# Genomic DNA from tissue

## Protocol at a glance (Rev. 17)

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<td></td>
<td>180 μL T1</td>
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<tr>
<td></td>
<td>25 μL Proteinase K</td>
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</tr>
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<td></td>
<td>56 °C, 1–3 h</td>
<td></td>
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<td>3 Lyse sample</td>
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<td>4 Adjust DNA binding conditions</td>
<td></td>
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<tr>
<td></td>
<td>210 μL 96–100 % ethanol</td>
<td></td>
</tr>
<tr>
<td>5 Bind DNA</td>
<td></td>
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</tr>
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<td></td>
<td>Load all</td>
<td></td>
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<td></td>
<td>11,000 x g, 1 min</td>
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<tr>
<td>6 Wash silica membrane</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1st wash</td>
<td>500 μL BW</td>
</tr>
<tr>
<td></td>
<td>2nd wash</td>
<td>600 μL B5</td>
</tr>
<tr>
<td></td>
<td>1st and 2nd</td>
<td>11,000 x g, 1 min</td>
</tr>
<tr>
<td>7 Dry silica membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11,000 x g, 1 min</td>
<td></td>
</tr>
<tr>
<td>8 Elute highly pure DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 μL BE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RT, 1 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11,000 x g, 1 min</td>
<td></td>
</tr>
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</table>
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1 Components

1.1 Kit contents

<table>
<thead>
<tr>
<th>REF</th>
<th>10 preps 740952.10</th>
<th>50 preps 740952.50</th>
<th>250 preps 740952.250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer T1</td>
<td>5 mL</td>
<td>20 mL</td>
<td>100 mL</td>
</tr>
<tr>
<td>Lysis Buffer B3</td>
<td>10 mL</td>
<td>15 mL</td>
<td>75 mL</td>
</tr>
<tr>
<td>Wash Buffer BW</td>
<td>6 mL</td>
<td>30 mL</td>
<td>150 mL</td>
</tr>
<tr>
<td>Wash Buffer B5 (Concentrate)*</td>
<td>6 mL</td>
<td>12 mL</td>
<td>50 mL</td>
</tr>
<tr>
<td>Elution Buffer BE*</td>
<td>13 mL</td>
<td>13 mL</td>
<td>60 mL</td>
</tr>
<tr>
<td>Proteinase K (lyophilized)**</td>
<td>6 mg</td>
<td>30 mg</td>
<td>2 x 75 mg</td>
</tr>
<tr>
<td>Proteinase Buffer PB</td>
<td>1.8 mL</td>
<td>1.8 mL</td>
<td>8 mL</td>
</tr>
<tr>
<td>NucleoSpin® Tissue Columns (light green rings)</td>
<td>10</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>Collection Tubes (2 mL)</td>
<td>20</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>User manual</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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

** For preparation of working solutions and storage, see section 3.
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
- Equipment for sample disruption and homogenization
- 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 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 regarding information about changes of the current user manual compared to previous revisions. Note: Buffer B3 is delivered premixed now.
2 Product description

2.1 The basic principle

With the NucleoSpin® Tissue method genomic DNA can be prepared from tissue, cells (e.g., bacteria), and many other sources. Lysis is achieved by incubation of the sample material in a proteinase K/SDS solution. Appropriate conditions for DNA binding to the silica membrane in the NucleoSpin® Tissue Columns are achieved by the addition of chaotropic salts and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by subsequent washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® Tissue** is designed for the rapid, small-scale preparation of highly pure genomic DNA from any tissue, cells, bacteria, yeast, forensic samples, serum, plasma, or other body fluids. It is also suitable for preparation of DNA from human or animal blood. The purified DNA can be used directly for PCR, Southern blotting, or any kind of enzymatic reactions.

- The kit allows purification of up to 35 μg of pure genomic DNA with an $A_{260}/A_{280}$ ratio between 1.7 and 1.9. The NucleoSpin® Tissue Column is capable of binding up to 60 μg of genomic DNA.

- For lysis of certain bacterial and yeast strains, additional enzymes may be necessary which are not part of this kit. See the relevant support protocol for details.

| Table 1: Kit specifications at a glance |
| Parameter | NucleoSpin® Tissue |
| Technology | Silica membrane technology |
| Format | Mini spin column |
| Sample material | < 25 mg tissue |
| | $10^2$–$10^7$ cultured cells |
| Typical yield | 20–35 μg |
| Elution volume | 60–100 μL |
| Preparation time | 20 min/prep (excluding lysis) |
| Binding capacity | 60 μg |
• **Forensic quality product:**
  NucleoSpin® Tissue is certified as forensic quality product. Consumables used in forensics need to be treated carefully to prevent DNA contamination. MACHEREY-NAGEL therefore 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 forensic quality 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](image_url)  
**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 Elution procedures

In addition to the standard method (recovery rate about 70–90 %), several modifications are possible to increase yield, concentration, and convenience. Use elution buffer for one of the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acid can be eluted.

- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be approximately 30 % higher than with standard elution. The yield of eluted nucleic acid will be about 80 %.

- **High yield and high concentration:** Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85–100 % of bound nucleic acid is eluted in the standard elution volume at a high concentration.

- **Elution at 70 °C:** For certain sample types, heating the elution buffer to 70 °C increases the DNA yield.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi use storage.
at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications. Note: Elution Buffer BE (5 mM Tris/HCl, pH 8.5) provided with the kit does not contain EDTA.

For optimal performance of isolated DNA in downstream applications, we recommend eluting with the supplied elution buffer and storage, especially long term, at -20 °C. Freeze-thaw cycles will have no effect on most downstream applications. Possible exceptions are detection of trace amounts of DNA or long-range PCR (e.g., > 10 kbp). Multiple freeze-thaw cycles or storing DNA at 4 °C or room temperature may influence detection sensitivities or reaction efficiencies due to DNA shearing or adsorption to surfaces.
3 Storage conditions and preparation of working solutions

Attention: Buffers B3 and BW contain chaotropic salt! Wear gloves and goggles!

CAUTION: Buffers B3 and BW contain 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 for at least one year.

- During storage, especially at low temperatures, a white precipitate may form in Buffer T1 or B3. Such precipitates can be easily dissolved by incubating the bottle at 50–70 °C before use.

Before starting any NucleoSpin® Tissue protocol, prepare the following:

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

- **Proteinase K**: Add the indicated volume of Proteinase Buffer PB to dissolve lyophilized Proteinase K. Proteinase K solution is stable at -20 °C for at least 6 months.

<table>
<thead>
<tr>
<th>NucleoSpin® Tissue</th>
<th>10 preps</th>
<th>50 preps</th>
<th>250 preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>740952.10</td>
<td>740952.50</td>
<td>740952.250</td>
</tr>
<tr>
<td>Wash Buffer B5 (Concentrate)</td>
<td>6 mL Add 24 mL ethanol</td>
<td>12 mL Add 48 mL ethanol</td>
<td>50 mL Add 200 mL ethanol</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>6 mg Add 260 μL Proteinase Buffer</td>
<td>30 mg Add 1.35 mL Proteinase Buffer</td>
<td>2 x 75 mg Add 3.35 mL Proteinase Buffer to each vial</td>
</tr>
</tbody>
</table>
4 Safety instructions

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

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

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

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Hazard contents</th>
<th>GHS symbol</th>
<th>Hazard phrases</th>
<th>Precaution phrases</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3</td>
<td>Guanidine hydrochloride 36–50 % Guanidinhydrochlorid 36–50 % CAS 50–01–1</td>
<td>☢️</td>
<td>302, 319</td>
<td>264, 280, 301+312, 305+351+338, 330, P337+313</td>
</tr>
<tr>
<td>Liquid Proteinase K</td>
<td>Proteinase K, liquid 90–100 % Proteinase K flüssig 90–100 % CAS 39450–01–6</td>
<td>☢️ ☢️</td>
<td>317, 334</td>
<td>261, 272, 280, 302+352, 304+340, 333+313, 342+311, 363</td>
</tr>
</tbody>
</table>

Hazard phrases

H 226 Flammable liquid and vapour. Flüssigkeit und Dampf entzündbar.

H 302 Harmful if swallowed. Gesundheitsschädlich bei Verschlucken.

H 317 May cause an allergic skin reaction. Kann allergische Hautreaktionen verursachen.

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 336 May cause drowsiness or dizziness. Kann Schläfrigkeit und Benommenheit verursachen.
Genomic DNA from tissue

Precaution phrases

P 210  Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P 233  Keep container tightly closed.
Behälter dicht verschlossen halten.

P 261  Avoid breathing dust / fume / gas / mist / vapours / spray.
Einatmen von Staub / Rauch / Gas / Nebel / Dampf / Aerosol vermeiden.

P 264  Wash … thoroughly after handling.
Nach Handhabung … gründlich waschen.

P 272  Contaminated work clothing should not be allowed out of the workplace.
Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.

P 280  Wear protective gloves / protective clothing / eye protection / face protection.
Schutzhandschuhe / Schutzkleidung / Augenschutz / Gesichtsschutz 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 BERÜHRUNG MIT DER HAUT: Mit viel Wasser / … waschen.

P 304+340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
BEI EINATMEN: Die Person an die frische Luft bringen und für ungehinderte Atmung sorgen.

P 305+351+338 IF IN EYES: Rinse cautiously with water for several minuts. Remove contact lenses, if present and easy to do. Continue rinsing.

P 330  Rinse mouth.
Mund ausspülen.

P 333+313 IF skin irritation or rash occurs: Get medical advice/attention.
Bei Hautreizung oder -ausschlag: 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 363  Wash contaminated clothing before reuse.
Kontaminierte Kleidung vor erneutem Tragen waschen.

