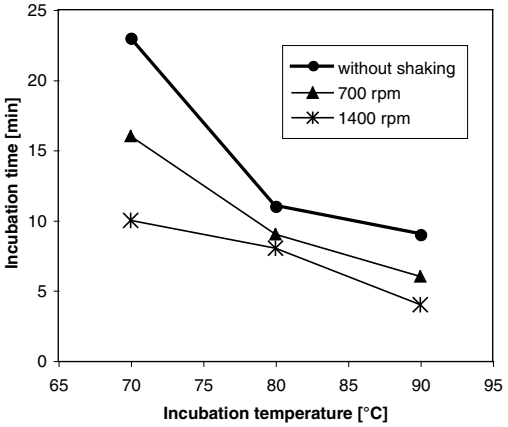

Figure 3 Removal of residual ethanol from the elution fraction by heat treatment.
In order to obtain highest PCR sensitivity, a heat incubation of the eluate is recommended. Heat incubation may be performed at temperatures of 70–90 °C in a heat block with or without shaking. Effective conditions (temperature, 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		
	10 preps	**50 preps**	**250 preps**
REF	**740901.10**	**740901.50**	**740901.250**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	6 mL Add 24 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.

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 ***Inhalt***	**Hazard contents** ***Gefahrstoff***	**GHS symbol** ***GHS Symbol***		**Hazard phrases** ***H-Sätze***	**Precaution phrases** ***P-Sätze***
B3	Guanidine hydrochloride 36–50 % *Guanidinhydrochlorid 36–50 %* CAS 50-01-1		Warning *Achtung*	302, 319	280, 301+312, 305+351+338, 330, 337+313
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert* 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

 The symbol shown on labels refers to further safety information in this section.

Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

Hazard phrases

H 302 Harmful if swallowed.
Gesundheitsschädich bei Verschlucken.

H 315 Causes skin irritation.
Verursacht Hautreizungen.

H 319 Causes serious eye irritation.
Verursacht schwere Augenreizung.

H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.

H 335 May cause respiratory irritation.
Kann die Atemwege reizen.

Precaution phrases

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+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/ doctor/…/if you feel unwell.
Bei Unwohlsein GIFTINFORMATIONSZENTRUM / 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/ doctor/…
Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /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***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**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

1st wash

Add **50 µL Buffer B5** to the 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

2nd 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***.

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

11,000 x *g*, 1 min

8 *<u>Optional:</u> 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 10^4 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

11,000 x *g*, 1 min

6 Wash silica membrane

1st 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

2nd 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***.

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

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

5.3 Protocol for paraffin-embedded tissue

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.

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.

+ 80 µL T1

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

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.

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

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 mi**n 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

1st 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

2nd 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

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**.*

Optional: 8 min, 90 °C

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

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^2 (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).
- Additional Buffer T1 and B3 might be necessary, see ordering information.

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 70 °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).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA yield	*Low DNA content of the sample* • The content of DNA depends very much on sample type, amount, and quality.
Column clogging	*Sample contains residual cell debris or cells* • The lysate may have contained residual particular matter. Make sure to proceed after the lysis step only with clear lysate before adding ethanol to create binding conditions.
No increase of PCR signal despite of an increased volume of eluate used as template in PCR	*Residual ethanol in eluate* • Please see the detailed description of removal of residual traces of ethanol in section 2.6.
Discrepancy between A_{260} quantification values and PCR quantification values	*Silica abrasion from the membrane* • Due to the typically low DNA content in very small samples and the resulting low total amount of isolated DNA, a DNA quantification via A_{260} 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 A_{260} quantification of small DNA amounts centrifuge the eluate for 30 s at > 11,000 x *g* and take an aliquot for measurement without disturbing any sediment. Alternatively, use a silica abrasion insensitive DNA quantification method (e.g., PicoGreen® fluorescent dye).
Unexpected A_{260}/A_{280} ratio	*Measurement not in the range of photometer detection limit* • In order to obtain a significant A_{260}/A_{280} ratio it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpected A_{260}/A_{280} ratios.

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® DNA FFPE XS	740980.10/.50/.250	10/50/250
Buffer T1	740940.25	50 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
NucleoSpin® Forensic Filters	740988.10/.50/.250	10/50/250 pieces
NucleoSpin® Forensic Filters (Bulk)	740988.50B/.250B / 1000B	50/250 / 1000 pieces
NucleoCard®	740403.10/.100	10/100
NucleoSpin® Filters	740606	50
Collection Tubes (2 mL)	740600	1000

Visit ***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.

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

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DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

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.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

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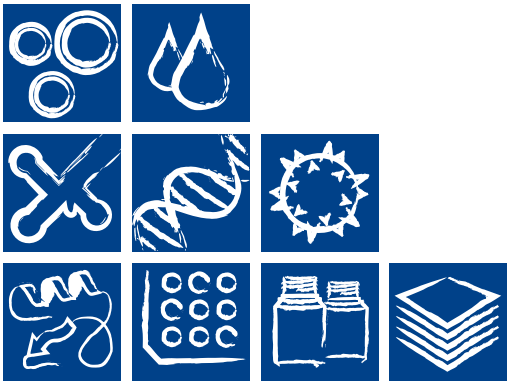

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