P 370+378 In case of fire: Use all extinguisher media to extinguish.
Bei Brand: Alle Löschmittel zum Löschen verwenden.

P 370+380 In case of fire: Evacuate area.
Bei Brand: Umgebung räumen.

P 403+235 Store in a well-ventilated place. Keep cool.
An einem gut belüfteten Ort aufbewahren. Kühl halten.

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

⚠️ 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.
5 Standard protocol for human or animal tissue and cultured cells

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Tissue

Cut 25 mg human or animal tissue into small pieces. Place the sample in a microcentrifuge tube (not provided). Proceed with step 2.

Samples that are difficult to lyse can be ground under liquid nitrogen or may be treated in a mechanical homogenizer (Polytron®, Ultra-Turrax®): Add 25 mg of tissue to a 1.5 mL microcentrifuge tube (not provided), add 50–75 μL phosphate buffered saline (PBS) and homogenize.

Cultured cells

Resuspend up to 10⁷ cells in a final volume of 200 μL Buffer T1. Add 25 μL Proteinase K solution and 200 μL Buffer B3. Vortex to mix and incubate the sample at 70 °C for 10–15 min. Proceed with step 4.

2 Pre-lyse sample

Add 180 μL Buffer T1 and 25 μL Proteinase K solution. Vortex to mix. Be sure that the samples are completely covered with lysis solution.

If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do not mix Buffer T1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digest in Buffer T1 without substrate.

+ 180 μL T1
+ 25 μL Proteinase K
Mix
Incubate at 56 °C until complete lysis is obtained (at least 1–3 h). Vortex occasionally during incubation or use a shaking incubator.

Samples can be incubated overnight as well. If RNA-free DNA is crucial for downstream applications, a RNase digest may be performed: Add 20 μL RNase A (10 mg/mL) solution (not included; see ordering information) and incubate for an additional 5 min at room temperature.

3 Lyse sample

Vortex the samples. Add 200 μL Buffer B3, vortex vigorously and incubate at 70 °C for 10 min. Vortex briefly.

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

4 Adjust DNA binding conditions

Add 210 μL ethanol (96–100 %) to the sample and vortex vigorously.

After addition of ethanol a stringy precipitate may become visible. This will not affect the DNA isolation. Be sure to load all of the precipitate on the column in the following step.

5 Bind DNA

For each sample, place one NucleoSpin® Tissue Column into a Collection Tube. Apply the sample to the column. Centrifuge for 1 min at 11,000 x g. Discard Collection Tube with flowthrough and place the column in a new Collection Tube (provided).

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6</strong> Wash silica membrane</td>
<td></td>
</tr>
<tr>
<td><strong>1st wash</strong></td>
<td>Add 500 μL Buffer BW. Centrifuge for 1 min at 11,000 x g. Discard flowthrough and place the column back into the Collection Tube.</td>
</tr>
<tr>
<td><strong>2nd wash</strong></td>
<td>Add 600 μL Buffer B5 to the column and centrifuge for 1 min at 11,000 x g. Discard flowthrough and place the column back into the Collection Tube.</td>
</tr>
<tr>
<td><strong>7</strong> Dry silica membrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centrifuge the column for 1 min at 11,000 x g. Residual ethanol is removed during this step.</td>
</tr>
<tr>
<td><strong>8</strong> Elute highly pure DNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Place the NucleoSpin® Tissue Column into a 1.5 mL microcentrifuge tube (not provided) and add 100 μL Buffer BE. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g. For alternative elution procedures see section 2.3.</td>
</tr>
</tbody>
</table>
6 Support protocols

6.1 Support protocol for mouse or rat tails

Before starting the preparation:
- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Cut two 0.6 cm-pieces of mouse tail and place them in a 1.5 mL centrifuge tube (not provided).

If processing rat tails, one 0.6 cm-piece is sufficient.

2 Pre-lyse sample

Add 180 μL Buffer T1 and 25 μL Proteinase K and vortex. Incubate at 56 °C overnight or until complete lysis is obtained. Lysis time can substantially be reduced down to approximately one hour if the tissue is broken up mechanically (e.g., if the tissue is cut into very small pieces before lysis).

Vortex occasionally during incubation or use a shaking water bath. To remove residual bones or hair, centrifuge for 5 min at high speed (e.g., 11,000 x g). Transfer 200 μL supernatant to a new tube.

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-digest in Buffer T1 without substrate.

3 Lyse sample

Add 200 μL Buffer B3 to the lysate and vortex vigorously.

Buffer B3 and ethanol (see step 4) can be premixed before addition to the lysate.

Adjust DNA binding conditions

Add 210 μL ethanol to the lysate and vortex vigorously.

Proceed with step 5 of the standard protocol (see section 5).
6.2 Support protocol for bacteria

Before starting the preparation:

• Check if Buffer B5 and Proteinase K were prepared according to section 3.
• Set an incubator or water bath to 56 °C.

1 Prepare sample

Up to 1 mL of bacterial culture can be used for the preparation depending on, for example, density of culture, culture medium, and bacterial strain.

Centrifuge up to 1 mL culture for 5 min at 8,000 x g. Remove supernatant.

2 Pre-lyse sample

Resuspend the pellet in 180 μL Buffer T1 by pipetting up and down. Add 25 μL Proteinase K. Vortex vigorously and incubate at 56 °C until complete lysis is obtained (at least 1–3 h). Vortex occasionally during incubation or use a shaking incubator.

Samples can be incubated overnight as well.

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 an additional 5 min at room temperature.

Hard-to-lyse bacteria: Some strains, especially Gram-positive bacteria, are more difficult to lyse. In such cases, a preincubation with a lytic enzyme is necessary: Resuspend the pelleted cells in 20 mM Tris/HCl; 2 mM EDTA; 1 % Triton X-100; pH 8 (instead of Buffer T1) supplemented with a final concentration of 20 mg/mL lysozyme or 0.2 mg/mL lysostaphin and incubate for 30–60 min at 37 °C. Add 25 μL Proteinase K, incubate at 56 °C until complete lysis is obtained.

Proceed with step 3 of the standard protocol (see section 5).
6.3 Support protocol for yeast

Before starting the preparation:

• Check if Buffer B5 and Proteinase K were prepared according to section 3.

• Check that sorbitol buffer and lyticase or zymolase (not provided with the kit) is available for sample pre-lysis.

• Set an incubator or water bath to 30 °C and 56 °C.

1 Prepare sample

Harvest 3 mL YPD yeast culture (OD₆₀₀ ≤ 10) by centrifugation for 10 min at 5,000 x g. Wash the cells once with 1 mL 10 mM EDTA, pH 8. Remove the supernatant and pellet the cells by centrifugation (5,000 x g, 10 min).

2 Pre-lyse sample

Resuspend the pellet in 600 μL sorbitol buffer (1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris/HCl pH 7.5; 35 mM β-mercaptoethanol). Add 50 U lyticase or zymolase*. Incubate at 30 °C for 30 min. This step degrades the yeast cell wall creating spheroplasts. Spheroplast formation may be checked microscopically. Centrifuge the mixture for 10 min at 2,000 x g remove supernatant and resuspend the pelleted spheroplasts in 180 μL Buffer T1. Add 25 μL Proteinase K solution and vortex vigorously. Incubate at 56 °C until complete lysis is obtained (at least 1–3 h). Vortex occasionally during incubation or use a shaking water bath.

Samples can be incubated overnight as well.

* Other protocols use 5–200 U lyticase or zymolase depending on enzyme quality or brand. Increasing the enzyme concentration may be required if spheroplasts are not formed.

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 an additional 5 min at room temperature.

Proceed with step 3 of the standard protocol (see section 5).
6.4 Support protocol for dried blood spots (e.g., NucleoCards, FTA® cards, Guthrie cards)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Cut out one or two dried blood spots as accurately as possible. Cut spots into small pieces and place them in a 1.5 mL microcentrifuge tube (not provided).

The area of the dried blood spots should be between 15 and 30 mm².

2 Pre-lyse sample

Add 180 μL Buffer T1 and mix by vortexing. Place the samples in a water bath or heating block and heat for 10 min at 94 °C. Let the sample cool down. Add 25 μL Proteinase K solution. Spin the samples briefly, vortex and incubate at 56 °C for 1 h. Vortex occasionally during incubation or use a shaking water bath.

Make sure that the samples are completely covered with lysis buffer during incubation.

3 Lyse sample

Add 200 μL Buffer B3, vortex vigorously to mix and incubate at 56 °C for 10 min.

Proceed with step 4 of the standard protocol (see section 5).
6.5 Support protocol for genomic DNA and viral DNA from blood samples

Before starting the preparation:

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

1 Prepare sample
   Not necessary

2 Pre-lyse sample
   Not necessary

3 Lyse blood sample
   Pipette 25 μL Proteinase K and up to 200 μL blood, buffy coat, or body fluid sample (equilibrated to room temperature) into 1.5 mL microcentrifuge tubes (not provided).

   For sample volumes less than 200 μL, add PBS to adjust the volume to 200 μL. If purifying DNA viruses, we recommend starting with 200 μL serum or plasma. If cultured cells are used, resuspend up to 5 x 10^6 cells in a final volume of 200 μL PBS.

   Add 200 μL Buffer B3 to the samples and vortex the mixture vigorously (10–20 s).

   Incubate samples at room temperature for 5 min. Mix.

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

   The lysate should become brownish during incubation with Buffer B3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.

4 Adjust DNA binding conditions
   Add 210 μL ethanol (96–100 %) to each sample and vortex again.

5 Bind DNA
   For each preparation, take one NucleoSpin® Tissue Column placed in a Collection Tube and load the sample. Centrifuge 1 min at 11,000 x g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000 x g). Discard Collection Tube with flowthrough and place the column in a new Collection Tube (provided).

   Proceed with step 6 (Wash silica membrane) of the standard protocol (see section 5).
6.6 Support protocol for hair roots

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Cut off the hair roots from the hair sample (up to 100) and collect them in a 1.5 mL microcentrifuge tube (not provided).

2 Pre-lyse sample

Add 180 μL Buffer T1 to the hair roots and freeze the samples in liquid nitrogen. Thaw samples in a 56 °C water bath. Repeat this procedure 4 times. Add 25 μL Proteinase K solution, mix by vortexing, and incubate 6–8 h or overnight at 56 °C. Use a shaking water bath or vortex occasionally.

Proceed with step 3 of the standard protocol (see section 5).
6.7 Support protocol for paraffin-embedded tissue

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check if xylene or n-octane is available.
- Set an incubator or water bath to 37 °C and 56 °C.

1 Prepare sample

Prepare small sections (up to 25 mg) 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.

Add 1 mL 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).

Proceed with step 2 of the standard protocol (see section 5).
6.8 Support protocol for genomic DNA from stool

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check if TE buffer is available.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Add **250 mg feces** to 1 mL TE buffer (10 mM Tris/Cl; 1 mM EDTA, pH 8). Resuspend the sample by vigorous vortexing (**30 s**).

Centrifuge the sample for **15 min** at **4,000 x g**. Discard supernatant.

Resuspend the pellet in **0.2–1 mL Buffer T1**. Use as much buffer as necessary for good resuspension of the sample.

*The prepared pellet contains, among other constituents, cells from the digestive tract and bacteria.*

Transfer **200 μL** of the **resuspended sample** to a new microcentrifuge tube.

Proceed with the addition of 25 μL Proteinase K in step 2 of the standard protocol (see section 5).

*Human cells, bacterial cells, and cells of pathogens in the stool lyse during the incubation step at 56 °C with Proteinase K with different efficiency. For the detection of cells that are difficult to lyse (e.g., some bacteria and parasites) it can be beneficial to perform an additional incubation at increased incubation temperature (up to 95 °C; 5–10 min). DNA yield will often be higher with such an additional incubation step at high temperature. However, note that the ratio of human to non-human DNA will typically change due to the increased release of bacterial/pathogen DNA.*
6.9 Support protocol for viral DNA (e.g., CMV) from stool

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Prepare 0.9 % NaCl.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Suspend the stool sample in **0.9 % NaCl** (ca. 0.5 g in max. 4 mL).

**Centrifuge** aliquots of the stool sample for **5 min** at **800 x g** at RT (e.g., 4 mL: 4 x 1 mL in a 1.5 mL microcentrifuge tube). Carefully reunite supernatant (do not touch the pellet).

Filtrate supernatant through 0.22–0.45 μm sterile filter. Fractionate the filtrate and centrifuge for **1 min** at **11,000 x g**.

2 Pre-lyse sample

Carefully remove the supernatant by decanting. Add **400 μL Buffer T1** and **35 μL Proteinase K** and mix by vortexing.

3 Lyse sample

Add **400 μL Buffer B3** and mix by vortexing. Incubate for at least **30 min** at 70 °C.

4 Adjust DNA binding conditions

Add **420 μL ethanol (96–100 %)** and mix by vortexing.

5 Bind DNA

For each sample, place one **NucleoSpin® Tissue Column** into a Collection Tube. Load the NucleoSpin® Tissue Column successively. Centrifuge for **1 min** at **4,500 x g**. Discard the flowthrough and place the column back into the Collection Tube.

*If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.*
6 Wash silica membrane

1st wash
Add 600 μL Buffer BW. Centrifuge for 1 min at 4,500 x g. Discard flowthrough and place the column back into the Collection Tube.

2nd wash
Add 600 μL Buffer B5 to the column and centrifuge for 1 min at 4,500 x g. Discard flowthrough and place the column back into the Collection Tube.

3rd wash
Add 600 μL Buffer B5 to the column and centrifuge for 2 min at 11,000 x g. Discard flowthrough.

7 Dry silica membrane
Place the NucleoSpin® Tissue Column into a new Collection Tube and incubate with open lid for 1–2 min at 70 °C.
Residual ethanol is removed during this step.

8 Elute highly pure DNA
Place the NucleoSpin® Tissue Column into a 1.5 mL microcentrifuge tube (not provided) and add 100 μL Buffer BE. Incubate with closed lid for 3–5 min.
Centrifuge for 1 min at 4,500 x g.

For alternative elution procedures see section 2.3.

Use 10 μL DNA extract for a 20 μL PCR reaction mix.
Add inhibition control mix (10 μL DNA extract with human DNA) and amplify with for example actin-/β-globin-/ or other human specific primer.
6.10 Support protocol for detection of *Mycobacterium tuberculosis* or *Legionella pneumophila* in sputum or bronchoalveolar lavage

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.
- Prepare N-acetyl cystein/NaOH (2 g NaOH; 1.45 g sodium citrate; 0.5 g N-acetyl cystein. Add water to 100 mL).

1 Prepare sample

Add 200–500 μL sputum or bronchoalveolar lavage to an equal volume N-acetyl cystein/NaOH. Vortex gently to mix.

Incubate the mixture for 25 min at room temperature with shaking.

Adjust the volume to 25 mL with sterile water.

Centrifuge for 30 min at 4,000 x g. Discard the supernatant.

Resuspend the pellet in 0.5–1 mL Buffer T1 (depending on sample viscosity).

Transfer 200 μL of the resuspended sample to a new microcentrifuge tube (not provided).

Proceed with step 2 of the standard protocol, see section 5 (addition of Proteinase K and incubation).
6.11 Support protocol for detection of EHEC bacteria in food (e.g., fresh cows‘ milk)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Prepare mTSB and 3.2 M sodium acetate.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

To a sterile 1 liter flask, add **25 mL milk** and **225 mL prewarmed (37 °C) mTSB medium** (supplied with Novobiocin). Incubate the mixture in a shaking water bath for **5–6 h** or overnight at 37 °C.

Preparation of mTSB medium: 30 g Tryptic Soy Broth (Gibco), 1.5 g bile salts No. 3 (Oxoid), 1.5 g KH2PO4. Add 900 mL H2O. Filter the medium and adjust the pH with 2 M NaOH to 7.4. Add water to 1 liter and autoclave for 15 min at 121 °C.

Centrifuge **100 mL culture** for **40 min** at **6,000 x g**.

Gently pour off the supernatant and resuspend the pellet in 2 mL sterile water. Centrifuge for **10 min** at **10,000 x g**.

2 Pre-lyse sample

Resuspend the pellet in **180 μL Buffer T1** and add **25 μL Proteinase K** solution.

Carry out the standard protocol, beginning with step 3 (see section 5).

After elution of the DNA, proceed with the following step.

Precipitate the DNA by adding **20 μL 3.2 M sodium acetate** and **400 μL ethanol** to **200 μL eluate**. Centrifuge for **30 min** at **11,000 x g**. Discard supernatant and wash the pellet with **1 mL 70 % ethanol** and resuspend in **10 μL sterile water**.
6.12 Support protocol for purification of bacterial DNA (e.g., *Chlamydia trachomatis*) from cultures, biological fluids, or clinical specimens

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Isolation of bacterial DNA from bacterial cultures or biological fluids: Pellet bacteria by centrifugation for 5 min at 13,000 x *G* and proceed with step 2 of the standard protocol, see section 5.

Isolation of bacterial DNA from eye, nasal or pharyngeal swabs: Collect samples, add 2 mL PBS containing a common fungicide, and incubate for several hours at room temperature. Pellet bacteria by centrifugation for 5 min at 13,000 x *g*. Discard supernatant.

Proceed with step 2 of the standard protocol (see section 5).
6.13 Support protocol for purification of bacterial DNA (e.g., *Borrelia burgdorferi*) from urine

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Centrifuge 1 mL urine sample at **13,000 x g for 30 min**. Discard supernatant, add again 1 mL urine sample to the pellet and centrifuge at **13,000 x g for 30 min**. This step a can be repeated up to three times.

*The sample material should be fresh. Storage at -20 °C to -80 °C is only recommended for a couple of days. After thawing incubate the sample at 40 °C until all precipitates are dissolved. Urine tends to form precipitates when stored at low temperatures.*

Proceed with step 2 of the standard protocol (see section 5).
6.14 Support protocol for purification of viral DNA (e.g., CMV) from urine

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Centrifuge aliquots of the urine sample for 10 min at full speed (e.g., 4 mL: 4 x 1 mL in a 1.5 mL microcentrifuge tube). Carefully decant supernatant.

*If frozen urine samples are used precipitates may appear after thawing, which must be dissolved before the centrifugation step. This can be done by a 30 min incubation step at 37–40 °C. If a complete solution is not achieved let the precipitate sediment and proceed with step 1 of the support protocol using only the supernatant.*

2 Pre-lyse sample

Resuspend the pellet in 180 μL Buffer T1 and 25 μL Proteinase K.

Resuspend the first pellet in 180 μL Buffer T1 and 25 μL Proteinase K. Transfer the resuspended solution of the first tube to the second tube and the resuspended solution of the second tube to the third tube and so on. Finally continue with step 3.

3 Lyse sample

Add 200 μL Buffer B3, vortex and incubate at least for 20 min at 70 °C.

4 Adjust DNA binding conditions

Add 210 μL ethanol (96–100 %) to the sample and vortex vigorously.

5 Bind DNA

For each sample, place one NucleoSpin® Tissue Column into a Collection Tube. Apply the sample to the column. Centrifuge for 1 min at 4,500 x g. Discard the flowthrough and place the column back into the Collection Tube.

6 Wash silica membrane

1<sup>st</sup> wash

Add 500 μL Buffer BW. Centrifuge for 1 min at 4,500 x g. Discard flowthrough and place the column back into the Collection Tube.

2<sup>nd</sup> wash

Add 600 μL Buffer B5 to the column and centrifuge for 2 min at 11,000 x g. Discard flowthrough and place the column back into the Collection Tube.
7 **Dry silica membrane**

Incubate with open lid for **1–2 min** at **70 °C**.

Residual ethanol is removed during this step.

8 **Elute highly pure DNA**

Add **70 μL Buffer BE**, close the lid and incubate for further **3–5 min**. Centrifuge for **1 min** at **4,500 x g**.
6.15 Support protocol for purification of genomic DNA from insects

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Homogenize not more than 50 mg insects under liquid nitrogen and transfer the powder into a 1.5 mL microcentrifuge tube (not provided).

Proceed with step 2 of the standard protocol (see section 5) with addition of Proteinase K.
6.16 Support protocol for purification of genomic DNA from dental swabs

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Place swab material (paper, cotton, brushes, plastic) in a 1.5 mL microcentrifuge tube (not provided).

2 Pre-lyse sample

Add 180 μL Buffer T1 and 25 μL Proteinase K to each sample. Close the microcentrifuge tube and spin briefly for 15 s at 1,500 x g in order to submerge the swab material completely. Incubate at room temperature for 5 min. Vortex the tube vigorously for 15 s and spin briefly for 15 s at 1,500 x g.

Incubate the tubes at 70 °C in an incubator for 10 min. Place a weight on top of the tube in order to prevent the caps from popping off. Shift the temperature to 95 °C for 5 min. Spin briefly for 15 s at 1,500 x g to collect any sample from the lids. Open the microcentrifuge tubes.

*Skip incubation at 95 °C, depending on the bacterial strain to be detected.*

2a Separate lysis solution from dental swabs

**Alternative A:**

Transfer the swab tip into a NucleoSpin® Forensic Filter (not provided; see ordering information). Cut off swab shaft. Centrifuge the NucleoSpin® Forensic Filter for 1 min at 11,000 x g. Discard the NucleoSpin® Forensic Filter. Continue with flowthrough.

**Alternative B:**

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 flowthrough.

**Alternative C:**

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.

Proceed with step 3 of the standard protocol (see section 5).
6.17 Support protocol for purification of genomic DNA from buccal swabs

Before starting the preparation:

• Check if Buffer B5 and Proteinase K were prepared according to section 3.
• Check if PBS is available.
• Set an incubator or water bath to 56 °C.

1 Prepare sample

Collect the samples with cotton, dacron® (Daigger), or C.E.P. swabs (Gibco BRL). Scrape firmly against the inside of 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 sample._

2 Pre-lyse sample

Place the dry swab material in 2 mL microcentrifuge tubes (not provided). Add 400–600 μL PBS and 25 μL Proteinase K solution to the swabs.

The volume of PBS is depending on the type of swab used: for cotton and dacron swabs, 400 μL are sufficient; for C.E.P. swabs, 600 μL are necessary.

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

2a Separate lysis solution from buccal swabs

**Alternative A:**

Transfer the swab tip into a NucleoSpin® Forensic Filter (not provided; see ordering information). Cut off swab shaft. Centrifuge the NucleoSpin® Forensic Filter for 1 min at 11,000 x g. Discard the NucleoSpin® Forensic Filter. Continue with flowthrough.

**Alternative B:**

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 flowthrough.

**Alternative C:**

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 Buffer B3** (400 or 600 μL; depending on the swab type/volume of PBS buffer used) and vortex vigorously. Incubate the samples at **70 °C** for **10 min**.

*Note: Depending on the number of preparations, additional Buffer B3 might be needed (see ordering information).*

4 **Adjust DNA binding conditions**

Add **one volume 96–100 % ethanol** (400 or 600 μL, depending on the swab type) to each sample and mix by vortexing.

5 **Bind DNA**

Transfer **600 μL of the samples** from the 2 mL microcentrifuge tubes into **NucleoSpin® Tissue Columns**. Centrifuge at **11,000 x g** for **1 min**. If the samples are not drawn through completely, repeat the centrifugation. Discard flowthrough.

Place the columns back into the Collection Tubes and repeat step 5 once or twice, depending on the lysis volume.

When all of the lysate has been applied to the columns, discard Collection Tube and place the column in a new Collection Tube. Proceed with step 6 of the standard protocol (section 5).
7 Appendix

7.1 Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause and suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete lysis</td>
<td>• Sample not thoroughly homogenized and mixed with Buffer T1/Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer T1.</td>
</tr>
<tr>
<td></td>
<td>• Decreased Proteinase K activity: Store dissolved Proteinase K at -20 °C for 6 months.</td>
</tr>
<tr>
<td>Reagents not applied properly</td>
<td>• Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add ethanol to the lysates before loading them onto the columns.</td>
</tr>
<tr>
<td>No or poor DNA yield</td>
<td>Suboptimal elution of DNA from the column</td>
</tr>
<tr>
<td></td>
<td>• For certain sample types, preheat Buffer BE to 70 °C before elution. Apply Buffer BE directly onto the center of the silica membrane.</td>
</tr>
<tr>
<td></td>
<td>• Elution efficiencies decrease dramatically, if elution is done with buffers with a pH &lt; 7.0. Use slightly alkaline elution buffers like Buffer BE (pH 8.5).</td>
</tr>
<tr>
<td></td>
<td>• Especially when expecting high yields from large amounts of material, we recommend elution with 200 μL Buffer BE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation.</td>
</tr>
<tr>
<td>Poor DNA quality</td>
<td>Incomplete lysis</td>
</tr>
<tr>
<td></td>
<td>• Sample not thoroughly homogenized and mixed with Buffer T1/Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer T1.</td>
</tr>
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<td></td>
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<tr>
<td>Problem</td>
<td>Possible cause and suggestions</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Poor DNA quality</td>
<td><em>Reagents not applied properly</em>&lt;br&gt;• Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add ethanol to the lysates before loading them on the columns.</td>
</tr>
<tr>
<td>RNA in sample</td>
<td><em>RNA in sample</em>&lt;br&gt;• If RNA-free DNA is desired, add 20 μL of RNase A solution (10 mg/mL; not supplied with the kit) before addition of Buffer B3 and incubate at 37 °C for 5 min.</td>
</tr>
<tr>
<td>Too much sample material used</td>
<td><em>Too much sample material used</em>&lt;br&gt;• Do not use more sample material than recommended (25 mg for most tissue types). If insoluble material like bones or hair remains in the lysate, spin down the debris and transfer the clear supernatant to a new microcentrifuge tube before proceeding with addition of Buffer B3 and ethanol. The use of the NucleoSpin® Filter prior column loading prevents column clogging.</td>
</tr>
<tr>
<td>Clogged columns</td>
<td><em>Incomplete lysis</em>&lt;br&gt;• Sample not thoroughly homogenized and mixed with Buffer T1/Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer T1.</td>
</tr>
<tr>
<td>Reagents not applied properly</td>
<td><em>Reagents not applied properly</em>&lt;br&gt;• Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add ethanol to the lysates before loading them on the columns.</td>
</tr>
<tr>
<td>Suboptimal performance of genomic DNA in enzymatic reactions</td>
<td><em>Carry-over of ethanol or salt</em>&lt;br&gt;• Make sure to centrifuge ≥ 1 min at 11,000 x g in order to remove all of ethanolic Buffer B5 before eluting the DNA. If, for any reason, the level of Buffer B5 has reached the column outlet after drying, repeat the centrifugation.</td>
</tr>
</tbody>
</table>
Genomic DNA from tissue

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause and suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suboptimal performance of genomic DNA in enzymatic reactions (continued)</td>
<td><strong>Contamination of DNA with inhibitory substances</strong></td>
</tr>
<tr>
<td></td>
<td>• Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer BE.</td>
</tr>
<tr>
<td></td>
<td>• If the $A_{260}/A_{280}$ ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume Buffer B3 plus 1 volume ethanol (96–100 %) to the eluate. Load the mixture onto a NucleoSpin® Tissue Column and proceed with step 5 of the standard protocol (see section 5).</td>
</tr>
</tbody>
</table>

### 7.2 Ordering information

<table>
<thead>
<tr>
<th>Product</th>
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<td>NucleoSpin® Tissue</td>
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<td>10/50/250 preps</td>
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<tr>
<td>NucleoSpin® Tissue XS</td>
<td>740901.10/.50/.250</td>
<td>10/50/250 preps</td>
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<td>NucleoSpin® DNA RapidLyse</td>
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<td>10/50/250 preps</td>
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<tr>
<td>NucleoSpin® Blood</td>
<td>740951.10/.50/.250</td>
<td>10/50/250 preps</td>
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<td>Buffer T1</td>
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<td>Buffer B3</td>
<td>740920</td>
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<td>Buffer B5 Concentrate (for 100 mL Buffer B5)</td>
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<td>20 mL</td>
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<td>Buffer BW</td>
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<td>Proteinase K</td>
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<td>NucleoSpin® Microbial DNA</td>
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<tr>
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<td>(40–400 μm glass beads; recommended for bacteria)</td>
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<td>(1–3 mm corundum; recommended for yeasts)</td>
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<td>NucleoSpin® Bead Tube Type D</td>
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<td>(3 mm steel balls; recommended for insects)</td>
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<td>(40–400 μm glass beads and 3 mm steel balls; recommended for hard-tolyse bacteria within insect or tissue samples)</td>
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<td>NucleoSpin® DNA FFPE XS</td>
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Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information.
7.3 Product use restriction / warranty

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

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

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 SUITABLE FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).**

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL’s sole obligation and the customer’s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or
components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

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The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

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